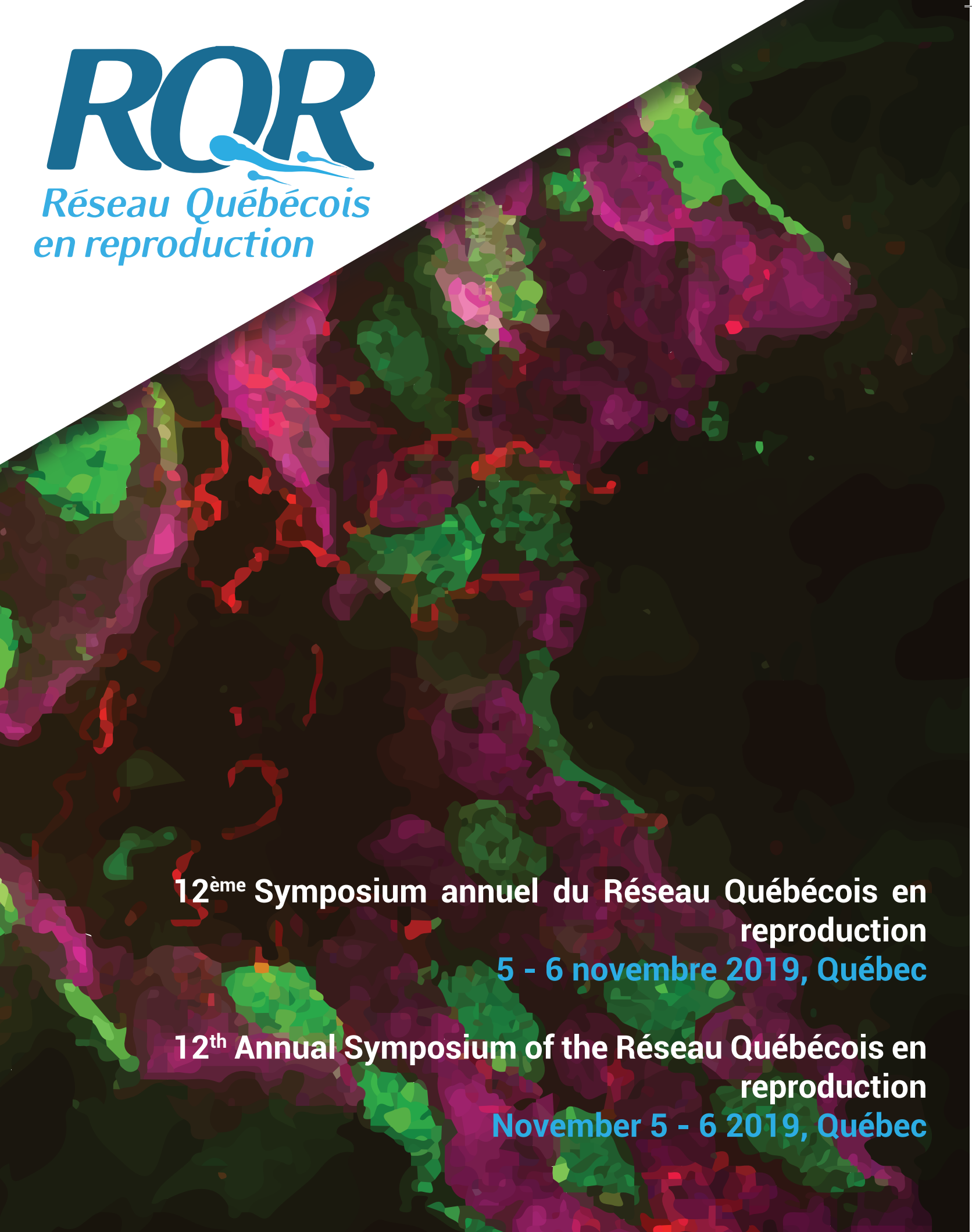
RQR

Réseau Québécois en reproduction



12ème Symposium annuel du Réseau Québécois en reproduction

5 - 6 novembre 2019, Québec

12th Annual Symposium of the Réseau Québécois en reproduction

November 5 - 6 2019, Québec

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

On behalf of the RQR Executive Committee, it is my pleasure to welcome you to the 12th Annual Symposium of the Réseau Québécois en reproduction (RQR). RQR, which is generously supported by the FRQNT, represents the largest collection of reproductive biologists in Canada and the Symposium is our signature event each year. It provides an opportunity for our community to come together to share our latest and most exciting results, to network, and to learn from distinguished colleagues from around the globe.

This year, the RQR is pleased to welcome a total of five invited speakers : Dr. Hélène Jammes (Institut National de Recherche Agronomique, France), the Axis 1 speaker, will discuss "Epigenetic biomarkers in cows". Representing Axis 2, Dr. Carole Yauk (Carleton University, Ottawa) will present, "The legacy of parental exposures to toxicants: Characterizing chemically-induced heritable genetic effects". Axis 3 invitee Dr. Richard Behringer (MD Anderson Cancer Center, Houston, TX) will describe his work on the "Genetic regulation of reproductive organ development".

At least year's Symposium, with the guidance of the Knowledge Transfer Committee, we launched a new initiative to host an annual lecture with a focus on KT in animal production. This year, we are pleased to welcome Dr. Olivier D'Amours (MAPAQ), who will deliver the Animal Production Seminar on "The adoption of innovation and the funding of research at the Quebec Ministry of Agriculture, Fisheries, and Food". Finally, with the collaboration of the RQR Diversity Committee, we are pleased to welcome our first lecturer on Equity, Equality, Diversity, and Inclusion. Dr. Line Chamberland (UQAM) will discuss, "Inclusion of sexual and gender diversity in the workplace: Access and challenges." In addition to the invited speakers, we look forward to learning from our trainees in 15 oral presentations and 58 posters.

Again, welcome to the RQR symposium 2019 and the Centre des Congrès in Québec City. We sincerely hope you will enjoy the next two days of science, networking, and collaboration.

All the best, Daniel Bernard, RQR Director

À tous les membres du RQR, étudiants et invités,

Au nom du Comité exécutif du RQR, il me fait plaisir de vous accueillir au 12ème Symposium annuel du Réseau Québécois en reproduction (RQR). Le RQR bénéficie du généreux soutien du FRQNT, ce qui rend possible cet évènement annuel phare. Notre symposium réunit le plus grand regroupement de biologistes de la reproduction au Canada. Il offre à notre communauté l'occasion de partager les résultats les plus récents et les plus intéressants, de développer des réseaux en plus d'apprendre de distingués collègues provenant du monde entier.

Cette année, le RQR est heureux d'accueillir cinq conférenciers-invités : Dre Hélène Jammes (Institut national de recherche agronomique, France), conférencière de l'axe 1, abordera les « Biomarqueurs épigénétiques chez la vache». Dre Carole Yauk (Université Carleton, Ottawa) présentera « L'héritage des expositions parentales à des substances toxiques » et Dr Richard Behringer (MD Anderson Cancer Center, Houston, TX), invité de l'Axe 3, décrira ses travaux sur la « Régulation génétique du développement des organes reproducteurs».

Lors du Symposium précédent, avec le Comité de mobilisation des connaissances, nous avions lancé une nouvelle initiative visant à organiser une conférence annuelle axée sur la production animale. Cette année, nous avons donc le plaisir de recevoir le Dr Olivier D'Amours (MAPAQ). Il animera le séminaire intitulé « Adoption de l'innovation et le financement de la recherche au ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec ». Enfin, avec la collaboration du Comité de la diversité, nous accueillons pour une première fois une conférencière sur les thématiques de l'équité, l'égalité, la diversité et l'inclusion : Dre Line Chamberland (UQAM). Dre Chamberland abordera « L'inclusion de la diversité sexuelle et de genre dans le lieu de travail: accès et défis».

En plus des conférenciers invités, nous attendons avec intérêt d'en apprendre davantage de nos étudiants, grâce 15 présentations orales et de 58 affiches qui nous seront présentées durant cet événement.

Une nouvelle fois, soyez les bienvenus au Symposium 2019 du RQR et au Centre des Congrès à Québec. Nous espérons sincèrement que vous apprécierez les deux prochains jours consacrés à la Science, au réseautage et à la collaboration.

Bon symposium ! Daniel Bernard, Directeur du RQR

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Program of the 12th Symposium of the Réseau Québécois en reproduction

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10h00 – 10h15	Mot de bienvenue *Welcome*
10h15 – 11h30	Présentations: Session I *Presentations: Session I*
11h30 – 13h00	**Dîner** ***Lunch***
13h00 – 14h00	Présentations: Session II *Presentations: Session II*
14h00 – 14h45	Séminaire en production animale/ *Animal production seminar* *** Olivier d'Amours (MAPAQ) *L'adoption de l'innovation et le financement de la recherche au ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec*
14h45 – 16h15	**Pause-café et session d'affiches I** ***Coffee Break and Poster Session I***
16h15 – 17h15	Conférencière invitée /Invited speaker *** Hélène Jammes (Institut National de Recherche Agronomique, France) *Biomarqueurs épigénétiques chez le bovin.*
17h15 – 17h30	Remise du Prix MdC du RQR *RQR KT Award Presentation*
17h30 – 19h00	**RQR Cocktail** ***RQR Cocktail***

Mercredi 6 novembre - *Wednesday November 6th*

9h00 – 10h30	**Session d'affiches II** ***Poster Session II***
10h30 – 11h30	Conférencière invitée/*Invited Speaker* *** Carole Yauk (Department of Biology, Carleton University) *The legacy of parental exposures to toxicants: characterizing chemically induced heritable genetic effects*
11h30 – 12h00	Atelier sur l'équité, l'égalité, la diversité et l'inclusion *** Line Chamberland, Département de sexologie, UQAM *L'inclusion de la diversité sexuelle et de genre en milieu de travail : Les acquis et les défis*
12h00 – 13h30	**Dîner** ***Lunch***
13h30 – 15h00	Présentations: Session III *Presentations: Session III*
15h00 – 15h15	**Pause-café** ***Coffee Break***
15h15 – 16h15	Conférencier invité/*Invited Speaker* *** Richard Behringer (University of Texas MD Anderson Cancer Center) *Genetic regulation of reproductive organ development*
16h15 – 16h30	**Remise des prix – Fermeture de la session** ***Awards presentation – Closing of session***

Présentations – Presentations : Session I
5 novembre – November 5th
10h15 – 11h30

Présidente – Chair : André Marques
Co-présidente – Co-Chair : Karla Elena Herrera Hidalgo

I. **Single cell RNA sequencing reveals developmental origins of mouse oviduct epithelial cell heterogeneity**
Matthew Ford, Postdoctoral Fellow, McGill University (Page 6)
10h15 – 10h30

II. **Screening for the safety of emerging plasticizers and flame-retardants**
Sarah Tardif, MSc Student, INRS-Institut Armand-Frappier, (Page 7)
10h30 – 10h45

III. **Hedgehog signaling is involved in Wolffian duct/epididymis development**
Maïra Bianchi Rodrigues Alves, Postdoctoral Fellow, Université Laval (Page 8)
10h45 – 11h00

IV. **Functional Studies of tribbles homolog 2 (TRIB2) in ovarian granulosa cells**
Aly Warma, PhD Student, Université de Montréal (Page 9)
11h00 – 11h15

V. **Primary cilia in efferent ductules**
Céline Augière, Postdoctoral Fellow, Université de Laval (Page 10)
11h15 – 11h30

Single cell RNA sequencing reveals developmental origins of mouse oviduct epithelial cell heterogeneity

Matthew J Ford[1], Alain Pacis[2], Helen Maunsell[1], Keerthana Harwalkar[1], Yu Chang Wang[2], Dunarel Badescu[2], Katie Teng[1], Nobuko Yamanaka[1], Ioannis Ragoussi[1,2], Yojiro Yamanaka[1]

[1]Goodman Cancer Research Centre, Department of Human Genetics, McGill University, Montreal, Quebec, Canada, [2]Genome Quebec Innovation Centre, McGill University, Montreal, Quebec, Canada

The oviduct, known as the fallopian tube in humans, is a conduit connecting the ovary to the uterus. Oviduct epithelial cells and their secretions provide an environment to support sperm transport, fertilization and preimplantation embryonic development. Histochemical analysis has identified two functional epithelial cell types, multi-ciliated and secretory. Considering the diverse array of events occurring along the length of the oviduct during reproduction it is highly likely further diversification of these two known cell types exists.

Using a *Pax2-GFP* BAC transgenic mouse line, which faithfully reports *Pax2* expression, we have identified the absence of *Pax2* expression in the distal region of the oviduct in adult mice and throughout development of the Müllerian duct. To investigate the regional heterogeneity further we conducted two single cell RNA sequencing experiments using epithelial cells isolated prior to epithelial differentiation at postnatal day 4 and cells from adult mice. Five epithelial cell types were identified in adult mice and located to different regions of the oviduct with differential expression of secreted proteins implicated in reproduction. The expression profiles of undifferentiated epithelial cells confirmed proximal-distal specification occurred before differentiation and identified regionally specific expression of *Wt1* complimentary to *Pax2* expression throughout Müllerian duct development.

In this study, we have identified heterogeneous gene expression within ciliated and secretory epithelial cells and spatially mapped these subtypes to different locations in the oviduct. Patterning of the distal and proximal regions of the oviduct from the initiation of Müllerian duct formation suggests two distinct origins of oviduct epithelial cells.

Screening for the safety of emerging plasticizers and flame-retardants

Sarah Tardif[1], Bernard Robaire[2], Géraldine Delbès[1]

[1]INRS, Armand Frappier Santé Biotechnologie, [2]McGill University, Department of Pharmacology and Therapeutic

The use of bis(2-ethylhexyl) phthalate (DEHP) and 2,2'4,4'-tetrabromodiphenyl ether (BDE-47), belonging respectively to the phthalate and the flame-retardant families, has been regulated, in part because of their endocrine disruption activities. New chemicals that are already detected in consumer products and human matrices are replacing them. However, their potential toxicity is poorly characterized.

We propose to screen the toxicity and endocrine disruptor potential of replacements chemicals and to compare their effects with those of legacy products. To this end, we use the organ culture of fetal rat testes, a tissue particularly sensitive to steroid hormone modulations. This method has already been used to evaluate the effects of MEHP, the main active metabolite of DEHP. Basal and LH-stimulated testosterone secretions have been measured from culture media by ELISA. Three replacements for DEHP/MEHP and two BDE-47 substitutes have been selected based on their cytotoxicity on testicular cell lines. Our results show that MEHP induces a significant decrease in basal and LH-stimulated testosterone secretions of fetal rat testes, while its replacements, 2,2,4-trimethyl 1,3-pentanediol diisobutyrate, diisononyl-phthalate and diisodecyl adipate, had no effect ($n \geq 3$). In parallel, neither BDE-47 nor its substitutes, (tributoxyethyl phosphate and isopropylated triphenyl phosphate), had any impact on testosterone secretions ($n \geq 4$). Morphology, number and proliferation rate of the main cell types of the testis will be analysed by immunofluorescence after a three- day culture period. This study will allow for the identification of less toxic alternatives and provide essential information regarding the need for their regulation.

Funded by CIHR.

Hedgehog signaling is involved in Wolffian duct/epididymis development

Maíra Bianchi Rodrigues Alves[1], Agathe Bernet[1], Denis Soulet[1], Barry Hinton[2], Clémence Belleannée[1]

[1]Université Laval, Canada, [2]University of Virginia, USA

Impairment of the epididymis-derived Wolffian duct (WD) during embryo development results in male infertility. While Hedgehog (Hh) signaling pathway is involved in organogenesis, its contribution to WD morphogenesis remains unknown. The Hh signaling is exclusively transduced through primary cilia (PC) in vertebrates. Recently, we showed that PC are present in WD from e16.5 mouse embryo of Arl13b-mCherry/Cetn2-GFP transgenic mice (which display endogenous fluorescence in primary cilia and centrioles) by using sophisticated methods of confocal microscopy imaging/3D reconstruction. Herein, we aimed to determine the role of Hh signaling pathway during WD development. In that regards, WD were collected from CD-1 e16.5 mouse embryo and cultured in air-liquid condition at 37ºC during 72 hours with different pharmacological treatments: (1) Cyclopamine: Hh inhibitor; and (2) Smoothened agonist: Hh activator. Images were taken every 24 hours on an inverted microscope. Duct elongation, intraluminal area and mesenchyme space were quantified on ImageJ. At the end of culture, WD were collected to deep-sequencing gene expression analysis. Our results indicate that blockade of Hh signaling impaired WD development by increasing the intraluminal area, while activation of Hh signaling increased mesenchyme space area. This suggested a role of Hh signaling in WD homeostasis and development. Microarray analyses revealed that genes involved in Hh signaling (*Ptch1*, *Ptch2, Gli1* and *Hhip*), WD development (*Inhba*, *Fgf5, Pkd1* and *Ar*) and secretion/absorption process (*Crispld1* and *Atp6v0d2*) were altered by the treatments. Our results provide insights on a possible mechanism involving Hh signaling mediated by PC in WD/epididymis morphogenesis and male fertility control.

Functional Studies of tribbles homolog 2 (TRIB2) in ovarian granulosa cells

Aly Warma[1], Jacques Lussier[1], Kalidou Ndiaye[1]

[1]Centre de recherche en reproduction et fertilité, Département de biomédecine vétérinaire, Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada.

Tribbles homolog (TRIB) 1, 2 and 3 represent atypical members of the serine/threonine kinase superfamily. We previously identified TRIB2 as a differentially expressed gene in granulosa cells (GC) of bovine preovulatory follicles. This study aimed to further investigate TRIB2 regulation and study its function in GC of bovine follicles.

