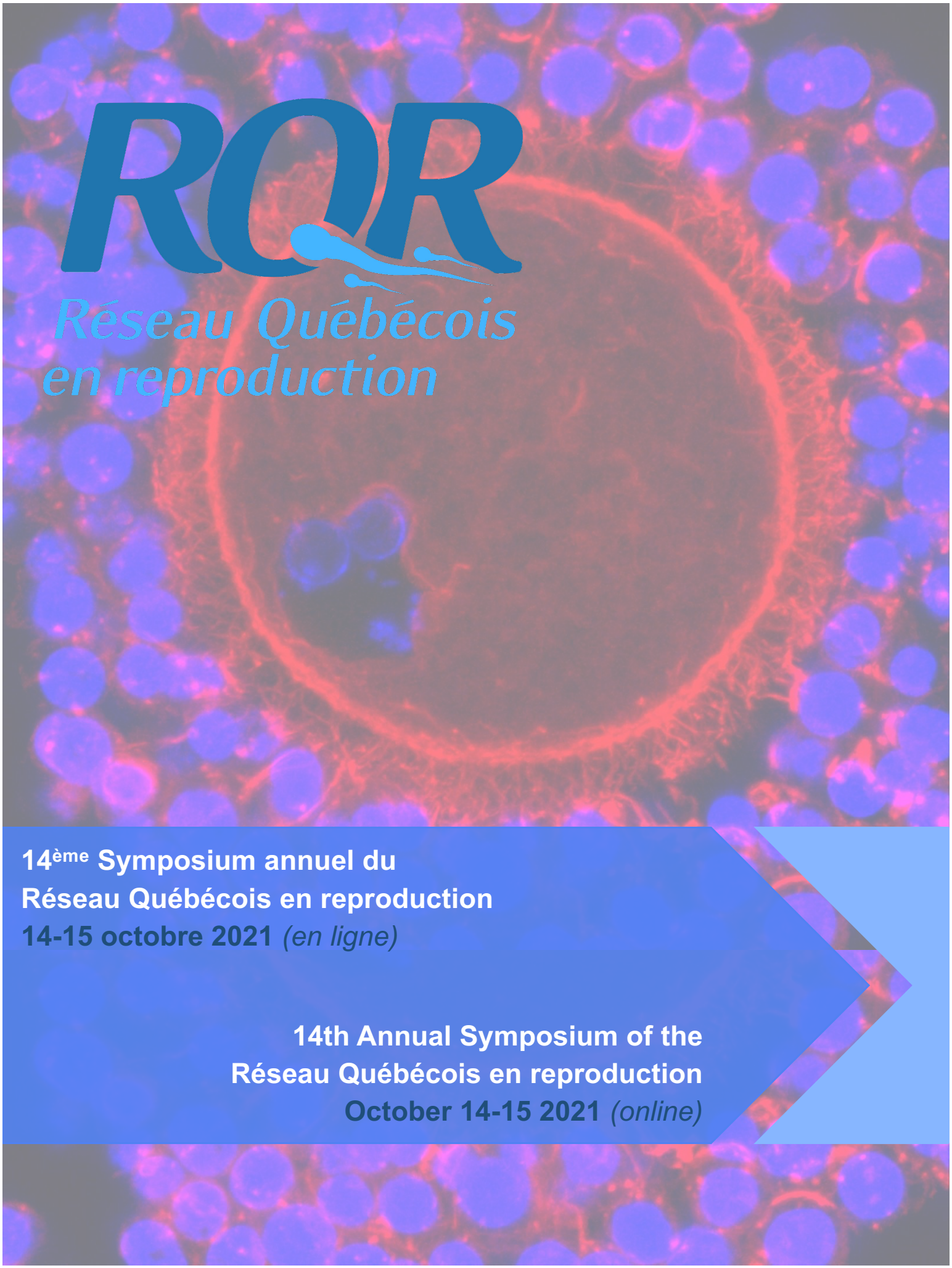


14ème Symposium annuel du Réseau Québécois en reproduction

14-15 octobre 2021 *(en ligne)*

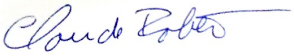
14th Annual Symposium of the Réseau Québécois en reproduction

October 14-15 2021 *(online)*

MOT DU DIRECTEUR

À tous les membres du RQR, stagiaires et autres participants,

Il me fait plaisir de vous souhaiter la bienvenue à cette 14ème édition du Symposium du RQR. Ce sera la deuxième édition utilisant le format virtuel. Dans un contexte pandémique qui semble s'améliorer, ce fut une décision difficile à prendre. Nous sommes tous conscients que ce n'est pas le format qui offre le meilleur niveau d'interaction entre nous. Ceci explique la décision tardive d'annuler l'événement en présentiel. Mais la décision devait se prendre afin d'éliminer toute chance que notre rencontre ne devienne le prochain épicentre de transmission. Espérons que la prochaine édition sera la bonne pour se retrouver en présentiel. Afin de voir les bons côtés des choses, le format virtuel permet de rejoindre les gens plus aisément et plus loin. Comme à l'habitude, nous avons un programme de grande qualité. Et comme vous le savez, le RQR c'est une communauté de recherche mais c'est aussi bien plus. Le RQR nous offre une vision large de la biologie de la reproduction de même que des opportunités uniques de nous informer et de nous éduquer vis-à-vis de nombreux enjeux sociétaux. À cet égard, nous aurons la chance d'assister à une conférence du Dre Lisa Greenhill qui nous présentera comment devenir beaucoup plus qu'un supporter mais plutôt un allié de l'équité, la diversité et l'inclusion. Au niveau de la physiologie de la reproduction, cette année le mouton sera à l'honneur lors de la première journée avec une conférence de Johanne Cameron portant sur la photopériode ainsi que celle du Dr Stéphane Fabre portant sur les déterminants génétiques de la folliculogenèse et de l'ovulation. Lors de la deuxième journée, ce sera au tour du génome d'être à l'avant plan pour nos conférenciers invités alors que Dre Deborah Bourc'his, nous présentera le rôle de la méthylation de l'ADN durant la spermatogenèse et Dr Bin Gu nous montrera comment modifier le génome tôt dans le développement embryonnaire afin de mieux comprendre la biologie du développement et produire des modèles pour l'étude des maladies. Bien entendu, le Symposium du RQR ne serait pas complet sans les présentations étudiantes. Comme ce sont eux qui souffrent le plus du manque de visibilité, je vous invite à participer grandement aux séances de présentations s'échelonnant sur les deux journées. Prenez le temps de les écouter et n'hésitez pas à les contacter pour poser vos questions.
Sur ce, je vous souhaite un excellent Symposium du RQR!



Claude Robert, Directeur du RQR

To all RQR members, trainees and other participants,

It is my pleasure to welcome you to this 14th edition of the RQR Symposium. This will be the second completely online edition. In the context of a pandemic that appears to be improving, this was a difficult decision to make. We are all aware that this is not the format that offers the best level of interaction. But the decision had to be made in order to eliminate any chance that our meeting meet face-to-face. On the positive side, being online makes it possible to reach people more easily and to do so across the globe. As usual, we have an excellent program. As you know, the RQR is a research community, but it is also much more. The RQR offers us a broad vision of reproductive biology as well as unique opportunities to inform and educate ourselves about many societal issues. In this regard, we will have the unique opportunity to attend a lecture by Dr. Lisa Greenhill, who will show us how to become much more than a supporter but rather an ally of equity, diversity and inclusion. In addition, this year sheep will be in the spotlight on the first day, with a conference by Johanne Cameron on the photoperiod as well as one by Dr. Stéphane Fabre on the genetic determinants of folliculogenesis and ovulation. On the second day, it will be the turn of the genome to take center stage for our guest speakers, as Dr. Deborah Bourc'his, will present the role of DNA methylation during spermatogenesis and Dr. Bin Gu will show us how to modify the genome early in embryonic development to better understand developmental biology and produce models for the study of diseases. Of course, the RQR Symposium would not be complete without the student presentations. As they are the ones who suffer the most from the a of visibility, I invite you to attend their presentation sessions in great numbers over the course of the two days. Take the time to listen to them and do not hesitate to contact them with comments and questions.
With that, I wish you an excellent RQR Symposium!

Claude Robert, RQR Director

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DOMESTIC ANIMAL DOHAD & EPIGENETICS QUÉBEC CITY • CANADA • 2021

Preliminary Program
Domestic Animal DOHaD and Epigenetics meeting (DOHaD satellite 2021)
**Jointly organized with the RQR Symposium*

Objective :
To foster the development of a new field of research based on the epigenetic programming of future health and phenotypes by gametes, embryos, fetuses and newborns in farm animals.