GC were obtained from follicles at different developmental stages: small follicles (SF), dominant follicles at day 5 of the oestrous cycle (DF), ovulatory follicles 24h post-hCG injection (OF), andcorpus luteum at day 5 of the oestrous cycle (CL). In addition to this *in vivo* model, an *in vitro* model of cultured GC was used for functional studies either via the CRISPR-Cas9 approach to inhibit TRIB2 or the pQE1 system to overexpress TRIB2.

RT-qPCR analyses showed greater expression of *TRIB2* in GC of DF as compared to OF and a downregulation of *TRIB2* mRNA abundance by hCG/LH. Specific anti-TRIB2 polyclonal antibodies were generated and western blot analyses confirmed TRIB2 downregulation by hCG at the protein level. Inhibition of *TRIB2* using CRISPR/Cas9 resulted in a significant increase in *PCNA* expression and an increase in steroidogenic enzyme *CYP19A1* expression, while TRIB2 overexpression tended to decrease GC proliferation. Western blot analyses showed reduction in ERK1/2 and p38MAPK phosphorylation levels following TRIB2 inhibition, while TRIB2 overexpression increased p-ERK1/2 and p-p38MAPK.

These results provide strong evidence that TRIB2 could be a modulator of MAPK signaling in GC and a regulator of GC proliferation and function, which could affect steroidogenesis in late stages of follicular development.

Primary cilia in efferent ductules

Céline Augière[1], Agathe Bernet[1], Denis Soulet[1], Hess Rex[2], Clémence Belleannée[1]

[1]Laval University, [2]University of Illinois

Efferent ductules are small tubules connecting the testis with the head of the epididymis. These tubules contribute to maintain a proper spermatocrite (ratio sperm/fluid) through mechanisms of fluid absorption, whose impairment result in male infertility. The efferent ductules encompass two types of cells: the ciliated cells that expose motile cilia stirring the sperm fluid, and the non-ciliated cells known to play a role in fluid reabsorption. Our recent discoveries indicated that the so-called "non-ciliated cells" expose at their surface a solitary primary cilia-like organelle. While in other model systems primary cilia are non-motile cilia involved in the control of organ development and homeostasis, nothing is known as regards to these atypical and unexplored structures in the efferent ductules. By confocal imaging and 3D reconstruction of the efferent ductules from a double transgenic mouse model, Arl13b-mCherry; Centrin2-GFP, we determined the presence of the ciliary markers Acetylated tubulin, IFT88 and IFT20 in these primary-like structures. Acknowledging the expanding importance given to primary cilia functions in all organ systems, we hypothesize that primary cilia from the efferent ductules play a role in the homeostasic control of its reabsorptive functions. In that regards, we developed a conditional KO to disturb cilia specifically in non-ciliated cells to decipher the role of primary cilia in male reproductive physiology. Ultimately, our research will provide new answers to the role of under studied primary cilia and might open new avenues to target efferent duct-specific ciliary components controlling male reproductive functions.

Présentations – Presentations : Session II
5 novembre – November 5th
13h00 – 14h00

Présidente – Chair : Céline Augière
Co-présidente – Co-Chair : Linda Ok

I. **Étude de la méthylation des ARN maternels dans l'ovocyte et des cellules somatiques**
Karine Dubuc, MSc Student, Université de Laval (Page 12)
13h00 – 13h15

II. **Distinct modes of chromosome lagging in meiosis-I predict aneuploidy in mouse oocytes**
Aleksandar Mihajlovic, Postdoctoral Fellow, Université de Montréal (Page 13)
13h15 – 13h30

III. **Synergistic cooperation between nuclear receptor NR2F2 and GATA4 regulate expression of the mouse Amhr2 gene in MA-10 Leydig cells**
Samir Mehanovic, PhD Student, Université Laval (Page 14)
13h30 – 13h45

IV. **Targeted disruption of Lats1 and Lats2 in mice impairs testis development and alters the fate of testis somatic cells**
Nour Abou Nader, PhD Student, Université de Montréal (Page 15)
13h45 – 14h00

Étude de la méthylation des ARN maternels dans l'ovocyte et des cellules somatiques

Karine Dubuc[1,2], Mathilde Marchais[1,2], Alexandre Bastien[1,2], Isabelle Laflamme[1,2], Renate K. Hukema[3], Robert Viger[1,5], Isabelle Gilbert[1,2], Maxim Maheux[4], Edward W. Khandjian[6], Claude Robert[1,2]

[1]CRDSI, [2]Département des sciences animales, [3]Department of Clinical Genetics, Erasmus MC, [4]Transbiotech, [5]Centre de recherche du CHU de Québec, [6]Département de psychiatrie et de neurosciences

Durant l'ovogenèse, les réserves emmagasinées sont utilisées afin de supporter les premières divisions cellulaires jusqu'à l'activation du génome embryonnaire. Parmi ces ressources, des ARNm sont utilisés pour soutenir la synthèse des protéines en l'absence de transcription nucléaire. Les transcrits emmagasinés présentent une demi-vie prolongée estimée en jours et le processus de leur stabilisation est peu connu. Nous avons émis l'hypothèse que les modifications post-transcriptionnelles jouent un rôle dans la stabilisation et la gestion des ARNm maternels. Actuellement, on peut trouver plus d'une centaine de modifications chimiques sur les molécules d'ARN dont la méthylation. L'objectif global du projet est de caractériser le potentiel de la méthylation de l'ARNm dans la gestion de la synthèse protéique. Relié aux concepts communs d'épigénétique, des *writers,readers*et *erasers* de la méthylation de l'ARN ont été détectés par microscopie au sein d'ovocytes et de coupes de tissus chez les espèces murine, porcine et bovine. Les *writers* d'intérêts sont DNMT2 pour r5mC et WTAP pour rN6mA, alors que le *reader* est MBD et les *erasers* sont FTO et ALKHB5. Les résultats démontrent la localisation sous-corticale de ces protéines. Nous avons également mesuré l'abondance de nucléotides modifiés (r1mA, rN6mA, r5mC et r7mG) par spectrométrie de masse à partir d'ARN des tissus somatiques totaux ainsi que des ARN maternels d'ovocytes. Les ovocytes avaient une abondance de r1mA et de rN6mA significativement plus faible que les ARN du foie utilisés comme référence. La présence de modifications post-transcriptionnelles de l'ARN dans l'ovocyte suggère l'existence de régulation épigénétique au niveau de l'ARN.

Distinct modes of chromosome lagging in meiosis-I predict aneuploidy in mouse oocytes

Aleksandar I. Mihajlovic[1], Caitlin Mehrotra[1], Greg FitzHarris[1]

[1]CRCHUM/ Université de Montréal

Background: The incidence of chromosome segregation errors that cause aneuploidy in the first meiotic division (MI) increases with age and is considered a major contributing factor of age-related decline in female fertility. Lagging chromosomes in anaphase are commonly observed defect, but whether they directly contribute to aneuploidy in aged oocytes is unclear. **Methods:** Here, we used confocal microscopy to monitor chromosome behavior and examine their attachments to spindle-microtubules during MI. **Results:** The incidence of lagging chromosomes increased in old versus young oocytes (58.8% vs. 26.7%). We identified two distinct types of lagging chromosomes we refer to as 'canonical', that originated from aligned bivalents, and 'non-canonical' that originated from mildly misaligned bi-oriented bivalents. The presence of 'canonical' lagging chromosomes, reminiscent of those in mitotic cells, strongly correlated with aneuploidy outcome of anaphase, while the presence of 'non-canonical' ones showed no correlation. The examination of chromosome attachment status shortly before anaphase revealed that in both young and old oocytes, the proportion of incorrect/merotelic attachments, considered responsible for producing lagging chromosomes, was negligible. However, the proportion of chromosomes lacking stable microtubule attachments remained constantly high (20-30%) throughout entire MI in old oocytes. At the anaphase onset, all chromosomes in both groups become stably attached to microtubules, suggesting that a large proportion of chromosomes in old oocytes rapidly stabilize their attachments just prior to anaphase. **Conclusion:** We propose that this sudden formation of stable microtubule attachment might be highly error-prone thus giving rise to 'canonical' lagging chromosomes that contribute to aneuploidy in old oocytes.

Synergistic cooperation between nuclear receptor NR2F2 and GATA4 regulate expression of the mouse Amhr2 gene in MA-10 Leydig cells

Samir Mehanovic[1], Robert Viger[1,2], Jacques J. Tremblay[1,2]

[1]Reproduction, Mother and Child Health, Centre de recherche du centre hospitalier universitaire de Québec—Université Laval, Québec, Canada, [2]Centre for Research in Reproduction, Development and Intergenerational Health, Department of Obstetrics, Gynecology, and Reproduction, Faculty of Medicine, Université Laval, Québec City, Québec, Canada

Leydig cells produce testosterone and insulin-like 3 hormones, which are essential for male sex differentiation and reproductive functions. Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII, NR2F2) belongs to the steroid/thyroid hormone nuclear receptor superfamily of transcription factors. Transcription factor GATA-binding protein 4 (GATA4) belongs to the GATA family of zinc finger proteins. COUP-TFII and GATA4 are expressed in the adult Leydig cells and are essential for their differentiation and steroidogenesis. Our hypothesis is that COUP-TFII and GATA4 cooperate to regulate gene expression in Leydig cells. To test our hypothesis, we first compared the gene lists from COUP-TFII- or GATA4-depleted MA-10 Leydig cells to better understand the roles of these two transcription factors. This revealed 24 commonly regulated genes including the anti-Müllerian hormone receptor (*Amhr2*) gene. The roles of the AMH/AMHR2 in males include regression of the Müllerian ducts during sex differentiation and regulation of Leydig cell differentiation and steroidogenesis. Although both COUP-TFII (4 fold) and GATA4 (4 fold) can independently activate the *Amhr2* promoter, their combination led to a stronger activation (12 fold). This functional cooperation is likely the result of an interaction between COUP-TFII and GATA4 in MA-10 Leydig cells as confirmed by co-immunoprecipitation. Furthermore, the results from chromatin immunoprecipitation (ChIP) assays validated COUP-TFII and GATA4 recruitment to the proximal *Amhr2* promoter region, which contains binding sites for both factors. Our results provide additional evidence strengthening the importance of a COUP-TFII/GATA4 cooperation in Leydig cells. Supported by CIHR (MOP-81387).

Targeted disruption of Lats1 and Lats2 in mice impairs testis development and alters the fate of testis somatic cells

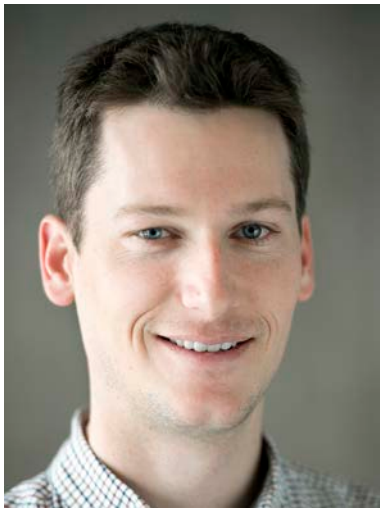
Nour Abou Nader[1], Amélie Ménard[1], Adrien Levasseur[1], Derek Boerboom[1], Gustavo Zamberlam[1], Alexandre Boyer[1]

[1]Centre de Recherche en Reproduction et Fertilité, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Canada

In the Hippo signaling pathway, the large tumor suppressor kinases 1 and 2 (LATS1/2) are functionally redundant serine/threonine-protein kinases that phosphorylate and inhibit the transcriptional coactivators YAP and TAZ, major players in the regulation of cell proliferation and differentiation during embryonic development. In order to investigate the role of Hippo signaling in testis development, we generated a mouse model (Lats1flox/floxLats2flox/flox;Nr5a1cre/+) in which Lats1 and Lats2 were conditionally deleted in testicular somatic cells (cKO). We report herein that developing testicular somatic cells adopt characteristics of mesenchymal cells in the cKO mice, resulting in testis cord dysgenesis and differentiation of interstitial cells into myofibroblasts. A marked accumulation of YAP and TAZ in the nuclei of the somatic cell populations, accompanied by increased expression of their transcriptional target genes in the testes of cKO animals, further suggests that the abnormal differentiation of the testicular somatic cells can be attributed in part to YAP and TAZ. Taken together, our results suggest that Hippo signalling is required to maintain proper cell fate of both the Sertoli and the Leydig cells.

Conférencier invité - Invited Speaker

Olivier d'Amours



Olivier D'Amours a effectué un baccalauréat en agronomie, puis une maîtrise et un doctorat en physiologie-endocrinologie à l'Université Laval. Lors de ses études graduées, M. D'Amours a étudié la fertilité mâle en collaboration avec l'industrie de l'insémination artificielle. Il agit maintenant à titre d'analyste de recherche en productions animales au ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec. Son mandat consiste notamment à effectuer une veille scientifique et technologique en sciences animales et en transfert de connaissances.

Il présentera un séminaire le mardi 5 novembre intitulé : ***L'adoption de l'innovation et le financement de la recherche au ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec.***

Session d'affiches I – Poster Session I
5 novembre – November 5th
14h45 – 16h15

1. Characterization of O-GlcNAcylation in granulsoa cells of bovine ovarian follicles. *Abigail M. Maucieri, MSc Student, Vermont University (Page 20)*

2. Examining the impact of mitosis duration on embryo development*. Adélaïde Allais, PhD Student, Université de Montréal (Page 21)*

3. Recellularisation of equine decellularized skin scaffolds with fibrolasts. *Adriana Raquel de Almeida da Anunciaçã, PhD Student, Université de Montréal (Page 22)*

4. Implication of mutated DNMT3A in the Pathogenesis of Tatton-Brown-Rahman Syndrome. *Alexandra Langford-Avelar, MSc Student, Université de Montréal (Page 23)*

5. Utilisation de la génomique pour gérer et protéger les populations de caribous. *Alexandra Carrier, PhD Student, Université de Laval (Page 24)*

6. Imaging oocytes: Optical clearing and mathematical modeling. *Alexandre Bastien, Research Professional, Université de Laval (Page 25)*

7. Mitochondrial cAMP compartimentalisation in ovarian follicular cells. *Amel Lounas, PhD Student, Université Laval (Page 26)*

8. Inhibition of sperm motility by STAT3 inhibitory compound V, Stattic, results from a decrease in reduced glutathione levels. *Andrée-Anne Saindon, Research Professional, Université de Laval (Page 27)*

9. Defining heritable epigenome dysregulatiosn at promoter regions in mouse embryonic stem cells. *Anthony Lemieux, BSc Student, Université de Montréal (Page 28)*

10. L'étrange potentiel traductionnel de l'ovocyte bovin. *Mallorie Trottier-Lavoie, MSc Student, Université Laval (Page 29)*

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Characterization of O-GlcNAcylation in granulsoa cells of bovine ovarian follicles

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Glucose is widely recognized as the preferred energy substrate for metabolism by granulosa cells (GCs). O-linked N-acetylglucosaminylation (O-GlcNAcylation) is a product of glucose metabolism in most cells, and occurs as the Hexosamine Biosynthesis Pathway (HBP) shunts O-GlcNAc sugars to serine and threonine residues of proteins. O-GlcNAcylation is evolutionarily-conserved, and together with the HBP is considered a nutrient sensor that regulates cellular processes such as metabolism, signal transduction, and proliferation. Yet relatively little is known about O-GlcNAcylation and its importance in GC function. The current study aimed to initially characterize O-GlcNAcylation in bovine GCs and determine if manipulating O-GlcNAcylation affects GC proliferation. Bovine ovary pairs were collected from an abattoir and those morphologically staged to the mid-to-late estrous period were used. Granulosa cells and follicular fluid were aspirated from small (3-5mm) and large (>10mm) follicles. Freshly isolated GCs of small follicles exhibited greater global O-GlcNAcylation and O-GlcNAc transferase expression than large follicles ($P<0.05$, n=7 ovary pairs). Glucose and lactate concentrations in the follicular fluid of these same follicles revealed less glucose in small follicles compared to large follicles ($P<0.05$, n=7 ovary pairs); whereas lactate concentration was greater ($P<0.05$, n=7 ovary pairs). In cell culture, exposure of GCs from small follicles to the O-GlcNAcylation inhibitor, OSMI-1 (50µM) impaired proliferation ($P<0.05$, n=3 expts.); whereas augmentation of O-GlcNAcylation via Thiamet-G (2.5µM) had no effect ($P>0.05$, n=3 expts.). The results indicate that O-GlcNAcylation in GCs of bovine follicles is readily evident, and disruption of this unique post-translational modification impairs GC proliferation.

Examining the impact of mitosis duration on embryo development

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The timing of cell divisions is a potential indicator of embryo health in fertility clinics. In somatic cells, extended mitosis can cause premature separation of chromatids known as "cohesion fatigue" (CF), which could cause aneuploidy. Separately, a mitotic clock checkpoint (MitClock) has been described wherein an extended mitosis duration can cause a subsequent G1/S arrest. We set out to determine whether CF can occur and whether the MitClock operates in mouse preimplantation embryos. We manipulated the duration of mitosis in two-cell stage mice embryos with APCin and performed live and fixed confocal imaging. Mitosis prolongation causes an increase of spindle length and a time-dependent loss of chromosome alignment. 4% of all sister pairs had individualized by 6hours, and 66% by 24hours. The loss of chromatid cohesion was preceded by an increase in inter-kinetochore distances from 0.59µm to 0.78µm ($p<0.0001$), consistent with CF. 24hours mitosis prolongation using a spindle poison did not trigger CF, suggesting it is spindle-tension-dependent. Strikingly, live imaging revealed that 6hours of mitosis prolongation does not prevent subsequent embryo development, but doubles the frequency of micronuclei per embryo (4.5 vs 2.7), suggesting that CF triggers aneuploidy. In contrast, 24hours mitotic arrest causes a cell cycle arrest. Preliminary data suggests that this arrest may require a critical level of CF. Moderately prolonged mitoses fail to activate a MitClock in embryos, but lead to appreciable increases in CF and chromosome segregation defects. Our data allude that CF could be a previously unappreciated cause of mosaic aneuploidy in the mammalian embryo.

Recellularization of equine decellularized skin scaffolds with fetal fibroblasts

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The aim of this study was to establish a protocol for equine skin decellularization that maintains the architecture of the extracellular matrix and to examine its potential to be recellularized for possible use as an in vitro model for construction of a horse injury skin substitute. For this, equine skin fragments were decellularized with EDTA, 2% SDS and 0.1% Triton for 25 days and sterilized with PBS and 1% antibiotic in ultraviolet light. To prove the efficiency of the decellularization protocol, decellularized samples were stained of Hematoxylin and Eosin (HE) and colloidal iron to locate possible remaining cell nuclei in the ECM and presence of proteoglycans, respectively. In addition, the level of remaining DNA was quantified after decellularization. After confirming decellularization, 3×10^5 fibroblasts were plated in untreated plates containing slices with $125mm^3$ of horse skin scaffolds for 7 days. After *in vitro* culture, scaffolds were stained with DAPI to confirm cell adhesion. Moreover, ECM components such as proteoglycans and collagen fibers remained present on the scaffold, indicating efficient decellularization and showing that the equine skin scaffold contains the functional properties for recellularization. As observed by confocal microscopy, fibroblasts proliferated rapidly on the surface and penetrated into the decellularized skin scaffold. Together, the presence of ECM in the decellularized skin scaffold and the ability to withhold and proliferate fibroblast are encouraging preliminary results, suggesting that decellularized skin scaffolds could eventually be used clinically for the construction of a horse injury skin substitute.

Keywords: Horse, scaffold, tissue repair

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:

Overall, 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.

Utilisation de la génomique pour gérer et protéger les populations de caribous

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Les populations de caribous du Québec sont en déclins, et certaines hardes sont même en voie de disparition. Bien qu'il soit connu que le cheptel fluctue, le nombre de têtes au Québec est à un niveau le plus bas depuis plus de 40 ans. Les menaces d'aujourd'hui pourraient limiter le retour de cet animal emblématique sur le territoire québécois.

Le ministère des Forêts, de la Faune et des Parcs du Québec (MFFP) a déjà mis en place plusieurs plans de protection du caribou et veut maintenant intégrer la génomique pour développer un outil qui permettra de suivre l'évolution des hardes en déterminant la structure des populations (nombre d'individus reproducteurs), leur niveau de consanguinité ou la possible érosion du patrimoine génétique. L'outil servira aussi à des fins judiciaires pour protéger les animaux contre le braconnage en déterminant leur harde d'origine.

Le premier volet de ce projet consiste à améliorer l'assemblage du génome de caribou existant pour développer efficacement une puce de génotypage qui permettra d'obtenir des données génétiques pour un grand nombre d'individus. Ensuite, ces données permettront de différencier génétiquement les individus et d'assigner un individu à une population grâce à son ADN.

À ce jour, 895 individus ont été séquencés afin de construire l'assemblage du génome et découvrir des polymorphismes d'un seul nucléotide (SNPs). De ce catalogue de SNPs, environ 50 000 seront choisis comme cibles sur la puce de génotypage. Une partie de ceux-ci serviront pour générer des métriques de diversité génétique alors que les autres permettront l'assignation populationnelle.

Imaging oocytes: Optical clearing and mathematical modeling

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Imaging oocytes is challenging as they are giant spherical cells with high turbidity. In CLSM, photons coming from the focal plane needs to converge at the pinhole in order to get detected. Organelles, lipid droplets and cytoplasm turbidity create refractive index (RI) mismatches that scatter the photons preventing them from being detected. Because of the large spherical shape of the oocyte, photons coming from cortical areas will be less scattered since they will cross less RI mismatches. This leads a common cortical artifact. In this study, we describe a method to evaluate the local scattering coefficients of denuded porcine oocytes compartments and we establish a mathematical model used to correct cortical artifacts. We also evaluate several optical clearing methods to reduce scattering and enable deep imaging within specimen using direct stains and immunofluorescence. Among the tested methods are Scale, BABB, passive CLARITY and simple Glycerol/H2O for dyes such as MitoTracker Orange, Hoechst 33342, SiR-Actin and Bodipy FL, and AF488 and AF555 secondary antibodies bind to primary against FMRP, TOMM20 and TUBA4A. Finally, we evaluate objective magnification and numerical aperture (NA) effect in both real-life and mathematical models. While high NA improves resolution and light collection, it also augments scattering which prevents deep imaging. Depending on the user needs, lower NA might be preferable. No single clearing agent works in all cases since they interact with particular dyes and antibodies. In addition, the clearing processes alter cellular composition such as lipids that can modify the natural distribution and localisation of targets.

Mitochondrial cAMP compartimentalisation in ovarian follicular cells

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Cyclic adenosine monophosphate (cAMP) is a ubiquitous second messenger that plays a central role in cell physiology. In recent year, several studies have demonstrated the compartmentalisation of cAMP signaling within the cell. This subcellular compartmentalisation is very important for the regulation of cellular responses. It has been observed that cAMP signaling is involved in dynamic mitochondrial regulation and biogenesis. In this study, we identified the molecular machinery of the cAMP signaling network in mitochondria of follicular ovarian cells. First, proteomic analysis by LC/MS-MS (Liquid chromatography-mass spectrometry) of isolated mitochondria from granulosa cells showed the presence of several proteins involved in cAMP signaling such as soluble adenylate cyclase (sAC), protein kinase A (PKA), exchange protein directly activated by cAMP (EPAC) and proteins A-kinase anchoring proteins (AKAPs). The results were validated by western blot analyses demonstrating the presence of this machinery in isolated mitochondria from granulosa cells. Our results support the mitochondrial subcellular compartimentalisation of cAMP signaling in follicular ovarian cells. Since mitochondria play a central role in the production of cellular energy, steroidogenesis and apoptosis, the regulation of mitochondrial function by cAMP signaling likely regulates ovarian cell function and will thus allow to further refine artificial reproductive technologies.

Inhibition of sperm motility by STAT3 inhibitory compound V, Stattic, results from a decrease in reduced glutathione levels

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STAT3 is a protein detected in membranes and in flagellar cytoskeletal fractions in human spermatozoa. It has been shown that Stattic (Stat3 inhibitory compound V) reduces sperm motility and cellular ATP levels, and depolarises the mitochondrial membrane. This causes oxidative stress in sperm; demonstrated by an increase of extracellular levels of reactive oxygen species (ROS). N-Acetylcysteine (NAc) prevents Stattic-induced detrimental effects on sperm such as immobilisation, production of the superoxide anion, depolarisation of the mitochondrial membrane, intracellular acidification, and oxidation of protein thiols. NAc is generally considered as an antioxidant although it most likely acts through the synthesis of glutathione (GSH), which may be the most important intracellular antioxidant able to prevent ROS-induced damage to cellular components. Therefore, GSH is a good indicator of the cell redox status. Our goal is to determine whether Stattic affects sperm motility through an effect on reduced GSH levels. Sperm GSH levels were measured using colorimetric and fluorometric assays. Both methods revealed a decrease in GSH in sperm treated with Stattic. This suggests that the deleterious effects of Stattic result from an oxidative stress caused by a decrease of reduced glutathione levels, therefore reducing its antioxidant activity. Furthermore, as ROS are produced in mitochondria, and since STAT3 is known to maintain proper mitochondrial activity, the effects of Stattic on mitochondrial activity were assessed by measuring the oxygen consumption rate. Our results show that mitochondrial activity is affected by Stattic; whether this effect occurs through the protein STAT3 remains to be determined.

Defining heritable epigenome dysregulations at promoter regions in mouse embryonic stem cells following a transient loss of DNMT1

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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 enhancers 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 18,192 identified promoters permanently lost their methylation after Dox treatment, altering gene regulation. We are presently analyzing 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.

L’étrange potentiel traductionnel de l’ovocyte bovin

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Les ribosomes sont des complexes ribonucléiques essentiels à toutes cellules. Dans les cellules somatiques, ils sont retrouvés attachés au réticulum endoplasmique (RE) et à l’enveloppe nucléaire et d’autres seraient libres dans le cytoplasme. Les mitochondries ont quant à elles leur propre contingent de ribosomes. Bien que les ovocytes bovins matures et les embryons précoces soient dans un état de silence transcriptionnel, ceux-ci ont un besoin intense en synthèse protéique afin de supporter les premières étapes de développement. Dans ces cellules, les profils d’ARN ribosomaux (ARNr) sont atypiques et demeurent ainsi jusqu’à l’activation du génome embryonnaire (AGE). Notre hypothèse est que les ovocytes et les stades précoces de développement embryonnaire utilisent différents types de ribosomes pour soutenir la synthèse protéique. Pour décrire la population d’ARNr, la quantification des transcrits dans l’ovocyte bovin, murin et porcin a été effectuée et démontre différentes abondances relatives d’ARNr comparativement aux cellules somatiques. En utilisant différents fluorophores, nous avons mis de l’avant une étroite relation entre les ribosomes, les mitochondries et le RE. L’étrange forme des mitochondries et leur intime relation avec le RE a été documentée il y plus de 40 ans sans être caractérisée davantage. L’identification de la traduction active de façon concomitante avec l’inhibition de différents types de ribosomes est en cours. Ce travail a pour but de fournir de nouvelles informations sur la machinerie traductionnelle particulière du gamète femelle et des blastomères précoces.

Identification of the protein phosphatase with EF-hand domain 1 (PPEF1) as a calmodulin binding protein in spermatozoa

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Capacitation, a process taking place in the female genital tract, is characterized by changes in the head and flagella of spermatozoa. These modifications enhance its ability to interact with the egg and to hyperactivate motility, two processes required for fertilization. Spermatozoa are haploid cells devoid of transcription and translation to regulate their activity. In this context, protein phosphorylation and dephosphorylation play important roles. PPEF1 (protein phosphatase with EF hand domain 1) was identified by mass spectrometry among sperm proteins enriched by calmodulin affinity pull-down. RdgC, the drosophila ortholog of PPEF1, is involved in dephosphorylation of rhodopsin. As rhodopsin and other opsin receptors are present in spermatozoa and have been shown to play a role in thermotaxis and motility, this suggests that PPEF1 is involved in the control of sperm motility. Our objective is to clarify the role of PPEF1 in sperm by 1) determining its localization in spermatozoa, 2) identifying the isoforms present in spermatozoa and 3) evaluating its phosphatase activity during capacitation. PPEF1 is detected in the neck, flagella and potentially in the acrosome of uncapacitated spermatozoa. Several predicted PPEF1 isoforms have been identified by PCR on testis mRNA and production of a recombinant protein is ongoing. Optimization of assays to measure phosphatase activity *in vitro*, after purification of PPEF1 from spermatozoa is currently ongoing. Overall, this project will help to further understand the role of calcium and calmodulin, and the importance of PPEF1 in sperm motility and fertilizing ability.

Involvement of cAMP efflux, through MRP4 transporter, in the acquisition of fertilizing ability of mouse spermatozoa

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To fertilize the oocyte mammalian spermatozoa must undergo a series of biochemical changes, altogether known as capacitation. Levels of cAMP are tightly regulated during this process by the ratio between its synthesis and degradation. However, in other cell types, cAMP efflux through the Multidrug Resistance Protein 4 (ABCC4/MRP4) contributes to modulate the availability of this nucleotide and provides cAMP to the extracellular space. We previously demonstrated that bovine spermatozoa possess functional MRP4 and the pharmacological blockade of MRP4 inhibits capacitation related events, whereas addition of extracellular cAMP (ecAMP) restores capacitation. In the present work we aim to further describe the role of MRP4 in mammalian sperm physiology, hence we investigated its participation in mouse sperm capacitation.

Results showed that mouse spermatozoa possess MRP4 and incubation with MK571 (50 µM), an MRP4 inhibitor, resulted in accumulation of intracellular cAMP and a decrease in ecAMP. Inhibition of MRP4 also produced a decrease in tyrosine phosphorylation, sperm motility, hyperactivation and in vitro fertilization, and addition of ecAMP (1 nM) failed to restore capacitation. However, unlike bovine, mouse spermatozoa showed increased Ca^{2+} levels with the MRP4 inhibitor. Furthermore, the absence of Ca^{2+} salts in the medium prevented the loss of motility induced by MK571. A similar response was observed in spermatozoa from *CatSper* KO mouse, supporting the association between MRP4 and Ca^{2+} homeostasis in this species.

Altogether, our results suggest that MRP4 plays a critical role in mammalian sperm capacitation, although the signaling pathways associated to this transporter differ between murine and bovine spermatozoa.

Genomic insight into IVF failure; Can we cluster negative patients into different failure causes?

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In average, about one in six couples will unfortunately experience infertility. Assisted reproductive technologies such as in vitro fertilization with embryo transfer (IVF-ET) gives these couples the chance to achieve pregnancy. However, the success rate of IVF remains quite challenging and more often than not, the patient is not achieving a pregnancy and IVF ends up in a cycle failure. Aiming to find markers of IVF success, numerous studies have been looking at gene expression in follicular cells These success markers could help to select oocytes or embryos. However, it does not provide any clues on what went wrong during the stimulation and when a cycle fails, we still don't know why. If we could characterize failure, we could adapt next cycle treatment based on why it previously failed and potentially increased the chances of success. This study aims to look if it is possible to distinguish different failure causes or different follicular responses among a population of negative IVF patients (patients who failed to conceive following an IVF cycle). Based on previous studies we selected a panel of markers indicative of 3 different failure causes. These markers were analyzed by RT-qPCR in a population of 135 negative patients. Hierarchical clustering analysis was performed, and the population was split into 3 clusters. Comparison of gene expression between those clusters showed that they were different regarding the 3 different types of markers and therefore that it was possible to cluster negative patients into different failures causes.

Discovering the function of IGSF1 and its role in the hypothalamic pituitary thyroid axis

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The hypothalamic-pituitary-thyroid (HPT) axis controls the synthesis and secretion of thyroid hormones (THs). THs have many actions throughout the body, but are best known for their regulation of growth and metabolism. Central hypothyroidism, a rare endocrine disorder, occurs when defects in the hypothalamus and/or the pituitary cause TH-deficiency. Mutations in the immunoglobulin superfamily, member 1 (*IGSF1*) gene are the most common cause of congenital central hypothyroidism. IGSF1 is a type 1 transmembrane protein of unknown function that is highly expressed in the pituitary gland. To define potential mechanism of IGSF1 action, we will identify its interacting partners using a new proximity interaction labelling method, BioID. The BioID method attaches a BirA* biotinylase onto the protein of interest (bait). In the presence of biotin, BirA* biotinylates interacting and proximal proteins. The biotinylated proteins (prey) are pulled down using streptavidin beads, which have a high affinity for biotin, and are identified using mass spectrometry. As we are most interested in intracellular signaling, we fused BirA* to the intracellular tail of IGSF1. 11 specific interacting proteins were identified in a preliminary BioID screen performed in a heterologous cell system. Several candidates identified in the BioID screen are expressed in thyrotrope cells, or have family members expressed in thyrotropes. Next, we will perform BioID in homologous cell lines. In the long-term, our work will define the function of IGSF1 in the pituitary, and may facilitate the discovery of novel causes of congenital central hypothyroidism.

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Caractérisation des Isoformes d'Akt dans l'ovaire et leurs impacts sur la Réserve Ovarienne

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La réserve ovarienne (R.O.) est un des déterminants de la fertilité. Son altération aboutit au vieillissement ovarien(V.O.) et à l'infertilité. Ces dernières décennies, les femmes tendent à repousser leur premier accouchement au-delà de 35 ans, l'infertilité due au V.O. va grandissante. Cet épuisement se fait par la croissance et l'atrésie folliculaires, phénomènes régulés par Akt. La thérapeutique concernant le V.O. appelle à élucider ses mécanismes. Les études établissent le rôle d'Akt dans la folliculogénèse mais pas ceux de ses trois isoformes. Cependant, elles peuvent avoir des rôles spécifiques. En cela, cette étude parait utile car pouvant ouvrir la voie à des thérapeutiques spécifiques pour les femmes atteintes de V.O. précoce et désireuses de concevoir. Notre objectif est de caractériser ces isoformes dans l'ovaire et déterminer leur impact sur la R.O. Avec des souris sauvages et K.O. utéro-ovariens spécifiques d'isoformes d'Akt obtenues grâce au système PR-Cre, nous avons étudié l'expression de ces isoformes, de certaines cibles d'Akt et comparé le nombre de follicules. Les résultats révèlent que ces isoformes montrent une variabilité d'expression (persistance de Akt2 au travers du cycle œstral), de localisation (nucléaire pour Akt1 ; cytoplasmique pour Akt2 et 3). La double annulation d'Akt1 et 3 altère la R.O. à 100 jours. Certaines cibles d'Akt (Bad, Fox03a) sont phosphorylées par Akt3 et Akt1 que par Akt2 pour protéger la R.O. Ces isoformes sont redondantes pour la phosphorylation de GSK3β en metestrus. L'implication spécifique des isoformes d'Akt sur la R.O. pourrait mener à la mise au point des cibles thérapeutiques.