October 12, 2021

9h00 – 9h10	**Welcome**
9h10 – 10h00	**Openning lecture** Sarah Kimmins (Canada) *"Epigenetic inheritance how did we get here and where are we going ?"*
10h00 – 10h20	**Break**
10h20 – 12h00	**Session I - Early programming by the gametes from both sexes** 10h20: Helene Kiefer (France) *"The epigenome of male germ cells in ruminants"* 11h00: Marc André Sirard (Canada) *"Periconceptual environmental impact on gametes and the resulting embryo epigenetic legacy"* 11h40: Jessica Ispada *"Metabolomic signature on culture medium of embryos with higher or lower levels of DNA methylation"*
12h00 – 13h00	**Lunch break**
13h00 – 14h30	**Online poster session I** **14h00: Question period for posters of session I**
14h30 – 16h10	**Session II - Embryo programming (cloning culture)** 14h30: Cindy Tian (USA) "*The quest continues: A hidden genomic code for DNA methylation?"* 15h10: Vilceu Bordignon (Canada) *"Chromatin role on embryo programming and reprogramming"* 15h50: Yahan Li *"Investigation of bovine chromosome architecture and its involvement in large offspring syndrome"*

October 13, 2021

8h30 – 10h10	**Session III - Health of the offspring's** 8h30: Pascale Chavatte-Palmer (France) *"Importance of DOHAD in the equine industry"* 9h10: Veronique Ouellet (Canada) *"Heat stress in dairy cows: why worry about future generations?"* 9h50: Maria Rabaglino *"Stimulated muscle development in healthy in vitro produced calves determined through a multi-omic approach"*
10h10 – 10h30	**Break**
10h30 – 12h00	**Online poster session II** **11h30: Question period for posters of session II**
12h00 – 14h00	**Lunch break**
14h00 – 16h00	**Session IV - Post natal programming** 14h00: Stephany McKay (USA) *"Examining the extent of environmental contributions towards DNA methylation and phenotypic variation"* 14h40: Eveline Ibeagha-Awemu (Canada) *"Epigenetic modulators of bovine mammary gland health and production: Enhancers or abaters"* 15h20: Romina Via y Rada *"Epigenetic programming of the rabbit preimplantation embryo in a diabetic context"* 15h40: Rhizlane El Omri-Charai *"Impact of a high-fat maternal diet during gestation on fetal germ cells DNA methylation"*
16h00 – 16h10	**Closure**

Noter que le programme est à l'heure de l'Est. (UTC-5)
Note that the meeting schedule is on Eastern time (UTC-5)

Conférencière invitée - Invited Speaker

Sarah Kimmins

Sarah Kimmins received her Ph.D. from Dalhousie University in 2003 and completed her post-doctoral training at the Institut de Génétique et de Biologie Moleculaire et Cellulaire in Strasbourg, France in 2005. She was appointed to the Department of Animal Science in the Faculty of Agricultural and Environmental Sciences in 2005 and is a tenured Associate Professor. She is an associate member of the Department of Pharmacology and Therapeutics, Faculty of Medicine at McGill. She held a Tier II Canada Research Chair in Epigenetics, Reproduction and Development (2011-2021), and was the Associate Director for the McGill Center for the Study of Reproduction (2014-2017). In 2014 she received the Young Andrologist Award from the American Society of Andrology, and in 2016 the Society for the Study of Reproduction Young Investigator award.

Sarah Kimmins will give the openning lecture on Tuesday, October 12th entitled: ***"Epigenetic inheritance how did we get here and where are we going"***.

Conférencière invitée - Invited Speaker

Hélène Kiefer

Hélène Kiefer obtained a permanent researcher position in 2010 at INRAE of Jouy-en-Josas. She joined a team working on livestock epigenetics (leader: Hélène Jammes), and developed several tools to study DNA methylation at a genome-wide scale in cattle: a Roche-NimbleGen microarray, and more recently RRBS. She collaborates with bioinformaticians and statisticians to develop pipelines for DNA methylation analyses. Her research interest is to understand how epigenetic modifications vary under the influence of both environmental and genetic factors and contribute to phenotype variability in cattle. She is particularly interested in bull sperm epigenome and its potential role as a mediator of intergenerational inheritance effects.

Hélène Kiefer will give a lecture on Tuesday, October 12th entitled: ***"The epigenome of male germ cells in ruminants".***

Conférencier invité - Invited Speaker

Marc-André Sirard

Marc-André Sirard spent all his professional life working in IVF. He used a laparoscopic approach to perform IVF in cattle and obtained the first test-tubes calves in 1985. During his post-doc in USA, Dr Sirard developed a method to produce bovine embryos by the hundreds using oocytes recovered from post-mortem cows. This extraordinary tool was then used in hundreds of laboratories to understand and improve all aspects of IVF in large animals and humans. Over the last 3 decades. He came back to Québec in 1987 and obtained an industrial chair to work on oocytes and sperm in 1990. He founded the Centre de Recherche en Biologie de la Reproduction in 1995 which has grown to include more than 100 people today. He obtained a senior Canadian Research Chair in 2000 on genomics applied to reproduction and has created an international effort to define the normal genomic program in early mammalian embryos which has become an NSERC strategic network, EmbryoGENE in 2008. He has published over 325 scientific papers and has been invited to give over 95 invited lectures in international meetings. His current research activities are focus on the epigenetic mechanism allowing information transfer from one generation to the next.

Marc-André Sirard will give a lecture on Tuesday, October 12th entitled: ***"Periconceptual environmental impact on gametes and the resulting embryo epigenetic legacy"***.

Metabolomic signature on culture medium of embryos with higher or lower levels of DNA methylation (#26)

Jessica Ispada[1], Camila Bruna de Lima[2], Erika Cristina dos Santos[1], Aldcejam Martins Fonseca Junior[1], Joao Victor Alcantara da Silva[1], Heloise Cale da Rocha[1], Patricia Kubo Fontes[1], Marcella Pecora Milazzotto[1]

[1]Universidade Federal do ABC, [2]Université Laval

Tricarboxylic acid (TCA) cycle metabolites have a direct role on epigenetic marks, coordinating the activity of enzymes responsible for genomic DNA methylations removal. This control also affects bovine early embryos, in which the culture media supplementation with α-Ketoglutarate or Succinate, respectively, increases or decreases DNA demethylation (Ispada et al, 2020). In this work, we describe the metabolic signature of the spent culture media of these embryos using Raman spectroscopy. For this, bovine embryos were produced using standard protocols with a semi-defined culture medium (Santos et al, 2021) without the presence of metabolite analogs (Control–CO) or with 4 mM α-Ketoglutarate (AKG) or 4 mM Succinate (SUC) until day 4. After that, embryos were cultured without metabolite analogs and, on day 7, five blastocysts (four replicates) were placed in a fresh 50 uL drop and cultured for 12 hours. This medium was evaluated by Raman spectroscopy. Thirty Raman bands were detected, and from that, 19 Raman bands were identified through multiples t test as differently expressed in at least one comparison (CO vs. AKG; CO vs. SUC). The spent culture media of AKG group presented 5 higher (494, 524, 627, 641 and 647 cm-1) and 12 lower intensity bands (830, 847, 874, 895, 914, 1037, 1068, 1082, 1159, 1306, 1447 and 1462 cm-1) when compared to the CO. On the other hand, SUC only had 4 Raman bands altered in relation to the CO ($P<0.06$), with 2 higher (627 and 647 cm-1) and 2 lower (1082 and 1159 cm-1). The comparison with AKG and SUC resulted in 13 different Raman bands ($P<0.06$), with one higher (1645 cm-1) and 12 lower in SUC (775, 830, 847, 874, 895, 914, 1037, 1068, 1082, 1306, 1447 and 1462 cm-1). The attribution of these Raman bands revealed that a variety of amino acids and glucose were differently consumed by blastocysts. Therefore, we conclude that blastocysts with different levels of DNA methylation induced by metabolic analogs treatment modify Raman metabolic signature on the bovine embryo culture media. This result might potentially be used to select embryos with different intensity of DNA methylation using a non-invasive approach.

DADE Session d'affiches en ligne I – Online poster session I
12 octobre – October 12th
13h00 – 14h30

Whole genome DNA methylation biomarker screening for bull fertility (#25). *Ying Zhang, PhD Student, Université Laval (Page 11)*

Allele-specific methylagtion editing of imprinting genes (#27). *Gilberto Duran Bishop, MSc Student, Université de Montréal (Page 12)*

Can the embryonic linker histone H1Foo modulate somatic cell reprogramming? (#30). *Mariana Priotto de Macedo, PhD Student, McGill University (Page 13)*

Variations in mtDNA copy number in oocytes cultured in different in vitro maturation conditions (#58). *Camila Bruna de Lima, Postdoctoral Fellow, Université Laval (Page 14)*

Studying the dynamic of DNA methylation in rat male germ cells during gametogenesis by Methyl-Seq capture (#60). *Rhizlane El omri-Charai, PhD Student, INRS - Armand Frappier (Page 15)*

Prediction of bull fertility based on sperm methylome (#68). *Valentin Costes, PhD Student, INRAE - Université Paris-Saclay (Page 16)*

Whole genome DNA methylation biomarker screening for bull fertility (#25)

Ying Zhang[1], **Marc-André Sirard**[1]

[1]Université Laval, Département des sciences animales

Nowadays, many efforts are being made to improve dairy cattle fertility particularly on females. However, bull fertility had been ignored, new methods and tools should be developed for improving male fertility independently. This study adopted enzymatic methyl sequencing to identify candidate DNA methylation biomarkers in sperm correlated with bull fertility. We filtered and selected 12 bulls (High Bull Fertility = 6; Low Bull Fertility = 6) with extreme bull fertility phenotypes according to the Bull fertility index via 6000 Artificial Insemination data from a private database. Libraries were prepared with Enzymatic Methyl-Seq kit following manufacturer's instructions. After sequencing, reads were processed in a bioinformatics pipeline for base calling and methylation detection. A total of 450 DMCs (Differentially Methylated Cytosines) presented a methylation difference higher than 20% and a significant q-value ($q<0.01$) between groups. Meanwhile, a 500 bp sliding bin size and a 100 bp step had been used to search for DMRs (Differentially Methylated Regions). Based on the top 70 candidate bins, 16 DMRs were screened after comparing the high bull fertility and low bull fertility groups when using a cutoff of $q<0.01$; 5 of these DMRs contain significant DMCs. Furthermore, 22 DMGs (Differentially Methylated Genes) could be mapped into reported specific genes. Interestingly, some of those genes (LOC781841, SPATA1, and MGC134232) have been correlated with spermatogenesis. In conclusion, this study was able to identify potential regions with differentially DNA methylation that could be used to complement the existing genetic methods and provide a better tool to predict bull fertility.

Allele-specific methylagtion editing of imprinting genes (#27)

Gilberto Duran Bishop[1, 2], **Elizabeth Elder**[1, 2], **Karine Doiron**[1, 2], **Serge McGraw**[1, 2]

[1]University of Montreal, [2]Centre de Recherche CHU Ste-Justine

Following fertilization, a major reprogramming wave removes most DNA methylation signatures across the genome to instigate the embryonic developmental program. Through mostly unknown mechanisms, parent-of-origin-specific methylation marks on one allele of imprinted genes (e.g., Igf2: Insulin-Like Growth Factor 2/H19 locus) are able to escape the embryonic reprogramming wave and retain their profiles via maintenance by the DNA methyltransferase 1 (DNMT1) family proteins. Failing to maintain proper genomic imprinting patterns during embryonic reprogramming leads to neurodevelopmental disorders characterized by developmental delay. It is still unclear how embryonic cells are able to re-establish DNA methylation profiles across most of the genome following a temporary lack of DNA methylation maintenance, while other regions such as imprinted loci become permanently dysregulated.Published and current data from the McGraw lab show that, for Igf2/H19 and Peg13 imprinting loci, a transient loss of DNA methylation maintenance in mouse embryonic stem cells leads to permanent loss of DNA methylation, decrease levels of repressive histone modification H3K9me3 associated with imprinting loci, increase levels of active mark H3K4me3 associated with actively transcribed genes, increase in H3K27ac associated with open chromatin, and increase in gene expression.What remains to be elucidated is whether we can permanently re-establish normal profiles of DNA methylation and subsequent histone modifications on imprinted (allele-specific) sequences following the loss of their DNA methylation imprinting status. To set the foundation for a clear understanding of the mechanisms behind re-establishing the loss of inherited methylation profiles, we hypothesize that we can re-establish specifically and permanently the lost DNA methylation imprints at specific loci by targeted epigenome editing in embryonic cells. This project will further the understanding of the fundamental complex biochemical mechanisms of epigenetic regulation that drive allele-specific gene expression in embryonic cells. It will reveal how such mechanisms are involved in imprinting maintenance during early embryogenesis and will elucidate possible causes leading to imprinting developmental disorders.

Can the embryonic linker histone H1Foo modulate somatic cell reprogramming? (#30)

Mariana Priotto de Macedo[1], Werner Giehl Glanzner [1], Karina Gutierrez[1], Luke Currin[1], Hernan Baldassarre[1], Serge McGraw[2], Vilceu Bordignon[1]

[1]Department of Animal Science, McGill University, QC, Canada., [2]Department of Obstetrics and Gynecology, Université de Montréal, QC, Canada.

The linker H1 protein family is the most diverse class of histones, and it regulates nuclear functions such as gene transcription through the modulation of the chromatin structure. H1 sits on top of the nucleosome, protecting and keeping it in place, while binding to the linker DNA. There are eleven variants of linker histones, which have different roles in chromatin organization and function. While the somatic variants have a repressive role on gene expression, the oocyte-specific variant, H1foo, is associated with cell totipotency. Induction of H1foo expression in embryonic stem cells maintained pluripotent genes expression and prevented cell differentiation. Although H1Foo seems to be completely reprogrammed in embryos produced by somatic cell nuclear transfer (SCNT), its involvement in cell reprogramming and embryo development remains unknown. This study aims to investigate if H1Foo expression in somatic cells alters nuclear function and improves reprogramming in SCNT embryos. Porcine fibroblasts were electroporated with 40ng/µl of synthetic bovine H1foo mRNA (sbH1Foo) or red fluorescent protein mRNA (RFP). Strong fluorescent signal for sbH1Foo was detected at 12h, 24h and 48h following electroporation in 83,9%, 88% and 89% of cells, respectively. Gene expression profiles of sbH1Foo and RFP transfected cells were compared by RNA-seq at 48h after treatment. A total of 447 differentially expressed genes were detected (272 upregulated and 175 downregulated) in the sbH1Foo group. Preliminary results revealed similar development of SCNT embryos to the blastocyst stage and total cell number in embryos derived from donor cells transfected with sbH1Foo 48h prior to nuclear transfer compared to SCNT embryos from control cells. These findings suggest that sbH1Foo expression induces functional changes in somatic cell nuclei, which are not associated with early development of SCNT embryos. However, further investigation should reveal the impact on nuclear reprogramming and embryo viability.

Variations in mtDNA copy number in oocytes cultured in different in vitro maturation conditions (#58)

Camila Bruna de Lima[1], **Marc-André Sirard**[1]

[1]Université Laval, Département des sciences animales

Mitochondrial DNA (mtDNA) plays an important role in regulating mitochondrial homeostasis, transcription and overall cell metabolism. Patterns of mtDNA vary among cell with different types and functions. In reproductive sciences, reports correlated low mtDNA copy number with poor oocyte quality and the occurrence of metabolic diseases in the offspring. It is also believed that mtDNA copy number changes according to the environment where oocytes are growing. In this context, here we investigated if the addition of different antioxidant molecules during in vitro maturation (IVM) could impact the number of mtDNA copies in bovine oocytes. For that, bovine ovaries were obtained from abattoir and oocytes of 3-5mm follicles were collected (GV; n=30). Next, IVM was performed in Control medium (n= 30) or in the presence of 2mM l-carnitine (Carn; n=30), 0.7uM melatonin (Mel; n=30) or a combination of both (C+M; n=30). Pools of 10 oocytes of each group were submitted to Proteinase K digestion 50mg/mL at 50°C for 20 min, followed by DNA extraction with QuickDNA Microprep Kit (Zymo Research) in 3 replicates. For Droplet Digital PCR, each 20 µL reaction contained 1× EvaGreen Supermix (Bio-Rad), 100nM primers for mitochondrial CYTB gene and DNA diluted to the equivalent of 1 oocyte. Samples were partitioned using a QX100 droplet generator (Bio-Rad) and emulsions were transferred to a 96-well plate. After ddPCR, fluorescence was measured using a QX100 droplet reader (Bio-Rad). The threshold for positive droplets was determined manually and the final output was in mtDNA copies/oocyte. Results were compared using ANOVA+Tukey's (=5%). The number of mtDNA copies significantly increased from GV-stage oocytes to mature Controls (GV=53,386 ± 5,519 vs. Control=71,844 ± 530). But when antioxidants were added to IVM culture media separately, this effect was not observed (Carn= 52,233 ± 4050 and Mel= 35,377 ± 6342). Surprisingly, the combination of both molecules, almost doubled the number of mtDNA copies (C+M= 82600 ± 3444). In conclusion, the environment surrounding the oocyte indeed impacts the number of mtDNA copies, but more tests are undergoing to understand how these variations will impact mitochondrial activity, and consequently oocytes' physiology and their potential to become viable embryos.

Studying the dynamic of DNA methylation in rat male germ cells during gametogenesis by Methyl-Seq capture (#60)

Rhizlane El Omri-Charai[1], Isabelle Gilbert[2], Mariana Gabriela Ghinet[3], Rebecka Desmarais[3], Julien Prunier[2], Claude Robert[2], Guylain Boissonneault[3], Geraldine Delbes[1]

[1]INRS- Armand Frappier, [2]Department of Animal Sciences, Université Laval, [3]Department of Biochemistry and Functional Genomics, Université de Sherbrooke

In mammals, DNA methylation (5mC) reprogramming in male germ cells (GC) during gametogenesis can be under the influence of environmental toxicants, leading to abnormal germline programming and transgenerational epigenetic inheritance. However, we lack the comprehensive understanding of 5mC reprogramming during gametogenesis in rats, the preferred animal model in toxicology. We hypothesize that the establishment of 5mC is dynamic during perinatal life and spermiogenesis and aim to provide the first developmental 5mC map of the rat male germline.GC were purified by FACS, using transgenic rats expressing GFP exclusively in GC, combined with DNA staining to gate for ploidy and nuclear compaction. We obtained 9 populations of GC (Purity≥ 80% (n=4/stage)): 1- proliferative gonocytes from gestational day 16 (GD16); 2: gonocytes entering quiescent phase at GD18; 3: quiescent GD20 gonocytes; 4: postnatal day 5 spermatogonia (PND5); 5: spermatids (stages 1-8); 6: spermatids 9-12; 7: spermatids 13-14; 8: spermatids 15-17; 9: cauda epididymal sperm. 5mC was assessed using the Agilent rat MethylSeq kit. Data were analyzed using Bismark Bisulfite Read Mapper and methylation-extractor.Global methylation levels show that 5mC is minimal at GD18 and is gradually restored with 5mC patterns being almost similar between PND5 spermatogonia and spermatozoa. Using the SMART2 tool to identify stage specific differentially methylated regions (DMR), we analysed 103,720 regions (mean size of 472bp) and identified 68,407 DMRs. These include 11,105 DMRs at GD16, 54,265 common at GD16/20 and 2,224 at PND5, most being hypomethylated compared to later stages. Only 53 DMR were identified during spermiogenesis suggesting only little change in 5mC pattern during post-meiotic differentiation. We have established the first map of the rat male germline methylome at different stages of development, which will ultimately help identify epigenetic signatures of exposure to chemicals, and test their specific sensitivity at critical stages of maturation.