Uncovering the Regulatory Domains of the Imprinted-like Xlr Gene Family Using CRISPR Epigenome Editing

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The *Xlr* (X-linked lymphocyte regulated) gene family in mice has sparked a lot of interest in the last years since the discovery that their regulation displays tissue-specific imprinted-like characteristics during early organogenesis and in later development. However, it is still unknown how or when they acquire imprinted-like methylation and their precise functions and regulatory domains are still to be determined. To study this phenomenon, we developed a mouse embryonic stem cell model (mESC *Dnmt1*$^{tet/tet}$) in which we can transiently deactivate *Dnmt1*, the enzyme that maintains DNA methylation, causing all methylation imprints to be lost since embryonic cells cannot recover these marks. We observed that all nine *Xlr* (*Xlr 3/4/5 a/b/c*) suspected promoter regions, that were initially all highly methylated, completely lose their methylation after *Dnmt1* deactivation causing a prolonged increase in expression, similarly to many imprinted genes (e.g. *H19/Igf2*, *Nnat*, *Meg3*). Furthermore, the *Xlr* promoters were at first mostly depleted in histone modifications and then acquired active marks (H3K4me3, H3K27ac) following the loss of DNA methylation/gain in expression. To investigate the *Xlr* regulatory domains, we transduce a CRISPR epigenome editing system (dCas9-*Dnmt3a* or *TET1*) coupled to *Dnmt3a*, the enzyme responsible for *de novo* DNA methylation, or TET1, an enzyme that removes DNA methylation, to specifically methylate/demethylate different CpG dense regions of each *Xlr* individually and observe the effect on gene expression (RT-qPCR) and on histone modifications (ChiP-qPCR). This project will lead us to discover the expression regulatory domains needed to elucidate how the *Xlrs* acquire tissue-specific imprinting mechanisms during development.

IGSF1 does not regulate FSH synthesis or secretion in vivo or in vitro

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Loss of function mutations in the human X-linked immunoglobulin superfamily, member 1 (*IGSF1*) gene result in central hypothyroidism, often associated with macroorchidism. *Igsf1*-deficient mice are also centrally hypothyroid due to impaired TRH action in the pituitary. The mechanisms underlying macroorchidism are unclear and disputed. IGSF1 was originally characterized as an inhibin B co-receptor. As inhibin B negatively regulates FSH secretion, it was hypothesized that loss of IGSF1 would impair inhibin B action, leading to elevated FSH levels and enhanced Sertoli cell proliferation during development. Yet, IGSF1 does not bind inhibin A or B in heterologous binding assays and our data further indicate that FSHβ subunit (*Fshb*) mRNA expression is similarly antagonized by inhibin A and B in pituitary cultures from wild-type and *Igsf1*-deficient mice. More recently, IGSF1 was proposed to inhibit activin type IB receptor (ALK4) signaling. As activins stimulate FSH, loss of this inhibition should lead to increased FSH. However, neither humans nor mice with IGSF1-deficiency have elevated FSH and IGSF1 expression in FSH-producing gonadotrope cells is negligible. Additionally, IGSF1-deficient mice have normal serum LH and inhibin B levels. Previous methods used to demonstrate IGSF1 regulation of a human *FSHB* promoter-reporter were conducted in a heterologous system in which such reporters lack activin/ALK4-dependent activity. We demonstrate that overexpression of IGSF1 in homologous LβT2 gonadotrope-like cells does not impair induction of endogenous *Fshb* by a constitutively active form of ALK4. Collectively, the available data fail to support a role for IGSF1 in FSH regulation by activins, inhibins, or otherwise.

YAP/TAZ-TEAD interaction is critical for the preovuatory EGF like cascade induced by LH in bovine granulosa cells

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The ovulatory process is initiated by a surge of LH that acts upon its receptors on granulosa cells (GC). This leads to activation of enzymes that cause the release of the membrane-bound proteins epiregulin and amphiregulin, members of the epidermal growth factor (EGF) family. These proteins then bind to the EGF receptor (EGFR) on mural and cumulus cells, which activates ERK1/2 and AKT signaling and stimulate the expression of critical genes for luteinization, COC expansion, oocyte maturation and ovulation. Interestingly, there is evidence in tumor cells that Hippo signaling effectors, YAP and TAZ, can modulate the EGFR-ERK and AKT signaling in a YAP/TAZ-TEAD-dependent manner. To determine the relevance of Hippo effectors to the preovulatory EGF-like cascade induced by LH in bovine granulosa cells, first cells were pre-treated with verteporfin (VP), a small molecule inhibitor that interferes with YAP/TAZ binding to TEAD family transcription factors, and challenged with LH. VP inhibited the expression of several key preovulatory genes required for LH-induced signaling, including EGFR. To explore whether Hippo effectors inhibition affects for EGFR-downstream signaling, we then challenged VP pre-treated cells with EGF. The results indicated that VP downregulated basal and EGF-induced EGFR expression and therefore EGF was not capable of activating ERK1/2 nor AKT, consequently VP also blunted the expression of important genes dependent on EGFR activation. Taken together, these results *in vitro* suggest that YAP/TAZ-TEAD interaction is critical the LH-induced ovulatory cascade in granulosa cells. Ongoing experiments are testing if the intrafollicular injection of VP affects induced ovulation *in vivo*.

Paternal age differentially affects the transcriptome of spermatozoa from the caput and cauda epididymidis of Brown Norway rats

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Increasing number of couples choose to start having children at a later age. Mounting evidence indicates that advanced paternal age is associated with decreased fertility and untoward effects on progeny outcome. To determine effects of advanced paternal age on sperm during epididymal maturation, we used a rat model to assess whether gene expression and epigenetic modification occur in aging male germ cells. We obtained spermatozoa from the caput and cauda regions of the epididymis of young (4-5 months) and aged (18-20 months) Brown Norway rats, isolated long and small RNA, and performed mRNA and small RNA sequencing. By screening for differently expressed genes between sperm from the caput and cauda epididymidis and between young and aged animals, we found a large number of differentially expressed RNAs. Some of the most striking changes were noted in the β-defensin family, a group of proteins with antimicrobial activity that are essential for innate immune system and expressed at high levels in the epididymis. While aging related germ cell expression pattern and regulatory mechanism of β-defensins has not been reported yet. We found 30 members of rat β-defensins and Sperm Associated Antigen 11a/b stably expressed in caput and cauda spermatozoa. Among these members, expression levels of Defb12, Defb22, Defb23, Defb25, Defb33, Defb41, Defb44 and Spag11a significantly decreased with age only in spermatozoa from the cauda epididymis. Further analysis of the RNAseq results should provide new insight into how aging modulates RNAs in spermatozoa as they transit therough the epididymis. Supported by CIHR.

Effet anti-tumoral de la mélatonine dans le choriocarcinome placentaire humain: Rôle du stress oxydatif et de l'activité mitochondriale

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La mélatonine exerce un pouvoir antioxydant et anti-apoptotique dans les cellules trophoblastiques saines. En revanche, elle induit la mort des cellules trophoblastiques tumorales. ***Hypothèse:*** la mélatonine augmente le stress oxydatif dans les cellules de choriocarcinome placentaire entraînant l'apoptose. ***Objectifs****:* Dans la lignée de choriocarcinome BeWo, déterminer l'effet de la mélatonine sur (1)-le taux d'espèces réactives de l'oxygène (EROs); (2)-l'expression des enzymes oxydantes (xanthine oxydase-XO) et antioxydantes (superoxyde dismutase-SOD, glutathionne peroxydase-GPx et la catalase-CAT); (3)-la peroxydation des lipides, la carbonylation des protéines et (4)-l'activité mitochondriale. ***Méthodes***: Les BeWo maintenues en normoxie (8%-O_2) ont été traitées à la mélatonine (0–1mM) et exposées ou non à un stress par une hypoxie (0,5%-O_2)-réoxygénation (8%-O_2) (H/R). Les niveaux des EROs ont été analysés par Carboxy-DCFDA et l'expression des enzymes par immunobuvardage. La peroxydation lipidique a été analysée par le test TBARS, la carbonylation des protéines par fluorimétrie et l'activité mitochondriale par SeahorseXF96. ***Résultats***: La mélatonine à 1mM augmente significativement le taux des EROs en normoxie et en H/R. Les taux protéiques de XO, sont augmentés par la mélatonine en normoxie, ainsi que les taux de SOD_2, GPx et CAT comparés au véhicule contrôle. La mélatonine augmente significativement la carbonylation des protéines, mais aucune différence n'a été observée au niveau de la peroxydation lipidique. Nos résultats préliminaires montrent que la mélatonine augmente la respiration mitochondriale maximale, mimant les effets de l'H/R. ***Conclusion***: Nos résultats démontrent que la mélatonine augmente le stress oxydatif dans les BeWo, suggérant que son effet anti-tumoral est médié par son action prooxydante.

Cytokinesis in early mouse embryo

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Early mammalian embryos frequently possess binucleated cells, suggesting a failure of cytokinesis, the final step of cell division causing the physical separation of the two new daughter cells. However, the mechanism of cytokinesis is unstudied in mammalian preimplantation embryos. In somatic cells, cytokinesis comprises the formation of a contractile ring that drives the ingression of the cleavage furrow to bisect the cell at the equator. At the end of the cytokinesis the contractile ring transforms into a so-called midbody structure also comprising stabilized microtubules to maintain contact between the newly nascent cells. Here by use of live cell imaging we demonstrate the recruitment of anillin in the plasma membrane during the cytokinetic furrow ingression, which ultimately forms a focused midbody structure on the intercellular microtubule bridge. When one of the daughter cells undergoes a subsequent division, the microtubule bridge disassembles, and concomitantly the anillin-positive structure is apparently expelled from the cell and forms an extracellular structure positive for plasma membrane components and the microtubule crosslinker PRC1. By tracking this extracellular structure in 4D live imaging, we have evidence that it can persist for at least 2 divisions before it disappears. In conclusion, in the preimplantation mammalian embryo after each blastomere division, the midbody remnant is being released before the onset of the next division. Future experiments will address the question whether the midbody and/or the midbody remnant carry transmittable signals that helps pattern the early embryo.

An environmentally relevant mixture of organophosphate ester flame retardants negatively impacts endochondral ossification

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Flame retardants (FRs) are applied to many consumer goods to slow their burning. With the global phase out of polybrominated diphenyl ether (PBDE) FRs, organophosphate esters (OPEs) have taken over the market. OPE levels in the environment have quickly surpassed those of PBDEs, but little is known about the safety of these new FRs. We previously showed that several individual OPEs, such as triphenyl phosphate (TPHP) and *tert*-butylphenyl diphenyl phosphate (BPDP), adversely impact bone formation in the *ex vivo* mouse limb bud culture model. However, real life exposure often involves mixtures. As such, in this study, we investigated whether exposure to an environmentally relevant mixture of OPEs, whose composition is based on dust, a major route of FR exposure, could affect bone formation. We tested this using the 6-day limb bud culture system and a strain of transgenic mice expressing fluorescently tagged collagen markers for the different stages of endochondral ossification: COL2A1-eCFP (chondrogenesis), COL10A1-mCherry (chondrocyte hypertrophy), and COL1A1-eYFP (osteogenesis). Limbs from gestation day 13 embryos were cultured in the presence of vehicle (DMSO), 1/1,000,000, 1/600,000, or 1/300,000 dilutions of the OPE mixture. Limb morphology scoring indicated that exposure to even the 1/1,000,000 dilution significantly decreased the extent of cartilage template development, compared to controls. The fluorescent markers revealed that exposure to the 1/600,000 dilution inhibited the progression of bone formation. This is the first evidence that an environmentally relevant mixture of OPEs may be detrimental to endochondral ossification. Supported by funding from CIHR, FRQS, RQR, CRRD, and McGill University.

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[1], Clémence Belleannée[1]

[1]Université Laval

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.

Variation génotypique de Spam1 chez les taureaux laitiers et bouchers

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SPAM1 est une protéine impliquée dans la liaison du spermatozoïde à la zone pellucide de l'œuf et possèdant une activité hyaluronidase permettant le passage du spermatozoïde au travers le cumulus. Des travaux visant à déterminer la séquence nucléotidique des transcrits encodant les isoformes de SPAM1 ont montré que, chez certains taureaux, SPAM1 possède un codon supplémentaire encodant une lysine dans la portion C-terminale, portion impliquée dans la liaison du spermatozoïde à l'ovocyte. Bien que la séquence de Spam1 soit bien conservée entre plusieurs espèces de bovins, cette variante pourrait provenir d'un allèle ancestral car, à l'instar de nos espèces domestiques (*Bos taurus*), elle est présente chez certains bovidés comme le bison (*Bison bison*) et le yak (*Bos mutus*). Comme les différentes races domestiques actuelles résultent de croisements et sélection génétiques afin de leur donner une vocation laitière et/ou bouchère, notre objectif est de déterminer si cette lysine supplémentaire dans la protéine SPAM1 se retrouve plus souvent chez certaines races. La présence ou absence du codon (AAG) codant pour la lysine a été déterminée par pyroséquençage de l'ADN isolé à partir des spermatozoïdes de taureaux provenant de 5 races laitières et de 5 races bouchères. Des différences importantes ont été observées quant à la présence de l'allèle portant une lysine supplémentaire chez les taureaux utilisés en insémination artificielle selon leur vocation bouchère ou laitière. Dans un prochain temps, nous déterminerons si la présence/absence de cette lysine supplémentaire est corrélée à la fertilité et à la qualité de la semence des taureaux.

Accuracy of corpus luteum color flow Doppler ultrasonography to diagnose non-pregnancy in dairy cows on day 21 after insemination

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The objective of this study was to validate the accuracy of corpus luteum color flow Doppler ultrasonography (CLCFDU) to diagnose non-pregnant dairy cows 21 days after insemination. Data from 1,632 Holstein cows (10 commercial herds) were used for the analysis in this prospective cohort study. During farm visits, cows were examined on day 21 after insemination using transrectal palpation and B-mode ultrasonography to quantify the presence and size of the corpus luteum and follicles. After identification of the corpus luteum, CLCFDU was performed and scored based on the proportion of the surface that was colored (D0 = none of the corpus luteum surface being colored; D1 = between 10 and 30 % of the surface being colored; D2 = between 40 and 60 % of the surface being colored; D3 = between 70 and 100 % of the surface being colored). Farmers were blinded to these findings and no intervention was performed following examination. On day 32 after insemination, the cows were examined by the regular herd veterinarian using transrectal palpation and B-mode ultrasonography to determine whether the cows were pregnant or not (reference test). The apparent prevalence, sensitivity, specificity, positive predictive value, and negative predictive value of CLCFDU for predicting non-pregnancy were 22.0, 36.6, 99.0, 98.1, and 52.0 %, respectively, when using D0 only as the diagnostic criterion; they were 47.2, 76.4, 94.8, 93.5, and 73.8 %, respectively, for D0+D1 criteria. In conclusion, the accuracy of CLCFDU to identify non-pregnant cows on day 21 after insemination was excellent.

Caractérisation de l'expression et de la localisation cellulaire des récepteurs MT1 et MT2 de la mélatonine dans le placenta de premiers trimestres de grossesse

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Introduction: Les récepteurs de la mélatonine (MT1, MT2) sont exprimés dans le trophoblaste villeux humain durant toute la grossesse. Cependant, la localisation tissulaire et intracellulaire des récepteurs de la mélatonine dans les trophoblastes extravilleux et villeux de placenta de premier trimestre de grossesse n'a jamais été étudiée.

Objectif: Cette étude visera à déterminer l'expression et la localisation intracellulaire des récepteurs MT1 et MT2 dans les trophoblastes de premier trimestre de grossesse.

Méthodes: L'expression génique et la localisation protéique des récepteurs de la mélatonine a été déterminée respectivement par RT-qPCR et immunocytochimie dans les cultures primaires de cytotrophoblastes extravilleux (CTev), de cytotrophoblastes villeux (CTv), ainsi que de syncytiotrophoblaste (ST). L'expression protéique des récepteurs de la mélatonine dans le tissu placentaire a été déterminée par immunohistochimie.

Résultats : L'immunohistochimie de villosités choriales de premier trimestre démontre que l'expression des récepteurs MT1 et MT2 est plus élevée dans les CTv et le ST que dans les CTev. Dans les cultures primaires de trophoblastes de premier trimestre, les niveaux d'ARNm confirment que les récepteurs MT1 et MT2 sont exprimés dans les CTev, les CTv et le ST. Les analyses d'immunocytochimie démontrent que les récepteurs MT1 et MT2 sont localisés dans le noyau pour les CTev et dans le cytoplasme pour les CTv ainsi que le ST.

Conclusion: L'expression différentielle des récepteurs de la mélatonine dans les cellules trophoblastiques issues de placentas de premier trimestre suggère que la mélatonine agit via des voies de signalisation différentes dans ces cellules.

Communication et partenariat entre cellules du cumulus et oocyte : rôle des protéines liées au X fragile

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[1]Université Laval, [2]Duke University

Le follicule ovarien est un syncytium complexe impliquant une interdépendance entre ses différents compartiments par des voies de communication servant à synchroniser la folliculogenèse et l'ovogenèse. Les cellules du cumulus adjacente à la zona pellucida produisent des projections cellulaires leur permettant un contact avec l'oolema. Nous avons montré que ces projections sont capables de transporter de gros cargos sous forme de granules riboprotéines pouvant être délivrées à l'oocyte. Notre hypothèse est que ces projections transzonale (TZPs) fonctionnent d'une manière similaire aux neurones. Pour caractériser le mécanisme par lequel ces cargos sont transporter le long des TZPs, nous avons étudier l'expression des trois membres de la famille des protéines liées au X Fragile (FMRP, FXR1 et FXR2) dans les bovins, les porcins et les souris. Nos résultats nous montrent une variation de l'abondance des ARNm et des protéines au cours de la folliculogenèse. Leur localisation et leur quantification dans les TZPs montrent que des trois protéines, FXR1 est le plus abondant. Ceci suggère différent patrons d'expression avant et après la formation des TZPs. Dans les souris Fmr1-KO, le réseau des TZPs diffère de celui des Wild-Type, la zona pellucida est plus fine et il semble avoir une compensation de la perte de FMRP par FXR2. Nos résultats supportent un modèle où FMRP serait impliqué dans l'établissement du réseau de TZPs tôt dans la folliculogenèse, FXR1 serait le plus abondamment impliqué dans le transport actif des granules de ARNm des cellules du cumulus à l'oocyte et FXR2 compenserait la perte de FMRP.

Loss of transzonal projections mediating germline-soma communication in the ovary is triggered by LHCGR-initiated signaling independently of oocyte meiotic maturation

Karen Carvalho[1,2], Laleh Abbassi[1,2], Qin Yang[2], Hugh Clarke[1,2]

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During oocyte growth in mammals, the surrounding somatic follicular granulosa cells elaborate actin-rich filopodia, termed transzonal projections (TZPs), that traverse the zona pellucida separating the two cell types and contact the oocyte plasma membrane, enabling transmission of signals from the granulosa cells to the oocyte. During meiotic maturation, the TZPs are lost, freeing the oocyte from direct maternal control in preparation for fertilization. The molecular mechanisms regulating TZP loss, however, are unknown. We show here that, when cumulus-oocyte complexes are removed from antral follicles, the TZPs slowly retract over a period of 12 to 16 hours. This retraction can be completely prevented by conditions that maintain high levels of intracellular cyclic GMP (cGMP), mimicking the condition within intact antral follicles. Following injection of human chorionic gonadotropin into 'primed' mice or activation of epidermal growth factor receptor (EGFR) signaling in cumulus-oocyte complexes in vitro, TZPs retract between 4 and 8 hours later – much more rapidly than under unstimulated conditions. Unexpectedly, TZP retraction can be experimentally dissociated from oocyte maturation, indicating that it is controlled exclusively by signaling within the cumulus granulosa cells.

Our results indicate that hormonal signals that initiate maturation also trigger TZP loss, underpinning the developmentally programmed loss of contact and communication between the maturing oocyte and its follicular microenvironment.

Supported by Canadian Institutes of Health Research.

Développement d'une nouvelle thérapie ciblée contre les cancers du sein hormono-dépendants et chimiorésistants à base des hybrides œstrogène-platine et des agents anti-inflammatoires

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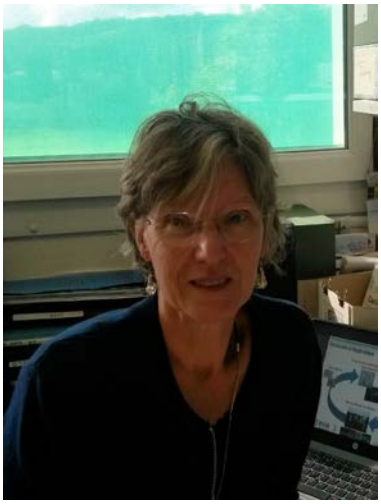
Au Canada, le cancer du sein est parmi les plus fréquents et les plus mortels chez la femme. Ce type de cancer reste difficile, voire impossible à traiter lors des récidives ou d'un diagnostic à un stade avancé en l'absence de thérapies efficaces. Malgré un succès très limité et une toxicité élevée sur les tissus sains, la chimiothérapie est encore largement utilisée pour tenter de limiter sa progression. Il est donc impératif de trouver de nouvelles thérapies plus innovantes et spécifiques pour améliorer le pronostic des patientes atteintes de cancers du sein. À noter, de multiples études suggèrent que l'inflammation chronique est associée avec la genèse des tumeurs hautement résistantes à la chimiothérapie et l'hormonothérapie. Dans cette optique, nos laboratoires ont caractérisé de nouveaux anti-inflammatoires dérivés de l'acide aminobenzoïque (DABs) ayant la capacité d'inhiber la croissance tumorale et la formation des métastases pulmonaires, sans présenter aucune toxicité sur les souris portant les tumeurs. D'autre part, de nouveaux composés anticancéreux hybrides œstrogène-platine (EP) ont été synthétisés et caractérisés pour cibler le récepteur des œstrogènes des cancers hormono-dépendants. L'objectif général du projet est de démontrer le principe préclinique de la mise en commun de ces différentes molécules prometteuses dans la thérapie moléculaire ciblée chez les patientes dont les traitements actuels ne permettent pas d'éviter les récidives tumorales, le risque de progression et encore moins l'éradication des tumeurs résistantes, récurrentes et métastatiques.

Conférencière invitée - Invited Speaker

Dre Hélène Jammes



Dr. Hélène Jammes, INRA DR2, is a biologist with more than 25 years of expertise in the cell biology, molecular biology, and reproductive biology as well as in epigenetics.

She started her career at INRA Jouy en Josas with a PhD thesis on the effects of gonadotrophins on ovine luteal cells in the CNRS' Laboratory of Physiology. She then joined the INRA's "Molecular Endocrinology Unit" and worked on GH signal transduction in mammary gland. In 2002, she joined the "Epigenetics and Genomics of Placental Pathologies Group" directed by Dr. Vaiman in Institut Cochin, (Paris), where she studied the epigenetic regulation of the imprinted genes in placenta in human and mice models. She also developed collaborations concerning DNA methylation alterations in sperm from infertile men.

After ten years, she joined the Biology of Development and Reproduction Unit (INRA, Jouy en Josas) and currently manages a team of 8 permanent personnel and students. The team is focused on DNA methylation as biomarkers of health and fertility in dairy cows and bulls.

On Tuesday, Dr. Hélène Jammes will deliver a talk entitled: **"*Biomarqueurs épigénétiques chez le bovin*"**.

Session d'affiches II – Poster Session II
6 novembre – November 6th
9h00 – 10h30

1. Morphological and cellular heterogeneity in the fallopian tube luminal epithelium. *Keerthana Harwalkar, PhD Student, McGill University (Page 53)*

2. Analysis of extracellular vesicle microRNA signatures from seminal plasma reveals pathogenic and protective mechanisms in varicocele patients. *Larissa Belardin, PhD Student, Université de Laval (Page 54)*

3. Primary cilia and Hedgehog signalling in the epididymis: a new role in the regulation of immune response in the epididymis highlighted by in vitro approaches. *Laura Girardet, PhD Student, Université de Laval (Page 55)*

4. Regulation of Dual Specificity Phosphatases (DUSPs) by Fibroblast Growth Factor 2 in bovine granulosa cells. *Lauriane Relav, PhD Student, Université de Montréal (Page 56)*

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Morphological and cellular heterogeneity in the fallopian tube luminal epithelium

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The fallopian tube (FT) is a tubular structure that extends from the uterus and connects to the ovary, it is involved in fertilization and preimplantation development. These functions are aided by two types of luminal epithelial cells, namely multi-ciliated and secretory cells. It is anatomically divided into four regions: the infundibulum, ampulla, isthmus and uterotubular junction, extending from the distal ovary to the proximal uterus. Interestingly, the distal FTE (infundibulum and ampulla) is implicated in an aggressive subtype of cancer, high grade serous ovarian cancer, where precancerous lesions are found restricted to the distal FT. In addition, we found that PAX2, which is considered a secretory cell marker in the fallopian tube, is not expressed in the distal fallopian tube epithelium, suggesting that two types of secretory cells, distal and proximal secretory cells, are present.

Using a combination of single-cell RNA-seq and immunofluorescence, we identified at least two types of secretory cells and two types of multi-ciliated cells based on regionality. We also cultured singularized distal or proximal FTE in extracellular matrix to allow for reorganization and organoid formation. Interestingly, only proximal FTE organoids turned on PAX2 expression, indicating distinct cellular identities. Taken together, our data highlights clear differences in epithelial cell subtypes of the FT, possibly conferring distinct functions in reproduction and distinct cancer susceptibilities.

Analysis of extracellular vesicle microRNA signatures from seminal plasma reveals pathogenic and protective mechanisms in varicocele patients

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It is important to identify microRNAs composition in seminal plasma of men with varicocele, once they are the major players in gene expression along the male reproductive tract. We aimed to identify microRNAs networks in seminal plasma extracellular vesicles in varicocele (VAR), PRE and POST-varicocelectomy men. MicroRNAs were extracted from seminal plasma extracellular vesicles and used to probe microarrays, using the Flashtag RNA. MicroRNAs were categorized in two classes: I) regulated in opposite directions between the comparisons PRE vs VAR and POST vs PRE, or II) regulated in the same direction between these same comparisons. We hypothesized that microRNAs from the class I would be related to pathogenic processes involved in male infertility. While microRNAs from class II could be enriched for protective mechanisms associated with male fertility. microRNA-mRNA regulatory networks were built using mirTarbase. Node degree was calculated for the microRNAs and mRNAs. Functional enrichment analyses were performed. In class I microRNAs network, the most connected microRNAs were hsa-miR-455-3p, miR-744-5p, miR-4793-3p, and miR-4668-5p. The 594 genes identified were associated with the regulation of transcription, cell proliferation, chromatin remodeling, RNA splicing, DNA damage response, and apoptosis. In the class II microRNAs network, the most connected microRNAs were hsa-miR-16-5p, miR-20a-5p, miR-193b-3p and miR-19b-3p. The 325 genes identified were associated with cell proliferation, regulation of apoptosis, cell migration, cell cycle, cytokine-mediated signaling pathway, cellular senescence, and DNA damage response. It was observed that varicocele is associated with pathogenic.

Primary cilia and Hedgehog signalling in the epididymis: a new role in the regulation of immune response in the epididymis highlighted by in vitro approaches

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Introduction. The primary cilium (PC) is a signalling antenna that plays an essential role in the transduction of the Hedgehog (Hh) signalling pathway, and whose dysfunction is associated with a broad spectrum of clinical features, including male infertility. Our research group has previously shown that epithelial cells of the epididymis expose a primary cilium whose function remains to be established in this organ. We aim to assess the role of epididymal PC as transducers of the Hh pathway in the control of epididymal functions.

Methods and Results. By immunofluorescence and western blot analysis, we found for the first time the presence Hh ligands and factors in different epididymal cell types in vivo and in vitro in immortalized epididymal epithelial cells (DC2). After pharmacological approaches with agonist and antagonist of Hh pathway on DC2 cells, the gene expression profiles were compared with controls from total RNA extracts by Affymetrix microarrays and ANOVA on Partek Software (n=3 per condition; Fold-change>1,5; p<0,005). We determined that ciliated DC2 cells were responsive to Hh agonist and antagonist by regulating the expression of 44 and 249 genes, respectively, including genes involved in migration, cell differentiation/proliferation and immune response pathways.

Conclusions. Our results showed that DC2 cell line respond to Hh signalling pathway through their PC. In combination with *in vivo* studies performed on a new mouse model disrupted for Hh pathway in epididymal epithelial cells, our research will shed light on the unexplored role of PC in the control of reproductive functions and male infertility.

Regulation of Dual Specificity Phosphatases (DUSPs) by Fibroblast Growth Factor 2 in bovine granulosa cells

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Intra-ovarian factors, such as fibroblast growth factor-2, regulate folliculogenesis. FGF2 stimulates proliferation of granulosa cells through MAPK, PKC and AKT pathways. A rapid and transient increase in MAPK3/1 phosphorylation is a typical mitogenic signal, and the intensity and duration of phosphorylation is regulated by DUSPs. In rats, DUSPs are implicated in the dephosphorylation of MAPK3/1 after FSH stimulation, but the regulation of their expression upon FGF signaling in the ovary is unknown. The aim of the present study was to identify which DUSPs are controled by FGF2 and to determine the pathways involved. Bovine granulosa cells were cultured under serum-free conditions and treated with FGF2 and/or specific inhibitors targeting MAPK, PKC and AKT pathways. Abundance of mRNA encoding DUSPs was assayed by RT-qPCR. Treatment with FGF2 significantly upregulated 3 of 9 DUSPs tested: *DUSP5* and *DUSP6* mRNA levels increased at all time points (2-6-8h), and *DUSP1* at 2-6h only. Inhibition of PI3K, involved in AKT pathway, with or without FGF2 treatment did not affect the expression of these 3 DUSPs. Similarly, we did not observe changes in their mRNA levels when targeting PKC. Disruptions of calcium signaling with a PLC inhibitor or an ionophore increased basal *DUSP1, DUSP5* and *DUSP6* mRNA levels but did not further increase FGF2-stimulated mRNA levels. Finally, inhibition of MAPK3/1 decreased FGF2-stimulated *DUSP1*, *DUSP5* and *DUSP6* expression.

We conclude that *DUSP1*, *DUSP5* and *DUSP6* are target genes of FGF2 and are controlled through calcium and MAPK3/1 pathways, which indicates a tight control of follicular growth.

Effect of Selective Serotonin Reuptake Inhibitors on the Serotonin System and Junctional Protein in Human Placenta

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Selective Serotonin Reuptake Inhibitors (SSRIs) are the most common pharmacological intervention for the treatment of antenatal depression. This type of antidepressant works by increasing the level of serotonin at the synaptic space by blocking the serotonin transporter (SERT; *SLC6A4*) on the post-synaptic neurons. The serotonin system, including SERT and monoamine oxidase A (MAOA), is expressed and functional in the placenta. We hypothesized that the exposure of primary placental cells to the SSRIs alter the expression of genes involved in placental serotonergic system and the junctional proteins associated with trophoblast cells fusion. Primary villous trophoblasts were isolated from normal full-term human placentas and were exposed at two concentrations (0.3uM and 0.03uM) of fluoxetine, norfluoxetine, sertraline, venlafaxine, or citalopram for 24-h. The mRNA level of *SLC6A4*, *MAOA* and Connexin 43 (*GJA1*) were analyzed by RT-qPCR. Overall, our preliminary data shows that all SSRIs tested tend to decrease the mRNA level of *SLC6A4*, *MAOA*, *GJA1* in primary trophoblasts, significantly in the cells treated with Citalopram, Fluoxetine and Sertraline. According to the results, SSRI alters the mRNA expression of the serotonin system and junctional proteins in human primary trophoblasts. The use of SSRIs during pregnancy poses an adverse effect on fetal development and may be associated with pregnancy complications such as gestational hypertension. Thus, pursuing the work is important to better understand the effect of SSRI on the placenta which is crucial for the maintenance of normal pregnancy and healthy fetal development.

The developmemtal constraint of bovine haploid androgenetic embryos is associated with an abnormal epigenetic regulation of X-chromosome inactivation and the KCNQ1 imprinted locus

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Haploid androgenetic embryos (hAE) show poor development compared to haploid parthenogenetic embryos (hPE), indicating that the chromosomes contributed by the sperm are developmentally restrictive when in a haploid condition. We hypothesized that the developmental limitation of haploid androgenotes is associated with paternal-specific regulatory long noncoding RNAs (lncRNAs) controlling X-chromosome inactivation (XCI) and maternally expressed imprinted genes. To exclude the effects caused by the lack of an X-chromosome, we produced bovine hAE by intracytoplasmic sperm injection (ICSI) using X-sorted sperm, which were compared to hPE, diploid parthenotes and ICSI-derived male and female fertilized embryos. Although no differences were observed at the 8-cell stage, XIST transcripts were significantly more abundant in hAE than in all other groups at the morula-stage. Nonetheless, PGK1 and HPRT mRNA levels in hAE did not differ from hPE, suggesting an ineffective XCI regardless of the high levels of XIST transcripts. Moreover, although transcripts levels of IGF2R did not differ among groups, transcripts of the KNCQ1OT1 lncRNA and the CDKN1C, PHLDA2, and TSSC4 imprinted genes were upregulated in hAE, suggesting a disturbed epigenetic regulation of the imprinted KCNQ1 locus. In conclusion, we show that the failure of haploid androgenetic embryos to develop to the blastocyst stage is associated with abnormal expression of key lncRNAs involved in XCI and genomic imprinting at the KCNQ1 locus, suggesting that their preimplantatory developmental constraint is regulated at an epigenetic level. This research was supported by a grant from NSERC Canada with L'Alliance Boviteq (LS) and a scholarship by Conicyt-Chile (LA).

Mice lacking ALK4 and ALK5 in gonadotropes are FSH deficient and hypogonadal

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Follicle-stimulating hormone (FSH) is an essential regulator of mammalian fertility. According to current dogma, activin B is the primary intra-pituitary stimulus for FSH synthesis. FSH is a dimeric glycoprotein,composed of a α subunit and a specific FSHβ subunit (product of the *Fshb* gene) that confers biological specificity. Activins signal via type II and type I serine/threonine receptor kinases to stimulate *Fshb* transcription *in vitro*. Mice lacking activin type II receptors (ACVR2A and ACVR2B) in gonadotropes are FSH deficient. However, mice lacking activin B (*Inhbb* knockouts) have elevated rather than reduced levels of FSH. Moreover, bioneutralizing activin A and B antibodies fail to affect FSH synthesis in murine pituitary cultures. The type I receptor inhibitor SB431542 fully suppresses FSH in the same context. Therefore, it is presently unclear whether activins or other members of the TGFβ family play necessary physiological roles in FSH production in mice. TGFβ ligands can bind to different type I receptors (ALK4, ALK5 and, ALK7). Here, we generated mice that lack ALK4 (*Acvr1b*) and ALK5 (*Tgfbr1*), alone and together, in pituitary gonadotropes. ALK4 conditional knockout (cKO) exhibited normal fertility and serum FSH levels. In contrast, serum FSH levels were reduced in ALK5 cKO mice relative to controls. ALK5 cKO females also had reduced ovarian follicle development and litter sizes. Double ALK4/ALK5 cKO were hypogonadal and FSH-deficient. These data challenge current dogma by showing that a non-activin TFGβ ligand that signals via ALK5 and, to a lesser extent, ALK4 is a main driver of FSH synthesis in mice.