Prediction of bull fertility based on sperm methylome (#68)

Valentin Costes[1,2,3], Aurélie Chaulot-Talmon[1], Eli Sellem[1,2], Jean-Philippe Perrier[1,4], Anne Aubert[1], Luc Jouneau[1], Charline Pontlevoy[1], Chris Hozé[2,3], Sébastien Fritz[2,3], Mekki Boussaha[3], Chrystelle Le Danvic[2], Marie-Pierre Sanchez[3], Didier Boichard[3], Laurent Schibler[2], Hélène Jammes[1], Florence Jaffrézic[3], Hélène Kiefer[1]

[1]Université Paris-Saclay, UVSQ, INRAE, BREED, Jouy-en-Josas, France, [2]R&D Department, ALLICE, 149 rue de Bercy, 75012, Paris, France, [3]Université Paris-Saclay, AgroParisTech, INRAE, GABI, 78350, Jouy-en-Josas, France, [4]Université Grenoble Alpes, Institute for Advanced Biosciences INSERM U1209, CNRS UMR5309, Grenoble, F38000, France

In humans and model species, alterations of sperm DNA methylation have been reported in cases of spermatogenesis defects, male infertility and poor developmental outcomes. This study was therefore designed to assess the potential of sperm DNA methylation in refining bull fertility predictions. A unique collection of 100 sperm samples was constituted by pooling 2-5 ejaculates per bull, for 100 Montbéliarde bulls of consistent ages, assessed as fertile or subfertile based on non-return rates after 56 days post insemination. DNA methylation profiles of these sperm samples were obtained by Reduced Representation Bisulfite Sequencing. Using methylKit, fertility-related differentially methylated cytosines (DMCs) were identified in 188 genes. Interestingly, 73 genes targeted by DMCs are involved in sperm differentiation and function, fertility and embryo development; out of which 5 were further analyzed by pyrosequencing. In order to evaluate the predictive ability of the fertility-related DMCs, the sperm samples were split into a training (n=66) and a testing (n=34) set. Using a Random Forest approach, a predictive model was built from the methylation values obtained on the training set at DMCs. The predictive accuracy of this model was 72% on the testing set, and 72% on individual ejaculates collected on an independent cohort of 20 bulls. This study, conducted on the largest set of bull sperm samples so far examined in epigenetic analyses, shows that the sperm methylome is a valuable source of fertility biomarkers. The next challenge is to combine these results with other data measured on the same sperm samples, in order to improve the quality of the model and better understand the interplay between DNA methylation and other molecular features in the regulation of fertility. Funding: Grant ANR-13-LAB3-0008-01 “SeQuaMol”, Apis-Gene and ANRT

Conférencière invitée - Invited Speaker

Cindy Tian

Dr. Xiuchun (Cindy) Tian is a professor of biotechnology and Interim Department Head of Animal Science at the University of Connecticut. She obtained her MS and PhD from Cornell University in reproductive endocrinology. As a recipient of the National Research Service Award from the NIH she did her post-docs in developmental genetics and molecular embryology. Dr. Tian's current research interests are 1) nuclear reprogramming by somatic cell nuclear transfer (cloning); 2) genetic engineering to improve production efficiency of domestic animals; 3) embryonic and induced pluripotent stem cells; 4) epigenetics of pre-implantation development. Dr. Tian has more than 110 primary publications in both premium journals such as Nature Genetics, Nature Biotechnology, PNAS, Stem Cells and specialty journals such as Biology of Reproduction and Cellular Reprogramming. She serves as a Section Editor for BMC Developmental Biology, associate editor for PLoS One, Scientific Reports, Journal of Reproduction and Development. She was on the Board of Govern for the International Embryo Technology Society from 2018-2021.

Cindy Tian will give a lecture on Wednesday, October 12th entitled: ***"The quest continues: A hidden genomic code for DNA methylation?".***

Conférencier invité - Invited Speaker

Vilceu Bordignon

Vilceu Bordignon, DVM, MSc, PhD, is Associate Professor of Animal Science and Director of the McGill Large Animal Research Unit. His main research interest is on reproductive and developmental biology. Current research in his lab is investigating epigenetic mechanisms regulating embryo development and cell reprogramming. His lab is also investigating how early stage embryos deal with stressful conditions by regulating coping mechanisms, and testing conditions to improve development of embryos derived by different in vitro technologies. His research program applies several cell and molecular technologies including in vitro embryo production, somatic cell nuclear transfer, interference RNA and genome-editing.

Vilceu Bordignon will give a lecture on Wednesday, October 12th entitled: ***"Chromatin role on embryo programming and reprogramming"*.**

Présentation orale étudiante - Student oral presentation Yahan Li

Investigation of bovine chromosome architecture and its involvement in large offspring syndrome (#21)

Yahan Li[1], Max Highsmith[2], Frimpong Boadu[2], Jianlin Cheng[2], Rocío Rivera[1]

[1]Division of Animal Sciences, University of Missouri, Columbia, [2]Department of Electrical Engineering and Computer Science, University of Missouri, Columbia

Large offspring syndrome (LOS) is a congenital overgrowth syndrome in cattle. In humans, this syndrome is known as Beckwith-Wiedemann syndrome. Our studies have identified global gene misregulation, aberrant DNA methylation, and global loss-of-imprinting in bovine day 105 LOS fetuses when compared with controls. However, aberrant DNA methylation in LOS could only explain ≤ 3% of altered expression of nearby (< 20kb) genes. DNA methylation can regulate gene expression in a long-range manner by altering chromosome architecture which defines the spatial organization of chromatin during interphase. We hypothesize that LOS-associated loss-of-methylation at the KvDMR1 and IGF2R imprinting control region (ICR) disrupts normal spatial chromosomal interactions regulated by the insulator protein CTCF. We employed circular chromosome conformation capture (4C) assays for KvDMR1 and IGF2R ICR, using cultured primary fibroblast cells established from skin of day 105 Bos taurus indicus X Bos taurus taurus F1 hybrid control and LOS fetuses. Additionally, we performed methylome analysis and CTCF chromatin immunoprecipitation (ChIP) in LOS and control cells. We identified normal allelic DNA methylation (i.e., methylated maternal allele and unmethylated paternal allele) in control cell samples and loss-of-methylation on the maternal allele in LOS cell samples. The bait region of each 4C assays contains in silico predicted binding sites for CTCF protein and a single-nucleotide polymorphism to differentiate the parental alleles in the sequencing reads. Preliminary 4C sequencing results for IGF2R ICR show differential chromatin interaction patterns between the parental alleles in controls, with the maternal allele having an ~130kb enrichment of contacts covering IGF2R and AIRN, while the enriched domain in the paternal allele is extended to ~220kb, covering at least four more protein coding genes. For LOS, this domain's loss-of-methylation results in the maternal allele behaving like the paternal allele in controls. Further, CTCF ChIP results show paternal allele enrichment at the predicted CTCF binding sites within IGF2R ICR in controls and reduced paternal/maternal allele ratio in LOS. For KvDMR1, initial observations did not identify allelic differences in control cells. Together, our results suggest that the allele-specific chromosome architecture of IGF2R imprinted domain is regulated by CTCF binding and affected by DNA methylation.

Conférencière invitée - Invited Speaker

Pascale Chavatte-Palmer

Pascale Chavatte-Palmer graduated as DVM in France in 1989 and specialized in animal reproduction in UK, USA and France, with a research focus in placental and perinatal development in horses. In 1999, she joined the Biology of Development and Reproduction (BDR) research unit at INRA in France and studied feto-placental and postnatal consequences of cloning and embryo technologies in cattle. In 2006, she started to develop biomedical and veterinary models for studying the developmental origins of health and disease (DOHaD), with an emphasis on placental function. Her group studied the programming effects of nutritional challenges, metabolic imbalance, embryo technologies and exposure to airborne and/or food pollutants, taking advantage of access to a large number of species, including horses, and developing multidisciplinary approaches. Since 2020, she is the director of the new Biology of Reproduction, Environment, Epigenetics and Development (BREED) research unit (succeeding BDR), that gathers expertise in animal and human reproduction and development. With this new responsibility, she decided to focus her own research mostly on the equine species.

Pascale Chavatte-Palmer will give a lecture on Wednesday, October 13th entitled: ***"Importance of DOHAD in the equine industry"***.

Conférencière invitée - Invited Speaker
Véronique Ouellet

Véronique Ouellet received her PhD in animal sciences from Laval University in Quebec City, Canada. During her graduate studies, she interned for Pr Wolfang Heuwieser and Dr Victor Cabrera at the Frei Universitat in Berlin and at the University of Wisconsin in Madison. After obtaining her PhD, she was hired as a postdoctoral fellow at the University of Florida in the lactation physiology laboratories of Dr Jimena Laporta and Dr Geoffrey Dahl. Her work at UF mostly focused on the maternal consequences of heat stress on the subsequent offspring. She recently joined the Department of Animal Sciences at Laval University as an Assistant Professor in dairy science. Her research program focuses on the effects of the environment on the performance of dairy cows. Through her research, her collaborations and involvement in in many transfers and extension activities, Véronique wishes to contribute to ensuring the sustainability and resilience of the Canadian dairy sector.