The Association of Follicular Fluid and Growth Factors Improve Oocyte Maturation and In Vitro Development of Porcine Embryos

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Most porcine oocytes used in research are sourced from the ovaries of females slaughtered at prepubertal ages. However, these oocytes are known to have significantly higher polyspermy and lower development rates following IVF, when compared to their adult counterparts. Therefore, we assessed whether the supplementation of in vitro maturation medium with growth factors and/or follicular fluid could help improve IVF rates in these oocytes. Building on previous literature, we used a cocktail of growth factors composed of 40 ng/mL FGF2, 20 ng/mL LIF and 20 ng/mL IGF1 (known as 'FLI'), which has been shown to be beneficial previously (Yuan et al. 2017, PNAS, 114:E5796). We assessed the effects of FLI alone (FLI), follicular fluid alone (pFF) or follicular fluid and growth factors (pFF + FLI). The pFF + FLI group resulted in significantly better maturation rates compared to pFF and FLI (92.9 ± 1.3 vs. 86.6 ± 4.7 vs. 79.73 ± 2.9 respectively. $P < 0.05$). There was no significant difference in polyspermy rates among groups, although FLI + pFF had the lowest rate compared to the FLI and pFF groups (22.2 ± 26.6 vs. 24.88 ± 11.5 vs. 30.82 ± 12.3, respectively $P > 0.05$). Finally, in terms of development, the FLI + pFF group had the best cleavage rate (65.6 ± 4.6 vs. 63.7 ± 9.4 vs. 49.02 ± 13.0, $P < 0.05$) and blastocyst rate/oocyte (27.96 ± 4.3 vs. 15.72 ± 9.15 vs. 15.58 ± 7.3, $P < 0.05$) compared with the pFF and FLI groups, respectively.

The inhibition of H3K9 methyltransferases suppresses porcine early embryo development

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Epigenetic marks regulate important cells processes during embryo development, including genome activation, cell programing and genome damage repair. Epigenetic marks are critical components of cell reprograming in embryos produced by somatic cell nuclear transfer. However, much remains to be done to uncover the effects of each epigenetic mark in early stage embryos. The methylation pattern of H3K9 is related to a repressive chromatin state and was identified as an important component of the epigenetic memory hindering complete cell reprogramming in SCNT embryos. H3K9 methylation also plays a role regulating DNA damage repair through homologous and non-homologous pathways in somatic cells, which include the formation of transitional repressive heterochromatin. To gain additional information on the role of H3K9 methylation during early porcine embryo development, we used Chaetocin, a chemical inhibitor of the H3K9me3 methyltransferases SUV39H1 and SUV39H2, to assess in which moment during development the increase in H3K9me3 is required. Parthenogenically-activated zygotes were treated with Chaetocin for 24h at different times during development. Cleavage rate was not affected by Chaetocin treatment. However, embryo development to blastocyst stage was completely suppressed by Chaetocin during the first 24h post-activation (D0-D1), and significantly reduced by exposure between D1-D2 (8,4%), D2-D3 (4,3%), D3-D4 (5,5%) and D4-D5 (22,4%), compared to control embryos (CT-50%). Blastocyst rates were not different between embryos exposed to Chaetocin between D5-D6 (45%) and CT embryos. These results indicate that increasing H3K9me3 during the first cell cycle and prior to embryo genome activation is critical for regulation of early development in porcine embryos.

Régulation des signaux pro-inflammatoires par les facteurs immunitaires gestationnels Oncostatine M (OSM) et Amphiréguline (AREG), dans les trophoblastes et les macrophages humains

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Il est vrai que le système immunitaire intra-utérin représente une certaine complexité mais reste néanmoins des plus fascinant. Chez la femme enceinte l'activité immune permet à la fois la protection et la défense immunitaire de cette dernière et de son enfant durant la grossesse et occupe une un rôle essentiel dans le support de la grossesse.

Cependant, une activité inflammatoire précoce ou soutenue à l'interface foeto-maternelle est néfaste pour le maintien de la grossesse. C'est pourquoi une communication cellulaire coordonnée et synchronisée est indispensable entre les cellules déciduales, trophoblastiques et immunitaires et ce par la sécrétion de facteurs placentaires ayant une activité immunomodulatrice.

L'étude s'intéresse particulièrement à l'activité immunomodulatrice de deux facteurs gestationnels dans les trophoblastes et les macrophages : l'OSM (*oncostatin M)* et l'AREG *(amphiréguline)*.

L'hypothèse est que les cytokines l'OSM et AREG, participent activement à la régulation de signaux pro-inflammatoires induits dans les cellules trophoblastiques et les macrophages. Ces cytokines gestationnelles sont requises pour la protection du placenta et de l'embryon en cas de stress inflammatoire rencontré au cours de la gestation.

Cependant l'ensemble des mécanismes moléculaires et cellulaires induits dans les trophoblastes par OSM et AREG en présence de facteurs pro-inflammtoires tels que l'IFN*γ* (*interféron gamma)* et le GM-CSF *(Granulocyte Macrophage colony stimulating factor)* reste peu connus.

Ainsi nos études s'appliquent à démontrer l'effet modulateur de l'OSM et de AREG dans les trophoblastes humains en présence de l'IFN*γ* et du GM-CSF par l'activation de voies anti-inflammtoires telles que JAK/STAT3 et ERK1/2.

Protection contre les effets d'une exposition à l'alcool chez le jeune embryon par une diète maternelle enrichie en donneurs de méthyles

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Introduction: La consommation d'alcool durant la grossesse peut avoir des conséquences irréversibles, menant à diverses anomalies du développement. L'alcool est reconnu pour altérer les mécanismes impliqués dans les processus menant à la méthylation de l'ADN (e.g. cycle de l'acide folique, actions des DNMTs). La méthylation d'ADN est très dynamique tout au long du développement de l'embryon, particulièrement lors des premières divisions alors que les profils de méthylation sont activement reprogrammés. Nos résultats démontrent qu'une exposition prénatale à l'alcool (EPA) pendant cette vague de reprogrammation altère les futurs profils de méthylation d'ADN du cerveau du jeune embryon et augmente le nombre de défauts morphologiques. Quelques études suggèrent qu'un régime alimentaire maternel enrichi en donneurs de méthyles, essentiels pour les réactions de méthylation de l'ADN, pourrait prévenir certaines de ces anomalies embryonnaires induites par l'alcool.

Objectif: Déterminer si une diète enrichie en donneurs de groupements méthyles atténuera les effets d'une EPA pendant la période de reprogrammation épigénétique.

Méthode: Des souris ont reçu la diète contrôle ou enrichie 4 semaines avant la gestation et durant celle-ci. Elles ont été soumises à une EPA au jour E2.5, puis nous avons récolté les embryons au jour E10.5 ainsi que E18.5 afin d'évaluer les défauts morphologiques.

Résultats: Nos résultats préliminaires démontrent qu'une diète enrichie diminue le nombre de défauts morphologiques, entres autres les défauts multiples, chez les embryons exposés à l'alcool.

Conclusion: La diète enrichie en donneurs de groupements méthyles procure un effet de protection aux embryons subissant une exposition prénatale à l'alcool.

Implication des isoformes d'Akt dans le processus de décidualisation d'un modèle murin PR-Cre

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L'une des principales causes d'infertilité est associée à une communication inefficace entre l'embryon et l'endomètre maternel qui permet normalement l'implantation. Afin que ce processus ait lieu avec succès, les cellules endométriales stromales doivent proliférer et se différencier lors de la décidualisation. La protéine kinase Akt est retrouvée sous trois isoformes différents, (1, 2, 3) chacun possédant des rôles physiologiques distincts qui demeurent peu étudiés. Récemment, un effet isoforme spécifique de l'activité d'Akt sur l'expression transcriptionnelle des marqueurs déciduaux prolactine et IGFBP1 dans un contexte *in vitro* a été démontré par notre laboratoire; toutefois, une meilleure compréhension de ces résultats est nécessaire. Notre objectif est donc d'étudier la contribution spécifique des isoformes d'Akt lors de la décidualisation grâce à un modèle *in vivo* novateur. En effet, nous avons développé un modèle murin KO simple et combiné pour chaque isoforme d'Akt spécifiquement dans les tissus exprimant le récepteur à la progestérone (ovaires, utérus et glandes mammaires). Par l'induction artificielle de la décidualisation, nous investiguons l'effet de l'expression, de la localisation intracellulaire ainsi que l'activité de chaque isoformes d'Akt sur l'expression des marqueurs déciduaux. Suite à ces résultats, la régulation isoforme spécifique de l'activité d'Akt sur ces cibles moléculaires en aval tel que p70S6K, IκBα, GSK3β et FOXO1 sera étudiée puisque des résultats préliminaires montrent une telle modulation lors d'un contexte non-gestatif, progestérone dominante. L'approfondissement de notre compréhension de l'implication de la voie de signalisation PI3K/Akt dans le processus de la décidualisation permettra d'élaborer de nouvelles cibles thérapeutiques pour le traitement de l'infertilité.

Analyse transcriptomique des voies de signalisation PKA, PKB et PKC dans les cellules de granulosa humaine en culture (KGN)

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Les fonctions reproductives chez la femme dépendent grandement de la coordination et de l'action chronologique des hormones gonadotrophines, la FSH et la LH. Ces deux hormones sont connues pour agir sur les cellules de la granulosa via les trois mêmes principales voies de signalisation soit les voies de la protéine kinase A (PKA), la protéine kinase B (PKB) et la protéine kinase C (PKC). Toutefois, la manière dont ces différentes voies sont régulées et leurs actions tout au long du processus de la folliculogenèse restent encore à élucider. Afin de fournir une image globale des principaux facteurs impliqués dans la signalisation de ces trois protéines kinase et de mieux comprendre leurs rôles respectifs, les cellules KGN, des cellules tumorales de la granulosa humaine, ont été traitées avec un activateur spécifique pour chacune des voies respectivement la forskoline, le SC79 et le PMA. L'analyse transcriptomique réalisée avec la technologie RNA-seq a permis de mettre en évidence 3864 gènes différentiellement exprimés entre les traitements (FC 1.5, p≤ 0.05). L'analyse fonctionnelle des facteurs clés pour chacune des voies suggère que PKA jouerait un rôle clé dans la différentiation précoce du follicule vers le stade folliculaire dominant alors que l'activation de PKC procurerait un signal fort de différentiation « avancée » et d'inflammation poussant le follicule vers les processus ovulatoires. Finalement, la voie PKB agirait quant à elle comme support nécessaire à l'expression des gènes PKA-dépendant et agirait sur la survie cellulaire tout au long de la folliculogenèse.

The role of KCNQ1OT1 on genomic imprinting of the bovine KCNQ1 locus

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In humans, the maternally methylated *KvDMR1* ICR regulates the expression of a cluster of maternally expressed genes associated with epigenetic disorders that resemble the ART-associated Large Offspring Syndrome in cattle. The long non-coding RNA *KCNQ1OT1* is a paternally expressed antisense transcript associated with the regulation of the KCNQ1 imprinting domain in humans. We hypothesized that the *KCNQ1OT1* expression is the main regulation of bovine KvDMR1, and that its impairment drives a dysregulation of allele-specific DNA methylation and monoallelic expression patterns in this imprinted locus. To investigate this hypothesis, we performed experiments aimed at characterizing the role of *KCNQ1OT1* in fetal fibroblasts by downregulating its expression using small interference RNAs (siRNAs). Three siRNAs targeting *KCNQ1OT1* and a scrambled siRNA, used as control, were used to transfect a crossbred (*Bos taurus* x *Bos indicus*) primary fetal fibroblast. After two rounds of transfection, fibroblasts were collected for real time qPCR analysis. *KCNQ1OT1* transcript levels were reduced by 60% with all three siRNAs, confirming their efficiency. In order to investigate the effect of *KCNQ1OT1* on imprinted gens in the *KCNQ1* locus, we also analyzed the expression of *CDKN1C* and *PHLDA2*. Our results showed that the *KCNQ1OT1* knockdown affected *CDKN1C* and *PHLDA2* expression, by either increasing or decreasing expression according to the siRNA type. These preliminary results confirmed the efficiency of our siRNA approach to downregulate *KCNQ1OT1* and its potential role in regulating the bovine *KCNQ1* imprinted domain. Future experiments will address whether *KCNQ1OT1* downregulation impacts on DNA methylation patterns of *KvDMR*.

Validation de la robustesse et reproductibilité d'une méthode de cartographie pangénomique des cassures bicaténaires post-méiotiques chez le spermatide murin

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Les mutations *de novo* (MDN) sont associées à l'incidence de désordre neurologiques chez l'enfant. Elles sont transmises quatre fois plus par le père que par la mère mais l'origine précise de ce biais demeure indéterminée. Il a été démontré que des cassures bicaténaires (DSB) de l'ADN se produisent transitoirement lors de la spermatogénèse, suivant la méiose. Leur réparation est alors susceptible de créer des mutations. Dans une ébauche de leur cartographie, en considérant seulement les gènes, ces cassures se sont révélées plus fréquentes dans les gènes neurodéveloppementaux.

Ainsi, la formation de cassures transitoires serait la principale source de MDN et toute augmentation des cassures chez les spermatides favoriserait statistiquement un risque de transmission de mutations aux gènes neurodéveloppementaux augmentant le risque de désordres cognitifs.

L'objectif est d'améliorer la robustesse de la méthode de cartographie pangénomique des DSB post-méiotiques (hotspots) chez la souris impliquant le tri des spermatides par FACS, la capture des DSB, la constitution de librairies, le séquençage à haut-débit et l'analyse de données.

La méthode de capture a été validée sur le génome HeLa-I-SceI-3L1 soumis à différentes conditions de fragmentations. La méthode de tri des populations de spermatides a été optimisée afin d'augmenter le rendement, ce qui augmentera la richesse des librairies.

La cartographie pangénomique des DSB permettra de confirmer la localisation des hotspots dans le génome des spermatides de souris, puis d'humain. Elle pourra être utilisée en contexte clinique afin d'évaluer le risque de transmission de MDN à la descendance et l'impact phénotypique. Subventionné par les IRSC(#PJT-159719).

Dynamique de méthylation d'ADN durant le développement des cellules germinales mâle du rat

Rhizlane El Omri[1], Mariana Gabriela Ghinet[2], Claude Robert[3], Guylain Boissonneault[2], Géraldine Delbès[1]

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Dans la lignée germinale mâle des mammifères, la méthylation d'ADN se reprogramme pendant la vie fœtale puis reste dynamique lors de la spermatogenèse. Des études ont suggéré l'influence des perturbateurs endocriniens sur l'établissement de cette méthylation d'ADN, ainsi qu'un lien entre l'altération des profils de méthylation d'ADN des spermatozoïdes et l'infertilité. Cependant, une cartographie du methylome des gonocytes jusqu'aux spermatozoïdes n'a encore jamais été établie chez le rat, espèce de choix en toxicologie, notamment à cause de la difficulté à purifier les différentes cellules germinales en développement. Notre étude vise à réaliser la cartographie de la méthylation du génome dans les cellules germinales mâle du rat, du stade fœtal à l'âge adulte. Grace à un modèle de rat exprimant spécifiquement la GFP dans les cellules germinales, combiné aux profils de ploïdie et de compaction de la chromatine, nous avons purifié 9 populations cellulaires par FACS (pureté de 85 à 100%): 1,2 : gonocytes à 16 et 20 jours de gestation, 3 : spermatogonies. 4 : spermatocytes, 5 : spermatides1-8, 6 : spermatides 9-12, 7-spermatides 13-15, 8 : spermatides 16-19, 9 : spermatozoïdes. L'analyse de la méthylation de l'ADN sera faite dans des zones ciblées du génome notamment, les promoteurs, les îlots CpG ainsi que les régions riches en GC tel qu'établie sur une plateforme commercialisée de methyl-seq pour le rat. Nous tracerons ainsi la dynamique du méthylome et identifierons les régions différentiellement méthylées au cours du développement. Ce méthylome de référence sera utilisé pour tester l'effet de perturbateurs endocriniens.

Analyse des gènes à empreinte d'embryons bovins issus des techniques de reproduction assitée

Simon Lafontaine[1], Rémi Labrecque[2], Patrick Blondin[3], Marc-André Sirard[4]

[1]Université Laval, [2]SEMEX Boviteq, [3]SEMEX Boviteq, [4]Université Laval

La production d'embryons bovins par maturation et fécondation in vitro devient 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 8 embryons (jour 12) provenant de vaches superovulées et inséminées artificiellement fut comparé à des embryons provenant d'ovocytes dérivés d'un protocole de stimulation ovarienne, récoltés par ponction transvaginale (OPU) et mis en culture avec (8 embryons) ou sans sérum fœtal bovin (FBS ; 8 embryons). L'ADN du disque embryonnaire et du trophoblaste de chaque embryon fut extrait séparément et traité au bisulfite de sodium pour en révéler les marques de méthylation. Les niveaux de méthylation pour 10 gènes à empreinte furent évalués par pyroséquençage. Pour chaque gène, nous avons observé une méthylation globalement moins élevée ainsi qu'une plus grande variabilité chez les deux groupes d'embryons produits in vitro. De façon générale, les embryons mis en culture avec du FBS démontrent une hypométhylation plus importante au site d'empreinte ce qui concorde avec la perturbation épigénétique observé précédemment dans la littérature ainsi que sont liens 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.*

Genome-wide analysis and predictive function of LRH-1 and its potential coregulators throughout folliculogenesis

Stéphanie Bianco[1], Bruce Murphy[2], Nicolas Gévry[1]

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Given the complexity of ovulation, the characterization of molecular events at the genome level is essential to understand the mechanisms governing the expression of specific genes in the rapidly differentiating follicle. The nuclear receptor liver receptor homolog 1 (LRH-1) is highly expressed in the steroidogenic granulosa cells at all stage of follicle development and luteal cells. Previous studies with conditional knockout (cKO) models have shown the important role of LRH-1 in the early and later stages of follicular development. We have recently demonstrated that, just after the LH signal that initiates ovulation, the granulosa cells undergo a major modification of the distal regulatory elements that induce reprogramming of the LRH-1 genome-wide DNA binding (cistrome). This cistromic reorganization correlates with important changes in gene expression, regulating in particular the cytoskeletal remodeling and cell migration, essential for ovulation and luteinization. The aim of our study is now to draw a global map of LRH-1 function and its potential coregulators throughout folliculogenesis. We will first determine and compare LRH-1 cistrome by chromatin immunoprecitation-sequencing (ChIP-seq) analysis at important time points during the early stage (proliferation phase) and the later stages (initiation of ovulation, ovulation, and corpus luteum) of folliculogenesis. We will then predict its specific function and its potential coregulators at each step by bioinformatics analysis.