Véronique Ouellet will give a lecture on Wednesday, October 13th entitled: ***"Heat stress in dairy cows: why worry about future generations?".***

Présentation orale étudiante - Student oral presentation Maria Rabaglino

Stimulated muscle development in healthy in vitro produced calves determined through a multi-omic approach (#49)

Maria Rabaglino[1], Jan Bojsen-Møller Secher[2], Poul Hyttel[2], Haja Kadarmideen[1]

[1]Technical University of Denmark, [2]University of Copenhagen

In cattle, in vitro produced (IVP) embryos are being generated at a high rate, even surpassing during the last years the production of embryos in vivo, i.e., after ovarian stimulation and AI (MOET). Despite the advantages of IVP technology, one of the most common undesirable consequences is fetal overgrowth and delivery of unusually heavy calves. Investigations have been performed to determine the origin of this pathology. However, changes at the molecular levels of IVP animals born healthy and with average body weight are unknown. The objective of this study was to determine transcriptomic and epigenomic alterations in the muscle of IVP calves compared to MOET calves at three months of age. IVP embryos were obtained after fertilization and culture of oocytes in serum-free media, which were generated through mild ovarian stimulation. MOET embryos were produced with a standard protocol. After delivery, birth weight and weight at sacrifice did not differ among IVP and MOET calves (n=4 per group). Samples from the semitendinosus muscle were collected at three months of age and subjected to RNA and gDNA extraction and sequencing through RNA-seq and WGBS. The resulting transcriptomic and epigenomic data were aligned with the bovine genome through STAR and Bismark software, respectively. Processed data were analyzed with the R environment. Differentially expressed genes (DEG, FDR<0.05) were determined through two approaches: (1) by negative binomial regression, applied only to the transcriptomic data (DESeq2 package) and (2) by holistically combining both omics data through a Non-Parametric Combination (NPC) methodology (STATegRa package), which takes into account correlations between both datasets. Enriched ontological terms associated with the DEG were identified with the clusterProfiler package. The 827 DEG with higher expression in the muscle of the IVP calves were strongly associated with the tricarboxylic acid (TCA) cycle / aerobic respiration (FDR=1.9x10-11), vasculature development (FDR=6.9x10-10), and striated muscle tissue development (FDR=2.3x10-06). Accordingly, the 315 DEG selected after 1000 permutations, considering both omics datasets as a whole, also enriched the TCA cycle (FDR=0.04). In conclusion, these results suggest that muscle development is more stimulated in IVP calves than MOET calves at three months of age and comparable body weights.

DADE Session d'affiches en ligne II – Online poster session II
13 octobre – October 13th
10h30 – 12h00

Developmental outcomes and therapeutic benefits of ultraviolet radiation during pregnancy (#4). *Peter Anto Johnson, MSc Student, University of Alberta (Page 24)*

Parental high carbohydrate/low protein diets in rainbow trout modulate DNA-methylation landscape in their progeny (#29). *Thérèse Callet, PhD, INRAE – AgroParis Techl (Page 25)*

Metabolic stressful environment drives epigenetic modifications in oviduct epithelial cells in an estrous cycle-dependent way (#59). *Patricia Fontes, Postdoctoral Fellow, Federal University of ABC (Page 26)*

Functional mutations in DNA Methyltransferase 3A (DNMT3A) leads to altered gene regulation in induced-pluripotent stem and neural progenitor cells (#63). *Karine Doiron, Postdoctoral Fellow, Université de Montréal (Page 27)*

Effect of inflammation on epigenetic markers and their persistence throughout successive lactations (#67). Elitsa Ivanova, PhD Student, INRAE – Université Paris-Saclay (Page 28)

Developmental outcomes and therapeutic benefits of ultraviolet radiation during pregnancy (#4)

Peter Anto Johnson[1], **John Christy Johnson**[1]

[1]University of Alberta

Objectives/Hypothesis: Sunlight exposure during pregnancy may be implicated in the physiological fetal development. Although several studies suggest the involvement of ultraviolet radiation-mediated vitamin D synthesis, current understandings of sunlight exposure effects during pregnancy remain incomplete. We aimed to (i) summarize the existing body of research on the influence of sunlight exposure on birth and long-term health outcomes and (ii) determine its implications for therapeutics and public health policy. Methods: We conducted a scoping review following PRISMA-ScR guidelines followed by a qualitative narrative synthesis. Databases including PubMed/MEDLINE, EMBASE and Google Scholar were screened, and no time, setting, or language restrictions were imposed on the search strategy. Primary research articles such as case studies, systematic reviews and meta-analyses, were included. Experimental and animal studies were excluded. Results: A total of 14 studies were included after screening and exclusion. Of the studies identified on birth outcomes, the majority (5/8) demonstrated an association between sunlight exposure and reduced adverse birth outcomes (e.g., low birth weight, preterm births, small for gestational age, etc.), 2/8 studies showed no association, and 1/8 suggested a negative association between sunlight exposure and reduction of these adverse birth outcomes. Of the studies examining long-term health outcomes, sunlight exposure during pregnancy was shown to promote skeletal growth and development (2/6) and reduce the incidence of multiple sclerosis (2/6), asthma (2/6) and pneumonia (1/6). However, several of these studies used different methodologies and populations making it difficult to compare and integrate findings. Based on these results, we examined: the importance of exposure at different stages of pregnancy, proposed mechanisms by which sunlight exposure could lead to optimal outcomes, epidemiological differences influencing the findings, and necessary practical considerations prior to the implementation of public health policy recommendations.Conclusion/Significance: While these associations are promising and suggest sunlight exposure intervention may have therapeutic benefits during stages of pregnancy, randomized controlled trials are warranted to support these recommendations.

Parental high carbohydrate/low protein diets in rainbow trout modulate DNA-methylation landscape in their progeny (#29)

Thérèse Callet[1], Cécile Heraud[1], Jingwei Liu[1], Hongyan Li[1, 2, 3], Vincent Véron[1], Stéphane Panserat[1], Lucie Marandel[1]

[1]INRAE, Université de Pau et des Pays de l'Adour, E2S UPPA, NUMEA, Saint-Pée-sur-Nivelle, [2]State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy , [3]University of Chinese Academy of Sciences, Beijing, 100049, China

For aquaculture sustainability, broodstock diet for carnivorous species will rapidly evolve toward a decrease of the protein-to-carbohydrate ratio, with the substitution of the protein-rich fishmeal by plant-derived carbohydrates (Callet et al., 2020). Numerous studies have reported that parental high carbohydrate (HC) - low protein (LP) nutrition could affect their progeny epigenetic landscape and subsequently their metabolism in various species (Gao et al., 2020). In carnivorous fish species, even though the effects of parental nutrition have already been demonstrated (Hou et al., 2020), the consequences of parental HC-LP have never been explored until now. To investigate this question, two-year old male and female trout were fed either a control diet (0% carbohydrate and 63.89% protein) or a diet containing a high proportion of carbohydrates (35% carbohydrate and 42,96% protein) for an entire reproductive cycle for females (10 months) and 5 months for males. Crossed-fertilizations were carried out in order to obtain four groups of fish. Samplings and DNA extractions were performed at different life stages in order to quantify the proportions of the four methyl-cytosine (5-mC) derivatives (5-hmC, 5-fC, 5-caC and C), by HPLC-UV analysis. Before the first feeding, the maternal HC-LP nutrition induced at the same time a decrease of 5-mC proportion and an increase of 5-caC proportion in their progeny (whole fry). These effects were enhanced by the paternal nutrition (Callet et al., 2021). The underlying mechanisms probably include the modulation of de novo methylation, as transcriptomic analyses revealed that dnmt3bbb relative expression was significantly reduced by the parental nutrition. Such outcome is maintained over time and regardless of the diet given, as the maternal HC-LP nutrition also triggered a decrease of both 5-mC and 5-hmC proportions in the liver, after a 9-month feeding trial.Interestingly, two previous studies have also reported a global DNA-hypomethylation in juvenile trout which were directly fed with a high carbohydrate diet (Marandel et al., 2016, Liu 2019). Overall, the data accumulation suggests that HC-LP nutrition, whether directly or indirectly via parental nutrition, highly modulates the DNA-methylation landscape in trout.

Metabolic stressful environment drives epigenetic modifications in oviduct epithelial cells in an estrous cycle-dependent way (#59)

Patricia Fontes[1], Heloise Rocha[1], João Silva[1], Aldcejam Fonseca Jr.[1], Marcella Milazzotto[1]

[1]Federal University of ABC, Santo Andre, SP, Brazil

The oviduct is responsible for providing a suitable microenvironment from gametes' final maturation until initial embryo development. Well-orchestrated functional changes are observed in the oviduct cells during the estrous cycle, mainly controlled by steroid hormones. These changes, however, may also be influenced by environmental changes, including metabolic stressful stimuli. Metabolic adaptations are dynamic processes that can even guide molecular modifications, known as metaboloepigenetics. Therefore, our objective was to investigate the epigenetic response of bovine oviductal epithelial cells (BOEC) submitted to a glycemic stressful environment, as well as evaluate the influence of the estrous phases in the outcome of this adaptation. For this, BOEC were isolated from oviducts of eight cows collected from a slaughterhouse (n=4 animals from follicular and mid-luteal phases) and cultured separately in physiological or glycemic stressful environments (respectively, 2.74 and 27.4 mM of glucose), resulting in four groups: Fol-Phy, Fol-Stress, Lut-Phy, and Lut-Stress. On day five of the culture, monolayer attached cells were submitted to analysis of mitochondrial membrane potential (MMP) and epigenetics markers (5-methylcytosine - 5mC and acetylation of lysine 9 histone 3 - H3K9ac). Five randomly selected areas were imaged per well and analyzed by evaluating fluorescence intensity using ImageJ software. Under physiological conditions, the MMP tended to be higher ($P=0.06$) in Lut-Phy than Fol-Phy, while MMP decreased in Lut-Stress compared to Lut-Phy ($P<0.05$), no change was observed between Fol-Phy and Fol-Stress. Yet, the stressful metabolic environment was enough to interfere with the epigenetic regulation, however in opposite manners estrous cycle-dependent: 5mC and H3K9ac were higher in Lut-Stress than Lut-Phy ($P<0.05$), while they were observed in higher levels in Fol-Phy compared to Fol-Stress ($P<0.05$). To our knowledge, these results provide the first evidence of a relationship between epigenetic reprogramming and energy metabolism in oviductal cells. Moreover, it is evident the influence of the estrous phase in the outcome of the cellular adaptation. Likewise, levels of metabolites in culture media may be crucial for cellular function and differentiation. Finally, this culture system might be a potent model to study the effects of systemic health conditions on the reproductive environment. Supported by FAPESP (2019/25982-7, 2020/02500-4).

Functional mutations in DNA Methyltransferase 3A (DNMT3A) leads to altered gene regulation in induced-pluripotent stem and neural progenitor cells (#63)

Karine Doiron[1], **Alexandra Langford-Avelar**[1, 2], **Serge McGraw**[1, 3]

[1]Centre de Recherche du CHU Ste-Justine, Montréal, Québec, Canada, [2]Département de Biochimie et Médecine Moléculaire, Université de Montréal, Québec, Canada, [3]Département d'Obstétrique et Gynécologie, Université de Montréal, Québec, Canada

Activity from the DNMT3A enzyme is required during development for establishing DNA methylation, a stable epigenetic mark crucial for the regulation of gene expression. During brain development, DNMT3A is strongly expressed to drive and regulate proliferation and differentiation of neuronal populations. In mice, complete lack of DNMT3A enzyme is lethal, whereas Dnmt3a+/− animals are normal and fertile. In humans, DNMT3A-deficiency in embryonic stem cells leads to loss of DNA methylation in a lineage and tissue specific manner as cells differentiate. Rare heterozygous mutations in functional DNMT3A domains cause an overgrowth intellectual disability syndrome called Tatton-Brown-Rahman Syndrome (TBRS). Currently, we do not know how functional mutations in the human DNMT3A protein can impair neurodevelopment. We postulate that pathogenic heterozygous DNMT3A mutations will lead to DNA methylation defects that will alter gene expression profiles during neurogenesis. To investigate such events, we derived 2 different induced-pluripotent stem (iPS) cell lines, each carrying a single mutation in the functional methyltransferase domain of DNMT3A, and reprogramed these cells into neural progenitors. We generated RNA-seq profiles from iPS and neural progenitor cells and performed bioinformatics analyses. Our results show that alteration in DNMT3A function leads to abnormal expression in iPS cells (n=1333 genes) and neural progenitors (n=3936 genes) in comparison to controls. While the top enriched genes in iPS mutant cells are associated to development and neurodevelopment, the top enriched genes in mutant neural progenitors are associated to embryogenesis and cell fate decisions. These results show that heterozygous DNMT3A mutations in the methyltransferase domain lead to altered gene expression profiles in iPS cells that are exacerbated during differentiation into neural progenitors. Further analyses are needed to understand the mechanisms by which pathogenic DNMT3A alters gene regulation, and how this can affect lineage specification of neural progenitor cells.

Effect of inflammation on epigenetic markers and their persistence throughout successive lactations (#67)

Elitsa Ivanova[1], Cathy Hue-Beauvais[1], Aurélie Chaulot-Talmon[2, 3], Johan Castille[1], Anne Aubert-Frambourg[2, 3], Hélène Kiefer[2, 3], Pierre Germon[4], Hélène Jammes[2, 3], Fabienne Le Provost[1]

[1]GABI, INRAE, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France, [2]Université Paris-Saclay, UVSQ, INRAE, BREED, 78350, Jouy-en-Josas, France, [3]Ecole Nationale Vétérinaire d'Alfort, BREED, 94700, Maisons-Alfort, , [4]INRAE, Université de Tours, ISP, F-37380, Nouzilly, France

Current strategies in animal husbandry and their consequences on animal health and welfare, and on the environment are important societal issues, in terms of both ethics and sustainability. Ruminant longevity depends on phenotype expression related to production characteristics (such as milk yield and quality), as well as characteristics related to health (such as mammary inflammation). Phenotype expression variability depends on genetic factors, but also epigenetic factors that reflect environmental influences. The goal of this project is to study epigenetic marks involved in mammary inflammation and their potential persistence throughout successive lactations in the hopes of discovering biomarkers of early inflammation. A proof of concept strategy was chosen in which a mouse model of early mammary inflammation was developed and in which differences in DNA methylation relating to inflammation and lactation, as well as the potential persistence of DNA methylation throughout successive lactations, were studied. Mammary glands from 1st or 2nd lactation, with or without inflammation were sampled and used to analyze DNA methylation (RRBS) and gene expression (RT-qPCR Fluidigm). Local inflammation during a 1st or a 2nd lactation allowed the identification of a similar number of CpGs (~400) with a differential methylation of 25% or more (DMC). In 1st lactation, 70% of DMCs were hypermethylated in inflamed mammary tissue compared to 100% hypomethylation in 2nd lactation. Thirty differentially methylated regions (DMR), targeting 23 genes, were identified in 1st lactation compared to 13 DMRs and 6 genes in 2nd, with one common gene between the two. Meanwhile, 18 genes related to mammary gland development, epigenetic regulation, and the immune response were found to be differentially expressed in 1st lactation compared to 2 in 2nd, with no common genes. These results show that inducing local inflammation quickly leads to specific transcriptional and DNA methylation modifications. Interestingly, these modifications differ when inflammation occurs in 1st compared to 2nd lactation. Results were acquired in a mouse model with the objective of transferring this knowledge to farmed species. From this work, new leads will emanate which could lead to proposals for new strategies to be used in breeding.

Conférencière invitée - Invited Speaker

Stephany McKay

Stephany McKay was born and raised in Texas where she attended Texas A&M University and received her undergraduate degrees in Biochemistry and Genetics. After receiving her M.S. degree at Texas A&M, Stephanie ventured to the University of Alberta in Edmonton, Alberta, Canada where she received her Ph.D. in 2007. Currently, Stephanie is an Associate Professor of Genetics and Genomics in the Department of Animal and Veterinary Sciences at the University of Vermont. The McKay lab performs genetic, genomic and epigenomic work with cattle and our primary interest is identifying epigenetic mechanisms driving epigenetic variation of complex traits.

Stephany McKay will give a lecture on Wednesday, October 13th entitled: ***"Examining the extent of environmental contributions towards DNA methylation and phenotypic variation".***

Conférencière invitée - Invited Speaker

Eveline Ibeagha-Awemu

Dr. Eveline Ibeagha-Awemu is a senior Research Scientist with Agriculture and Agri-Food Canada. She has established national and international reputation for her research in animal genomics and epigenomics for dairy health management and production. Her research supports the animal science community to develop strategies that contribute to the production of safe and affordable food. **Dr. Ibeagha-Awemu's** research focuses on applying OMICs and emerging technologies to unravel the molecular mechanisms of lactation, detect genetic/epigenetic markers of production and health traits, uncover the contribution of epigenetic factors to phenotypic variation, unravel the molecular mechanisms underlying bovine diseases, and develop next generation biomarkers/therapeutics for bovine health management. She is among pioneer scientists to characterise regulatory elements in the bovine genome and to create awareness of the important contributions of epigenetic factors to phenotypic outcomes in livestock health and production traits.

Eveline Ibeagha-Awemu will give a lecture on Wednesday, October 13th entitled: "***Epigenetic modulators of bovine mammary gland health and production: Enhancers or abaters*?"**

Présentation orale étudiante - Student oral presentation Romina Via y Rada

Epigenetic programming of the rabbit preimplantation embryo in a diabetic context (#2)

Romina Via y Rada[1], Catherine Archilla[1], Anne Aubert-Frambourg[1], Luc Jouneau[1], Nathalie Daniel[1], Véronique Duranthon[1], Sophie Calderari[1]

[1]INRAE, BREED, France

Type 2 Diabetes, with an increasing prevalence worldwide, affects younger populations nowadays, including women of childbearing age. The development in utero under impaired maternal metabolic status, such as imbalanced glucose-insulin homeostasis is associated with the programming of the offspring to a higher risk of metabolic diseases in adulthood, as described by the Developmental Origins of Health and Disease (DOHaD). Epigenetic mechanisms constitute a candidate player behind the establishment of programming. A particularly sensitive stage of development corresponds to the preimplantation period in which thigh epigenetic regulation is crucial for the proper development of the fetus and the placenta, an organ prone to programming. In addition, glucose metabolism, essential for blastocyst formation, is at the core of the crosstalk between chromatin and metabolism by producing co-factors required for chromatin modifications. We hypothesize that exposure to high glucose and/or insulin during preimplantation development could deregulate epigenetic mechanisms and impact gene expression programs, contributing to programming. Hence, 1-cell rabbit embryos were developed in vitro with high glucose and/or high insulin until the blastocyst stage. To characterize the potential effects, the blastocyst's embryonic compartments: the inner cell mass (ICM), future individual, and the trophectoderm (TE), future placenta were separated by immunosurgery and we performed genome-wide transcriptome profiling by RNA-seq.ICM and TE transcriptomes showed overnutrition (e.g. cell proliferation, apoptosis, mTOR signaling) and metabolic stress signatures (e.g. cell proliferation, ROS production) when embryos were developed with high glucose or high glucose and high insulin respectively. Interestingly, high glucose TE showed to be impacted to a greater extend with overrepresented processes including chromatin remodeling (differential expression of e.g. BPTF, SMARCD3, KDM5A). Surprisingly, high glucose and/or high insulin ICM showed the ectopic expression of TE genes (e.g. PLET1, GATA3) suggesting a potential perturbation in cell lineage commitment. We show that preimplantation development under an in vitro pre-diabetic environment impacts energy metabolism, cell proliferation-apoptosis balance, and cell differentiation, especially in the TE, an extra-embryonic lineage essential for proper development and function of the placenta. A deeper understanding of how glucose and/or insulin influences the epigenetic regulation of gene expression in the ICM and TE is to be explored.