This study will provide a better understanding of ovulation and luteinization processes by thoroughly characterizing the imperative and indispensable function of LRH-1. The development of molecules targeting LRH-1 would offer considerable potential for the treatment of infertility.

Androgens Influence the Differentiation of the Epididymal Stem Columnar Cells in the Rat

Sylvie Pinto[1], Tifen El Belaidi [1], Laurie Pinel[1], Julie Dufresne[1], Mary Gregory[1], Daniel Cyr[1]

[1]INRS-Institut Armand Frappier

The epididymis is responsible for sperm maturation and fertility. Epididymal epithelium differentiation occurs postnatally in rodents but limited information exists on the regulation of this process. In young rats, the epididymis is comprised of undifferentiated columnar cells (UCS) that further differentiates into principal, basal, clear, narrow and apical cells, suggesting that UCS represent a stem/progenitor cell population. Our objectives were to determine if UCS are a stem/progenitor cell population and if cellular differentiation is under endocrine regulation. Microarray analyses of epididymides from young (7-day-old) versus adult rats indicated that over 3000 genes were differentially expressed in young rats, including EGFR, estrogen and androgen receptor signaling. 3D culture of epididymal cells from 7-day-old rats showed that cells could form organoids and differentiate in vitro, as determined by basal (TP63) and principal cell (AQP9) markers. Since UCS expressed ITGA6, these cells were isolated using ITGA6 antiserum and magnetic separation, and cultured in 3D. Only the purified fraction formed organoids. To assess the role of androgens on differentiation of UCS-derived organoids, cells were cultured with increasing concentrations of DHT (0, 10, 100, 200 nM). Analysis of organoids by electron microscopy showed that androgens stimulated differentiation in a dose-dependent manner. At lower doses, differentiation of basal cells was noted. At the highest DHT concentration, cells exhibited polarization, apical microvilli, well-defined tight junctions, and clearly delineated lumens. These results provide evidence that UCS represent a stem/progenitor cell population and that they can differentiate in vitro under the regulation of androgens. Supported by NSERC and CIHR.

Role of WNT and HIPPO Pathways in the Differentiation of the Epididymal Epithelium

Tifen El Belaidi [1], Sylvie Pinto[1], Julie Dufresne[1], Laurie Pinel[1], Mary Gregory[1], Daniel Cyr[1]

[1]INRS-Institut Armand Frappier

WNT and HIPPO pathways are involved in adult stem cell homeostasis. We have demonstrated the presence of adult basal stem cells in the epididymal epithelium. These cells can form organoids under 3D culture and can differentiate into principal cells. Developmentally, basal cells are derived from undifferentiated columnar cells present in the epididymis of young animals. The hypothesis is that the differentiation of columnar cells into other epididymal cell types is regulated, in part, by the WNT and HIPPO pathways. Microarray analyses of rat epididymides from 7-day old animals versus adults indicated that WNT9b was expressed 27-fold higher in young animals, while expression of its receptor, Frizzled-10, was 5-fold higher, suggesting that WNT signaling occurred early in postnatal development. However, CTNNB1 immunolocalization in young animals showed that it was present at the plasma membrane and did not localize to the nucleus, suggesting that WNT9B signaling may be modulated by a non-canonical WNT pathway. Immunolocalization of CTNNB1 in organoids derived from epididymal cells of 7-day old rats confirmed that CTNNB1 did not localize to the nucleus, supporting the notion of a non-canonical WNT signaling pathway. Immunolocalization of YAP, a component of the HIPPO pathway, indicated that during development YAP localized to the cytosol and nucleus. In adults, YAP was present in the nucleus of basal cells. These data support the role of WNT and HIPPO pathways in the differentiation of epididymal epithelial cells and in the regulation of epididymal stem cells. Supported by CIHR.

Investigating the post-meiotic DNA double-strand breaks formation in male gametes and their genetic consequences

Tiphanie Cavé[1], Guylain Boissonneault[1]

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The paternal inheritance of *de novo* mutations have been linked to neurodevelopmental disorders. Although meiotic recombination allows for the reshuffling of alleles and independent assortment of chromosomes, the genetic consequences of the post-meiotic maturation of the haploid male gamete are yet unknown despite a striking remodeling chromatin structure and an important surge in DNA double-strand breaks (DSBs). DSBs formation in the haploid context of spermatids may lead to genetic instability and variation. Meiosis in the fission yeast, *Schizosaccharomyces pombe*, shares similarities with that of male mammals and we have observed post-meiotic DNA DSBs during sporulation pointing to the highly conserved nature of this mechanism and providing a powerful genetic model to study the underlying molecular mechanism of DSBs formation and repair. In-gel nuclease digestion assays combined with mass spectrometry allowed the identification of Pnu1, an endonuclease potentially involved in the process. We have shown that Endonuclease G, the mammalian functional homolog of Pnu1, is expressed in mouse spermatids as well. Interestingly, generation of Pnu1 deletion mutant in the synchronizable Pat1-114 strain prevented the post-meiotic DNA fragmentation demonstrating a key role in the process. In addition to yielding information regarding the role of Pnu1 in the post-meiotic DSBs formation and sporulation, results from these experiments could lead to evidences of post-meiotic DSBs contribution to mutagenesis and adaptation, and direct further analyses in mammalian spermatids. New hypotheses regarding the paternal origin of genetic disorders can emerge from this work. Funded by the Canadian Institutes of Health Research (#PJT-159719).

Transzoanl projections may mediate GDF9 signalling from oocyte to granulosa cells

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Bidirectional signaling and communication between the oocyte and its surrounding granulosa cells is crucial for oocyte and follicular growth. One such signaling interaction depends on the activation by oocyte-produced growth-differentiation factor (GDF) 9, a member of the transforming growth factor (TGF)-β superfamily, of type I-type II TGFβ dimeric receptors on the plasma membrane of the granulosa cells. However, the mechanics of this essential ligand-receptor interaction remain poorly understood. During follicular growth, actin-containing filopodia-like structures known as transzonal projections (TZPs) extend from the granulosa cells, pass through zona pellucida, and attach to oocyte. We speculated that TZPs, which mediate signaling from the granulosa cells to the oocyte, might also mediate the GDF9 signaling in the opposing direction. By using immunostaining and 3D reconstruction of confocal serial sections, we show that ALK4, the type I receptor of GDF9, is abundant at the tips of TZPs. Intriguingly, when granulosa cell-oocyte complexes were disaggregated and then re-constructed, ALK4 became localized at the tips of the newly generated TZPs. Moreover, when granulosa cells were cultured in vitro, ALK4 became exclusively localized in the membrane region of intercellular contact, suggesting that its localization may be induced by cell-cell contact. Our findings identify an unexpected regionalization of the granulosa cell plasma membrane and suggest that the direct membrane contact between oocyte and granulosa cells may induce ALK4 to concentrate at tips of TZPs, possibly enabling efficient GDF9-dependent signaling from the oocyte to the granulosa cells.

Effects of environmentally-relevant mixtures of organophosphate ester (OPE) flame retardants on KGN cells, a human granulosa cell line

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OPE flame retardants are found ubiquitously in the environment. Previous studies suggest that exposure to individual OPEs may be detrimental to female fertility. Since ovarian granulosa cells play key roles in female reproduction, we tested the hypothesis that an environmentally relevant OPE mixture will adversely affect their function. KGN immortalized human granulosa cells were exposed for 48h to one of two OPE mixtures. The first (total mixture) was composed of 13 OPEs detected in Canadian house dust; the second triaryl-OPE mixture, was a subset of 7 OPEs with 3 phenyl moieties in their structure. Cells were exposed to vehicle or 1/1,000,000X – 1/3,000X dilutions of these mixtures, where 1X represents the OPE concentrations in 6.32g of dust. Effects on cell survival, lysosomes, ROS production, and lipid droplets were determined using fluorescent dyes and high content imaging. At dilution of 1/10,000X, the total and triaryl mixtures decreased cell survival by more than 80% and 50%, respectively. At non-toxic dilutions, only the triaryl-OPE mixture decreased the number of lysosomes/cell. Only the total mixture increased ROS production in cells at dilutions that were not cytotoxic. Both mixtures significantly increased the total area of lipid droplets at exposures as low as 1/300,000X;the total mixture induced this increase to a greater extent. Together, these data show that the total and triaryl OPE mixtures affect specific endpoints differentially. We propose that exposure to “house dust” OPE mixtures may have adverse effects on female reproductive health. Acknowledgements: CIHR, McGill University and Mike Wade (Health Canada).

Follicle stimulating hormone (FSH) does not impact rankl induced osteoclastogenesis in raw 264.7 cells

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Postmenopausal osteoporosis has been attributed to decreased estradiol levels. In the hypothalamus-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 serum FSH levels. It was recently proposed that this increase in FSH also contributes to postmenopausal osteoporosis by stimulating differentiation and activation of bone-resorbing osteoclasts cells. Our objectives are to determine whether FSH has direct actions on osteoclast differentiation *in vitro* and, if so, its mechanism of action. First, a murine leukemic monocyte macrophage cell line, RAW 264.7, was differentiated into osteoclasts by treatment with receptor activator of nuclear factor kappa-B ligand (RANKL, 50 ng/ml) for seven days. As expected, we observed the appearance of osteoclasts, characterized as tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells. Also, RANKL treatment induced gene expression of established osteoclast differentiation markers, including *Rank*, *Trap*, cathepsin K (*Ctsk*), and matrix metalloproteinase-9 (*Mmp-9*). The mRNA expression of FSH receptor (*Fshr*), however, was very low and mostly undetectable before and after osteoclast differentiation. Second, RAW 264.7 cells were co-treated with FSH (35, 70 and 140 IU/L) during RANKL-induced osteoclastogenesis. FSH did not impact the expression of *Rank*, *Trap*, *Ctsk, Mmp-9,* and *Fshr*. In conclusion, FSH did not further induce osteoclast differentiation in the presence of RANKL; nonetheless, more experiments will be performed to determine whether and how FSH might directly regulate bone resorption.

Jagged-notch signaling between the oocyte and granulosa cells during follicular growth within the ovarian follicle

Herthana Kandasamy[1,3], Qin Yang[1,3], Hugh J. Clarke[1,2,3]

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Oocyte and follicular development depend on bi-directional communication between the oocyte and neighbouring somatic cells. The specific pathways that mediate this crucial communication are, however, only partly understood. The Jagged-Notch pathway is a highly conserved signaling pathway, where membrane-bound Jagged binds to membrane-bound Notch, triggering cleavage of Notch, releasing its intracellular domain, which migrates to the cytoplasm and ultimately to the nucleus. Previous studies have shown that Jagged-Notch signaling is required for the formation of primordial follicles. We hypothesize that it may also act later; specifically, to regulate growth of the oocyte or follicle. RT-PCR confirmed that Jagged1 mRNA is expressed at all stages of oocyte growth. Conversely, using an antibody specific for cleaved NOTCH2, immunofluorescence of granulosa cell-oocyte complexes (GOCs) isolated at mid- and late follicular growth revealed strong cytoplasmic staining in the granulosa cells. This suggests that oocyte-derived JAGGED1 activates granulosa cell-derived NOTCH2 throughout growth. Unexpectedly, anti-JAGGED1 immunoblotting of oocytes revealed a band at ~20 kDa instead of the predicted 150 kDa. Following overnight incubation of oocytes as intact GOCs or in the absence of granulosa cells, bands migrating at ~20 kDa or 150 kDa, respectively, were observed. These results suggest that activation of Jagged-Notch signaling in the ovarian follicle leads not only to cleavage of the Notch receptor in the granulosa cells, but also to cleavage of the Jagged ligand in the oocyte. Future work will address the potential role of the Jagged cleavage product.

Supported by CIHR (HJC) and by an RQR fellowship to Herthana Kandasamy.

Developmental Genome-Wide DNA Methylation Asymmetry Between Mouse Placenta and Embryo

Karine Doiron[1], Lisa-Marie Legault[1], Anthony Lemieux[1], Maxime Caron[1], Donovan Chan[2], Flavia Lopes[3], Guillaume Bourque[4], Daniel Sinnett[1], Serge McGraw[1]

[1]Centre de recherche du CHU Sainte-Justine, [2]McGill University Health Centre, [3]Sao Paulo State University, Brazil, [4]Genome Quebec Innovation Centre

In early embryos, DNA methylation is remodelled to initiate the developmental program but for mostly unknown reasons, methylation marks are acquired unequally between embryonic and placental cells. To better understand this, we generated high-resolution DNA methylation maps of mouse mid-gestation (E10.5) embryo and placenta. We uncovered specific subtypes of differentially methylated regions (DMRs) that contribute directly to the developmental asymmetry existing between mid-gestation embryonic and placental DNA methylation patterns. We show that the asymmetry occurs rapidly during the acquisition of marks in the post-implanted conceptus (E3.5-E6.5), and that these patterns are long-lasting across subtypes of DMRs throughout prenatal development and in somatic tissues. We reveal that at the peri-implantation stages, the *de novo* methyltransferase activity of DNMT3B is the main driver of methylation marks on asymmetric DMRs, and that DNMT3B can largely compensate for lack of DNMT3A in the epiblast and extraembryonic ectoderm, whereas DNMT3A can only partially palliate in the absence of DNMT3B. However, as development progresses and as DNMT3A becomes the principal *de novo* methyltransferase, the compensatory DNA methylation mechanism of DNMT3B on DMRs becomes less effective.

Transcription factors RUNX1, NR5A2 and TBX3 are upregulated in bovine early embryos and show no sexual dimorphism

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The initiation of gene expression in embryos is referred to as embryonic genome activation (EGA) and, as a part of maternal-to-embryonic transition, is a key regulatory event during pre-implantation development in mammals. Although the bovine EGA is known to occur around the 8 cell stage, the molecular mechanism that trigger transcriptional activation remain poorly understood. To verify whether transcription of regulatory genes are upregulated during EGA and to determine whether differences levels are found in male and female embryos, we produced bovine oocytes and embryos by *in vitro* fertilization using X- and Y-sorted spermatozoa. Quantitative RT-PCR was used to measure the transcript amounts of the transcription factors NR5A2, RUNX1 and TBX3 at different stages of early development. Oocytes contained negligible amounts of all three transcripts, indicating absence of cytoplasmic carryover in post-fertilization embryonic stages. No differences in transcript amounts were found between male and female embryos at any stage of development, indicating sexual monomorphism for these transcription factors. NR5A2 and RUNX1 transcripts were more abundant at the 8 cell than at the blastocyst stage, suggesting possible involvement during the early stages of EGA. On the other hand, TBX3 transcript levels were lower at the 8-cell stage and increased at the morula and blastocyst stage, suggesting a potential role after EGA. Together, these results demonstrate that the levels of transcripts for regulatory transcription factors do not vary among sexes but vary dynamically during bovine early development, suggesting they could play a role during and immediately after EGA in this species.

Identification de nouvelles cibles de la kinase AMPK dans les cellules de Leydig par une approche protéomique quantitative à haut débit

Zoheir B. Demmouche[1], Houssein S. Abdou[1], Jacques J. Tremblay[1]

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Les cellules de Leydig produisent la testostérone, une hormone essentielle à la différenciation sexuelle masculine et à la spermatogènese. Dans ces cellules, l'hormone hypophysaire LH stimule la production de testostérone en induisant une augmentation d'AMPc intracellulaire, ce qui conduit à l'activation de différents facteurs de transcription et ultimement une modulation de l'expression de gènes impliqués dans la stéroïdogenèse. L'AMPc est par la suite dégradé en AMP et une concentration intracellulaire accrue en AMP active la kinase AMPK. Cette dernière réprime stéroïdogenèse en phosphorylant des protéines présentement inconnues, modulant ainsi l'expression génique. L'objectif de la présente étude est d'identifier de nouvelles cibles de la kinase AMPK impliquées dans la régulation négative de la stéroïdogenèse. Pour identifier les protéines phosphorylées par l'AMPK, une approche quantitative à haut débit par chromatographie liquide couplée à de la spectrométrie de masse en tandem après un enrichissement en phosphopeptides a été réalisée à partir d'extraits protéiques provenant de cellules de Leydig MA-10 où la stéroïdogenèse et l'activité AMPK ont été ou non stimulées. La validation de ces cibles potentielles sera alors réalisée par des essais de phosphorylation *in vitro* et le rôle de ces phosphoprotéines dans la stéroidogenèse sera étudié par une approche de déplétion par siRNA dans les cellules de Leydig. AMPK étant la première kinase identifiée réprimant la stéroïdogenèse, la mise en évidence de ses cibles ouvrira de nouvelles voies thérapeutiques pour les pathologies hormono-dépendantes. Subventionné par les IRSC (PJT-148738).