Présentation orale étudiante - Student oral presentation Rhizlane El Omri-Charai

Impact of a high-fat maternal diet during gestation on fetal germ cells DNA methylation (#46)

Rhizlane El Omri-Charai[1], Alexandra Langford[2], Arlette Rwigemera[1], Isabelle Gilbert[3], Lisa-Marie Legault[2], Claude Robert[3], Deborah Sloboda[4], Serge McGraw[2], Geraldine Delbes[1]

[1]INRS - Armand Frappier Santé Biotechnologie, [2]Department of Obstetrics and Gynecology, Faculty of Medicine, Université de Montréal, [3]Department of Animal Sciences, Université Laval, [4]Department of Biochemistry and Biomedical Sciences, McMaster University

In the mammalian germline, epigenetic reprogramming consists of an erasure of DNA methylation (5mC) in primordial germ cells (PGCs) followed by progressive de novo 5mC. This establishes the epigenome of mature gametes that will, in part, guide embryo development. Early life signals including diet, change the in utero environment and may interfere with 5mC erasure in PGCs; this could have consequences on the gametes and transgenerational inheritance. Maternal high fat diet (HFD) intake in pregnant rats, has been shown to produce offspring with impaired fertility. We hypothesise that maternal HFD will impair the 5mC in male and female PGCs of the developing offspring which could explain impaired fertility.Germ cell-specific GFP transgenic Sprague Dawley dams were fed control diet (18% kcal from fat) or HFD (45% kcal from fat), (n=5/group) from the day after mating (GD0). Fetal gonads were collected and pooled per sex at GD16, a time when DNA demethylation is expected to be complete and PGCs have not entered meiosis. GFP-positive PGCs were purified by FACS (purity≥ 89%) and 5mC was assessed using the Agilent rat MethylSeq kit. Data analysis and identification of differentially methylated regions (DMRs) with at least 10% methylation difference were done using Methyl-seq pipeline and Methyl-kit.HFD significantly increased maternal weight gain without effecting the number and weight of fetuses/litter, their viability, the sex ratio, or the histology of the gonads. GD16 control gonads had higher global 5mC level in male PGCs (12.63% ± 0.86) compared to females (5.8% ± 1.98). We identified DMRs after HFD, mostly hypomethylated, in both males and females, the majority being in intronic and intergenic regions. Interestingly, more DMRs were identified in males compared to females and only 21% of DMRs linked to coding genes are common between both sexes.Maternal HFD and obesity are associated with fetal adaptations that are linked to adult chronic disease conditions, including reproductive dysfunction. Our data established the impact of HFD in the rat germline during DNA demethylation. Identification of genomic elements in DMRs will inform our understanding of the impact of dietary exposure and predict long-term effects on the gamete epigenome.

Programme du 14e Symposium du Réseau Québécois en reproduction
Agenda of the 14th Symposium of the Réseau Québécois en reproduction

Jeudi 14 octobre – *Thursday October 14th*

8h45-9h00	**Connexion sur la plateforme Zoom** ***Connexion on Zoom platform***
9h00 – 9h15	**Mot de bienvenue** ***Welcome***
9h15 – 10h30	**Présentations - Session I / *Presentations – Session I*** 5 présentations d'étudiant(e)s / 5 *student présentations*
10h30 – 10h45	**Pause** ***Break***
10h45 – 11h45	**Séminaire en production animale / *Animal production seminar*** *** Johanne Cameron, Consultante indépendante spécialisée en production ovine « *Utilisation d'un programme lumineux intensif pour produire à l'année chez les ovins - Un succès québécois* »
11h45 – 13h00	**Dîner** ***Lunch***
13h00 – 14h00	**Présentations - Session II / *Presentations – Session II*** 4 présentations d'étudiant(e)s / 4 *student présentations*
14h00 – 15h30	**Pause-café - session d'affiches I** ***Coffee Break - Poster Session I***
15h30 – 16h30	**Conférencier invité / Invited speaker** *** Stéphane Fabre, INRAE *« Genetic regulation of ovulation rate and ovarian follicular development in sheep »*
16h30 – 16h45	**Remise du Prix MdC du RQR** ***RQR KT Award Presentation***

Vendredi 15 octobre – *Friday October 15th*

9h00 – 10h30	**Session d'affiches II** ***Poster Session II***
10h30 – 11h30	**Conférencière invitée / Invited speaker** *** Deborah Bourc'his, Institue Curie *« The dichotomy of DNA methylation is spermatogenesis: protect or program »*
11h30 – 12h15	**Atelier EDI / DEI workshop** *** Lisa Greenhill, Association of American Veterinary Medical Colleges *« Be More than a DEI Supporter, Be an Ally »*
12h15 – 13h30	**Dîner** ***Lunch***
13h30 – 15h00	**Présentations - Session III / *Presentations – Session III*** 6 présentations d'étudiant(e)s / 6 *student présentations*
15h00 – 15h15	**Pause** ***Break***
15h15 – 16h15	**Conférencière invitée / Invited speaker** *** Bin Gu, Michigan State University *«2-Cell Based Genome Editing: From Embryonic Development to Disease Modeling »*
16h15 – 16h30	**Remise des prix et mot de la fin** ***Awards presentation and closing***

Noter que le programme est à l'heure de l'Est (UTC-5)
Note that the meeting schedule is on Eastern time (UTC-5)

Présentations orales - Session I / Oral presentations – Session I
14 octobre - October 14th
9h15 - 10h30

Président / Chair : Guillaume St-Jean

I. **The role of intercellular bridges in preimplantation embryo development (#13)**
Filip Vasilev, Postdoctoral Fellow, Université de Montréal (Page 36)
9h15 – 9h30

II. **Cell size and polarization determine cytokinesis furrow ingression dynamics in mouse embryos (#14)**
Lia Paim, PhD Student, Université de Montréal (Page 37)
9h30 – 9h45

III. **Early preimplantation alcohol exposure induces sex-specific DNA methylation dysregulations in late gestation placentas (#28)**
Lisa-Marie Legault, PhD Student, Université de Montréal (Page 38)
9h45 – 10h00

IV. **Profiling the guinea pig preimplantation development by protein expression of lineage markers: looking for a model for human development (#51)**
Jesica Canizo, Postdoctoral Fellow, Université de Montréal (Page 39)
10h00 – 10h15

V. **Steroidogenic factor 1 regulates transcription of a novel inhibin B co-receptor in pituitary gonadotrope cells (#69)**
Yeu-Farn (Claire) Li, PhD Student, University of Missouri (Page 40)
10h15 – 10h30

The role of intercellular bridges in preimplantation embryo development (#13)

Filip Vasilev[1], **Gaudeline Rémillard-Labrosse**[1], **Greg FitzHarris**[1, 2]

[1]Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Montreal, Canada, [2]Department of Obstetrics and Gynecology, Université de Montréal, Montréal, Canada

Cytokinesis is the final step of cell division that causes the physical separation of the two daughter cells, a process that is generally assumed to occur relatively normally in the embryo. Here, using a photo-activatable GFP to track cell-cell connectivity, we show that dividing blastomeres in mouse embryo in fact undergo an incomplete cytokinesis, where the two sister cells remain physically connected through a narrow intercellular bridge throughout almost the entirety of the subsequent cell cycle. We show that the cytoplasmic bridge is rich with microtubules and associated proteins such as AuroraK-B, PRC1, and TEX14 - a protein known to lead to bridge formation in some cells. Maintenance of the bridge is AuroraK-B-dependent, as inhibition with Hesperadin prevents the transfer of fluorescent molecules from one sister cell to another. The cytoplasmic bridges are not a result of an abscission checkpoint, such as has been described in somatic cells, since chromosome bridges induced by the topoisomerase IIα inhibitor ICRF193 delays but does not prevent the eventual closure of the bridge, and bridge closure under these circumstances causes chromosome breakage. We hypothesized that cytoplasmic bridges might allow synchronization of the cell cycle in sister cells. Accordingly, analysis of 4C and 8C embryos showed that sister cells have similar interphase length and synchronous cell-cycle transitions, whereas cell cycles differ markedly between ‘cousin’ cells within the same embryo. However, premature bridge abscission in the 8C embryos with Hesperidin does not interfere with the interphase length in the sister cells, suggesting the cytoplasmic connectivity between sister cells is dispensable for the cell-cycle synchrony. Notably however, whilst premature bridge abscission in the 8C embryos does not interfere with the rate of blastocyst formation, it does significantly reduce the total cell number count, suggestive of an important role for cytoplasmic bridges in early development. Taken together, our data reveal a surprising feature of early mammalian development, wherein incomplete cytokinesis allows sister cells to share molecules, which is apparently beneficial for healthy early embryogenesis.