Decreased expression of the vitamin D signaling components is associated with aging-related prostate disorders including cancer

Gabriel Henrique Campolina-Silva[1]; Bruna de Toledo Maria[1]; Hipácia Werneck-Gomes[1]; Maria Clara Barata[1]; Germán Arturo Bohórquez Mahecha[1]; Cleida A. Oliveira[1]

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Preclinical studies have proposed protective roles for vitamin D in prostate cancer (PCa). However, PCa clinical trials using vitamin D or its active metabolite calcitriol as therapeutic targets have obtained unsatisfactory outcomes. These conflicting data could be due to a limited ability of the altered prostate to mediate the vitamin D signaling. Therefore, herein we investigated the expression pattern of the vitamin D receptor (VDR) and its heterodimeric partner RXR, as well as of the key enzymes involved in the local activation (CYP27B1) and catabolism (CYP24A1) of vitamin D, in the prostate of young adult to senile Wistar rats, a suitable *in vivo* model for studying aging-related prostatic disorders. We unraveled that both receptors and enzymes are highly expressed in the prostate epithelium. However, as the animals aged, the expression of VDR, RXR, and CYP27B1 drastically reduced in punctual areas exhibiting benign, precancerous and tumors lesions. On the other hand, CYP24A1 expression remained unaltered from adulthood to senility, including in lesion areas. Moreover, classic downstream targets of vitamin D signaling, such as the calcium transporters PMCA, CaBP-D_{28k}, and TRPV6, also had their expression altered in those prostatic lesions that formed naturally with increasing age. Concurrently, lower intraprostatic levels of calcitriol were detected at senescence in parallel with higher proliferative activity. Taken together, our findings point that vitamin D activation and responsiveness is limited in the aged prostate, and this imbalance on the intricate mechanism of tissue regulation by the vitamin D responsive system may be involved in prostatic carcinogenesis.

Conférencière invitée - Invited Speaker

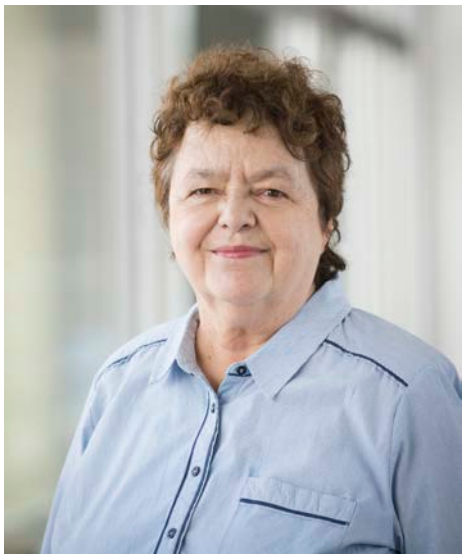
Dre Carole Yauk

Dr. Carole Yauk obtained a PhD in Biology from McMaster University and went on to do an NSERC post-doctoral fellowship at the University of Leicester in England. She joined Health Canada in 2002 as a research scientist in the Environmental Health Science and Research Bureau. She currently leads the Genomics Laboratory at Health Canada and is an adjunct professor of Biology at Carleton University. Her research is focused on the development of genomic approaches for chemical risk assessment and on improving regulatory evaluations to identify chemicals causing heritable genetic effects. She has over 180 publications in these research fields. She is co-chair of Health Canada's Modernized Approaches to Risk Sciences (MARS) Working Group, a Canadian delegate to the OECD's Extended Advisory Group for Molecular Screening and Toxicogenomics, and vice president of the North American Environmental Mutagenesis and Genomics Society.

On Wednesday, Dr. Carole Yauk will deliver a talk entitled: ***"The legacy of parental exposures to toxicants: Characterizing chemically-induced heritable genetic effects"***.

Conférencière invitée - Invited Speaker

Dre Line Chamberland



Dre Line Chamberland, sociologue de formation, est professeure au département de sexologie de l'Université du Québec à Montréal (UQAM) depuis 2009 et titulaire de la Chaire de recherche sur l'homophobie depuis 2011. Elle a réalisé plusieurs recherches sur les différentes formes d'exclusion sociale des personnes faisant partie de groupes minorisés en raison de leur orientation sexuelle ou de leur identité de genre. Elle s'intéresse notamment aux préjugés et aux pratiques discriminatoires ayant cours dans un contexte institutionnel (éducation, milieu de travail, services sociaux et de santé). Elle dirige actuellement le projet de recherche *Savoirs sur l'inclusion et l'exclusion des personnes LGBTQ* (savie-lgbtq.uqam.ca), mené en collaboration avec des partenaires associatifs, syndicaux et gouvernementaux

Elle présentera un séminaire le mercredi 6 novembre intitulé : ***"L'inclusion de la diversité sexuelle et de genre en milieu du travail : Les acquis et les défis"***.

Présentations – Presentations : Session III
6 novembre – November 6th
13h30 – 15h00

Présidente – Chair : Karine Doiron
Co-présidente – Co-Chair : Yassine Oufqir

I. **TGFβ/SMAD signaling regulates granulosa cell proliferation and TZP generation during early follicular development**
Sofia Granados Aparici, Postdoctoral Fellow, McGill University (Page 84)
13h30 – 13h45

II. **Epididymal Basal Cells Expressing LGR5 Participate in the Self-Renewal of the Epithelium of the Epididymis**
Laurie Pinel, PhD Student, INRS Institut Armand-Frappier (Page 85)
13h45 – 14h00

III. **Sperm miRNA – a potential mediator of bulls age and early embryo development**
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TGFβ/SMAD signaling regulates granulosa cell proliferation and TZP generation during early follicular development

Sofia Granados Aparici[1], Qin Yang[1], Hugh J. Clarke[1]

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During initiation of follicular development, the somatic cells surrounding the oocyte, termed granulosa cells (GCs), start to proliferate and cuboidalise. Shortly after, an extracellular layer called the zona pellucida starts to form and physically separates the oocyte from the GCs. Since GC-oocyte contact-dependent communication is essential for oocyte development, GCs generate transzonal projections (TZPs), filopodia-like structures that penetrate the zona pellucida and establish contact with the oocyte plasma membrane. We investigated whether the canonical TGFβ/SMAD signalling pathway regulates the rate of GC proliferation and generation of TZPs. The tamoxifen-inducible *Cre-ER*/floxed *Smad4*$^{+/+}$ knockout (KO) system was used in neonatal ovaries and granulosa cell-oocyte complexes (GOCs) in culture to delete the *Smad4* gene in non-growing and growing follicles. Whole ovaries and GOCs were exposed to tamoxifen (1ug/ml) for 1 and 2 days, respectively, followed by additional days in tamoxifen-free conditions. Wholemount staining of isolated follicles and GOCs was performed and confocal images were taken to quantitatively analyse the GC number, oocyte diameter and TZP number using Image J software. Single-layered follicles isolated from Cre^{+} ovaries after culture showed a significant decrease in GC number per oocyte diameter when compared to Cre^{-} controls. Cre^{+} GOCs showed a significant decrease in the number of TZPs whereas oocyte diameter remained unchanged. Our results suggest a new role for the canonical SMAD signalling on the molecular mechanisms regulating early GC proliferation and germ line-somatic communication in preantral follicles which may help identify novel markers of follicle/oocyte quality and provide insight into the causes of infertility.

Epididymal Basal Cells Expressing LGR5 Participate in the Self-Renewal of the Epithelium of the Epididymis

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The epididymis is lined with a pseudostratified epithelium comprised of various cell types. These include principal cells, which are the most abundant and line the lumen, and basal cells, located at the base of the epithelium. Our laboratory has demonstrated recently the existence of a basal cell population capable of proliferation, self-renewal, and differentiation *in vitro*. These basal cells could also form organoids which displayed the morphology and some functions of the epididymis. The present objective is to identify a specific marker of this population of progenitor cells in the epididymis. We observed that LGR5, a receptor involved in the WNT signaling pathway, was specifically expressed in selected number of basal cells. LGR5 is expressed by undifferentiated columnar cells of the epithelium at PND7. As the epithelium differentiates, expression of LGR5 decreases and becomes associated with basal cells. LGR5 is expressed in all regions of the epididymis, and co-localization of LGR5 with a basal cell marker TP63 in the adult epididymis indicates the existence of 3 basal cell sub-types: $LGR5^{+}/TP63^{-}$, $LGR5^{+}/TP63^{+}$ and $LGR5^{-}/TP63^{+}$. The localization of LGR5 in basal cells was confirmed using a transgenic mouse model that expresses LGR5-GFP. Lineage tracing studies indicated that $LGR5^{+}$ basal cells can differentiate into other cells types and permit the renewal of the epithelium of the cauda epididymidis within one month. Together, these data suggest that epididymal basal cells have an important and active role in the preservation and regeneration of epididymal tissue.

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Sperm miRNA – a potential mediator of bulls age and early embryo development

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Sperm miRNAs have been reported to regulate spermatogenesis and early embryonic development. Nowadays, the breeding industry tends to collect semen from younger bulls under high selection pressure, while the production at this period is suboptimum compared to adults. Whether the patterns of spermatic miRNAs are varied by paternal age and further impact on the early embryogenesis is not clear. Hence, we created the small non-coding RNA libraries of sperm collected from bulls at age of 10, 12 and 16 months, regarding 16 months as control. A total of 1080 and 1075 bovine miRNAs were identified in 10 vs 16 months and 12 vs 16 months groups, respectively. Amongst them, 10 sperm specific miRNAs were significantly differentially expressed in younger bulls (2 up-regulated and 2 down-regulated in 10 vs 16 months contrast, 3 up-regulated and 3 down-regulated in 12 vs 16 months contrast). Ingenuity pathway analysis of the targets of these miRNAs demonstrates potential influences on the developmental competence of 2-cell embryo and further on the metabolism of blastocysts. The results show that miRNAs pattern in sperm is affected by bull's age and will possibly mediate the paternal age effects on the early embryonic development.

Steroidogenic Factor 1 is essential for reproductive organ function in mature female mice

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As a growing population of women choose to have children later in life, the lack of information on the causes of age-related infertility represents a major gap in our knowledge of reproductive biology. The orphan nuclear receptor steroidogenic factor-1 (SF-1, Nr5a1) has been identified as an indispensable regulator of factors involved in the hypothalamic-pituitary-gonadal (HPG) axis, implicated in the transcription of gonadotropin subunits and steroidogenic genes. While it has been shown that granulosa cell-specific depletion of SF-1 in mice results in hypoplastic ovaries, impaired ovulation and infertility, its specific role in female reproductive processes remains to be determined. Using a progesterone receptor-driven recombinase-Cre (Pgr), we have developed a murine model characterized by conditional depletion of SF-1 in the pituitary gland and ovary of the mature female (PgrCre-SF1f/f; cKO, SF1f/f; CON). Reproductive evaluation, histological analysis and gene and protein expression measurements of mature cKO female tissues demonstrated that SF-1 depletion in peri-ovulatory events leads to absence of ovulation, extended estrus, reduced gonadotropin subunit synthesis, and infertility. Neither exogenous delivery of hormones to induce ovulation nor ovarian transplantation of CON ovaries in ovariectomised cKO mice is sufficient to successfully restore fertility in these cKO females. This indicates the importance of SF-1 in regulating both the pituitary gland and ovarian function, controlling the normal reproductive activity of mature female mice. This novel model of female infertility demonstrates the critical role that SF-1 plays in reproductive organ function, identifying it as a potential target for development of new targeted infertility treatments for women.

Characterisation of the epigenetic reprogramming in male rat germ cells and its sensitivity to ethinylestradiol exposure

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Epigenetic reprogramming is a key event of perinatal germ cells' development. Indeed, it guarantees genomic imprinting and cell differentiation. However, the kinetics and actors of this reprogramming are poorly characterized in rats. Moreover, it could be targeted by endocrine disruptors inducing long-term fertility disorders. Our study aims to 1- characterize the epigenetic dynamics in rat gonocytes during perinatal development; 2- test if this reprogramming is affected by xenoestrogen. Using transgenic rats expressing GFP specifically in germ cells, we purified gonocytes by FACS at various stages of perinatal development and established the transcriptomic profile of 165 chromatin remodeling enzymes. In parallel, we determined the dynamics of DNA methylation (5mC) and six histone modifications by immunofluorescence on testes sections. Our results highlight a transient chromatin remodeling involving histone modifications during DNA remethylation. In parallel, we studied the effect of exposure to ethinylestradiol, a xenoestrogen, in a rat fetal testes culture model that reproduces epigenetic reprogramming. During DNA remethylation, ethinylestradiol does not affect the number of gonocytes. However, DNA methylation analysis of exposed gonocytes by Reduced Representation Bisulfite Sequencing highlighted 4080 differentially methylated regions of which 80% are hypomethylated. Additionally, transcriptome analysis demonstrated that the expression of none of the enzymes studied above is affected in exposed gonocytes. But it revealed that genes associated with olfactory transduction, mRNA translation, and cell adhesion were significantly affected. Our study established the kinetics of epigenetic reprogramming in male rat gonocytes and suggested that xenoestrogens may affect gene expression in these cells, independently of chromatin remodeling dynamics.

Tetraploidy causes chromosomal instability by altering kinetochore-microtubule dynamics in the early embryo

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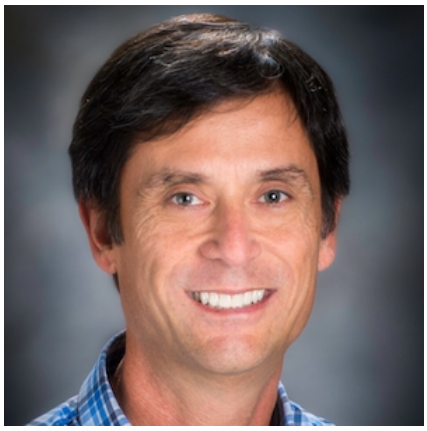
Embryos with binucleated (tetraploid) blastomeres are seen in human fertility clinics, but the impact of tetraploidy has been obscure. We recently showed that embryo tetraploidy leads to chromosomal instability (CIN) and aneuploidy. However, the mechanism by which tetraploidy leads to CIN had not been elucidated. We applied high resolution imaging, advanced 4D live centromere tracking, fluorescence photoactivation, and protein overexpression techniques to elucidate the mechanisms by which tetraploidy induces CIN. Centromere tracking experiments demonstrated that tetraploid embryos more frequently display chromosomes that fail to maintain alignment, increasing the likelihood of misalignments at anaphase. Interestingly however, these misaligned chromosomes do not seem to be the major contributors to anaphase lagging chromosomes. Experiments using photoactivatable-GFP-tubulin demonstrated that tetraploidy leads to increased kinetochore-microtubule (kMT) half-life (3.38 +/- 0.33 min in tetraploids vs. 2.28 +/- 0.12 min in controls), which is a direct measure of the ability to correct erroneous kMT-attachments. Consistent with this notion, tetraploid embryos display increased rates of kMT-misattachments (7%) as compared to controls (0.9%) and decreased kinetochore localisation of the error correction protein MCAK (94.53 +/- 8.85 a.u. in tetraploids vs 120.86 +/- 5.71 a.u. in controls). Strikingly, ectopic overexpression of MCAK:GFP decreased the rates of chromosome segregation errors (50% in GFP-injected vs. 25% in MCAK:GFP-injected). Our results suggest that tetraploidisation reduces the available pool of MCAK, decreasing MT turnover and increasing kMT-misattachments which in turn cause CIN. This novel route from tetraploidy to CIN contributes to embryo mosaicism, and may even participate in the generation of CIN in cancer.

Conférencier invité - Invited Speaker

Dr Richard Behringer



Dr. Richard Behringer is a Professor in the Department of Genetics and Ben F. Love Chair for Cancer Research at the University of Texas M.D. Anderson Cancer Center in Houston, Texas. Dr. Behringer's research focuses on mammalian developmental genetics, including organogenesis, stem cells, and evolution. Dr. Behringer also conducts field studies on Kangaroo Island in Australia and on the Caribbean island of Trinidad. Previously, he was the Director of the *Molecular Embryology of the Mouse* course at the Cold Spring Harbor Laboratory and Director of the *Embryology* course at the Marine Biological Laboratory in Woods Hole. He is one of the editors of the 3rd and 4th editions of *Manipulating the Mouse Embryo: A Laboratory Manual* and co-author with Dr. Virginia Papaioannou of *Mouse Phenotypes: A Handbook of Mutation Analysis* both published by CSHL Press. Through social media (Twitter @rrbehringer), he is an advocate for developmental biology, genetics, and reproductive biology.

On Wednesday, Dr. Richard Behringer will deliver a talk entitled: **"*Genetic regulation of reproductive organ development*"**.

Notes :

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Crédit photo **: Céline Augière, Ph.D., Post-Doctoral fellow in Clémence Belleannée's research group**

Atypical primary-cilia like organelles extend from the surface of the so-called "non-ciliated cells" in the efferent ductules.
The primary cilium is a sensory organelle composed of an axonemal extension (Arl13b, red) and a modified centriole (Centrin2, green), which are visualized by confocal microscopy in the efferent ductules of a double transgenic Arl13b-mCherry; Centrin2-GFP mouse model. Singular and long primary cilia-like structures are present in Villin-positive non-ciliated cells (purple) that are adjacent to multi-centriolar ciliated-cells. While primary cilia are involved in the development and homeostasic control of most biological systems, the contribution of these organelles to reproductive biology remains to be established.

Un cil primaire atypique à la surface des cellules "non ciliées" des vas efferents.
Le cil primaire est un organite sensoriel composé d'une extension axonémale (Arl13b, en rouge) et d'un dérivé du centriole (Centrin2, en vert) qui sont visualisés par microscopie confocale dans les vas efferents d'une souris double transgénique Arl13b-mCherry; Centrin2-GFP. Ce cil atypiquement long est présent à la surface des cellules non-ciliées marquées par la Villine (en violet) qui sont adjacentes aux cellules multi-centriolaires ciliées. Bien que le cil primaire soit impliqué dans le développement et le contrôle homéostatique de presque tous les organes, la contribution de cet organite dans le système reproducteur mâle reste inconnu.

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