Cell size and polarisation determine cytokinesis furrow ingression dynamics in mouse embryos (#14)

Lia Paim[1], **Greg FitzHarris**[1, 2, 3]

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Cytokinesis is achieved by the constriction of an actomyosin ring that separates the daughter cells. Understanding cytokinesis regulation is key, as its failure leads to binucleation - a common feature of human embryos in IVF clinics that compromises embryo health. Cells halve in size with each division in embryos, and how the actomyosin ring adapts to changing cell size is unknown. We applied confocal imaging and micromanipulation to investigate the role of cell size and developmental changes in constriction speed (CS) in mouse embryos. CS was similar from the 1-cell through 8-cell stages (1C:9.73+/-0.69µm/min; 2C:9.40+/-0.66 µm/min; 4C:10.15+/-0.45µm/min; 8C:9.65+/-0.63µm/min) and significantly reduced at the 16- (6.29+/-0.33µm/min) and 32-cell stages (5.65+/-0.42µm/min). Artificially halving cell size significantly decreased CS only when cytoplasmic size was equivalent to that of a 16-cell blastomere (control: 11.22+/-0.55µm/min; reduced: 8.55+/-0.30µm/min). Interestingly, at the 16-cell stage, we observed a further decrease in CS of outer cells (5.39+/-0.15µm/min) as compared to inner cells (7.71+/-0.42µm/min). This reduction was not due to cell-cell adhesion, as outer cells from embryos devoid of adhesion still displayed reduced CS (outer:6.01+/-0.39µm/min; inner:7.42+/-0.27µm/min). Instead, we observed a furrow ingression bias, in which the basal side ingressed twice as quickly (17.37µm+/-2.30) as the apical side (8.71µm+/-0.57). Moreover, Anillin and p-Myosin were substantially underrepresented where the contractile ring overlapped with the apical domain (Anillin – apical:15.88+/-5.23 a.u.; basal:71.78+/-14.44 a.u.; p-Myosin – apical: 35.41+/-5.56 a.u.; basal:91.15+/-8.94 a.u.). Depletion of apical polarity reversed these phenotypes, with constriction speed of outer cells increasing (outer:7.04+/-0.20µm/min; inner:6.65+/-0.20µm/min), the basal-to-apical ingression bias being abolished (apical side:10.88µm+/-0.73; basal side:13.28µm+/-0.90), and cytokinesis components becoming evenly distributed around the contractile ring (Anillin - apical:47.04+/-12.79 a.u.; basal:52.76+/-12.44 a.u.; p-Myosin - apical:51.01+/-8.63 a.u.; basal:67.71+/-8.50 a.u.). Our results demonstrate that the emergence of apical polarity locally inhibits the recruitment of cytokinesis components and negatively regulates furrow ingression specifically on one side. We propose that the apical domain acts as an obstacle for cytokinesis progression potentially providing an explanation for the occurrence of binucleation in embryos in the clinics.

Early preimplantation alcohol exposure induces sex-specific DNA methylation dysregulations in late4 gestation placentas (#28)

Lisa-Marie Legault[1, 2], Mélanie Breton-Larrivée[1, 2], Alexandra Langford-Avelar[1, 2], Serge McGraw[1, 2, 3]

[1]Centre de Recherche du CHU Sainte-Justine, Montréal, Canada, [2]Département de Biochimie, Faculté de Médecine, Université de Montréal, Canada, [3]Département d'Obstétrique-Gynécologie, Faculté de Médecine, Université de Montréal, Canada

Prenatal alcohol exposure is known to alter the epigenetic profiles of cells during brain development and is part of the molecular basis underpinning Fetal Alcohol Spectrum Disorder (FASD) etiology. However, the impact of alcohol exposure on the future epigenetic (DNA methylation) profiles and function of the placenta remains mostly unknown, especially when this exposure occurs during the initial stages of embryo development (preimplantation period)Hypothesis : Our research hypothesis is that an alcohol exposure during preimplantation will initiate DNA methylation dysregulations during the embryonic epigenetic reprogramming wave and cause abnormal establishment of DNA methylation profile in the developing placenta.Objective : Our objectives are to identify if early embryonic ethanol-exposure leads to DNA methylation errors in the placenta at late-gestation and how these dysregulations can be related to abnormal brain development and associated DNA methylation patterns.Method : To model early embryonic alcohol exposure, we subjected pregnant mouse females to ethanol at 2.5 days (E2.5), corresponding to embryos at the 8-cell stage. We collected E18.5 embryos and dissected the placentas of ethanol-exposed and control (saline) embryos. We then established genome-wide quantitative DNA methylation profiles of placentas samples (controls; 3 males and 3 females, ethanol-exposed; 5 males and 5 females) by Methyl-seq and performed bioinformatics analyses. Results: Based on our previous works, we analyzed male and female samples independently, uncovering 991 differentially methylated regions (DMRs) in male placentas, 1309 DMRs in female placentas and 21 regions commonly affected in both sexes. In all our analyses, we observed a majority of DMRs with increased methylation in ethanol-exposed placenta. Interestingly, gene ontology analysis of genic DMRs in male placentas are related to synaptic transmission, neuron development and morphogenesis, whereas in female genic DMRs gene ontology reveals implication in inflammation, cell morphogenesis and cytoskeletal organization. Conclusion: Our results show that an early acute alcohol exposure generates long-lasting sex-specific DNA methylation perturbations in the developing placenta. Since there is no diagnostic test for FASD, specific DNA methylation dysregulation in the placenta could be used as a potential biomarker for prenatal alcohol exposure.

Profiling the guinea pig preimplantation development by protein expression of lineage markers: looking for a model for human development (#51)

Jesica Romina Canizo[1, 2], **Katherine Vandal**[1, 2], **Savana Biondic**[1, 2], **Sophie Petropoulos**[1, 2, 3, 4]

[1]Centre de Recherche du CHUM, [2]Department of Molecular Biology, Université de Montréal, [3]Department of Medicine, Université de Montréal, [4]Department of Clinical Science, Investigation and Technology, Karolinska Institutet

Background: In Canada, 1 in 6 couples experience infertility. Knowledge of the molecular and cellular processes that control human preimplantation development would help to understand and treat infertility. Mouse embryos have been used to model reproduction, but caution is warranted as several discrepancies with the human exist during preimplantation. As guinea pigs represent a superior animal model in terms of placentation and brain development compared to the mouse; we speculate that they may also represent a good model to study preimplantation development. Objective: To characterize the guinea pig preimplantation embryo and compare its development to the human. Methods: In vivo guinea pig embryos were flushed on embryonic days (E)3-E5.75. Immunofluorescence of Sox2 (epiblast (EPI) marker), Sox17 and Gata6 (primitive endoderm (PE) markers) and Cdx2 and Gata3 (trophectoderm (TE)) was performed to establish their dynamics of expression. In parallel, we are performing scRNA-seq of guinea pig embryos from E3-E5.75 (work in progress). Results: At compacted morula stage (late E4.25-E4.75) we observe Sox2, Gata6 and Gata3, with no detection of Sox17 and Cdx2. In early blastocysts (E5), expression of Sox17 and Cdx2 emerge and by mid-blastocysts (E5.25-E5.75), a high number of cells co-express Sox2/Sox17, with no cells co-expressing markers of all three lineages, Sox2/Sox17/Cdx2. At E5.5 expanded blastocysts, specification of all three lineages is achieved and no cell co-expression of Sox2/Sox17/Cdx2 is observed. Conclusion: Preimplantation guinea pig embryos appear to better recapitulate the human embryo compared to the mouse. Similarities include: 1) the preimplantation period consists of ~ 6 days, 2) compaction occurs after the 16 cells stage and 3) the blastocyst forms between E5 to E5.25. In contrast to the human, the TE-ICM segregation occurs first, followed by further specification into EPI/PE/TE. In-depth single-cell analysis of gene expression dynamics will be used in parallel to further validate our findings (in progress). Further, we plan to perform single-molecule FISH to compare the status of X-chromosome inactivation in the guinea pig to that in the human. Having a small animal model that more accurately capture human preimplantation development is of importance in the fields of Reproduction, Development, ART and Stem cell.

Steroidogenic factor 1 regulates transcription of a novel inhibin B co-receptor in pituitary gonadotrope cells (#69)

Yeu-Farn (Claire) Lin[1], Gauthier Schang[1], Hailey Schultz[2], Evan Buddle[1], Ulrich Boehm[3], Daniel Bernard[1]

[1]Department of Pharmacology and Therapeutics, McGill University, [2]Department of Anatomy and Cell Biology, McGill University, [3]Department of Experimental Pharmacology, Center for Molecular Signaling, Saarland University School of Medicine

Inhibin A and B are gonadal hormones that suppress synthesis of follicle-stimulating hormone (FSH) by pituitary gonadotrope cells. Both inhibins were previously thought to act through the same co-receptor, betaglycan, to mediate their actions. However, inhibin B action was maintained in gonadotrope-specific betaglycan knockout mice, suggesting that it might act via a distinct co-receptor. We recently discovered a novel inhibin B co-receptor, which we will refer to here as inhibin B binding protein (IBBP). The co-receptor is selectively and highly expressed in gonadotropes in both mice and humans. Here, we describe our initial characterization of mechanisms controlling Ibbp transcription. We mapped the murine Ibbp transcription start site by 5' RACE and identified two putative steroidogenic factor 1 (SF-1) cis-elements in the proximal promoter. We demonstrated SF-1 binding to both sites using DNA precipitation assays. We next ligated ~1 kb of the murine Ibbp flanking sequence upstream of luciferase in a reporter plasmid. In heterologous HEK293T cells, SF-1 over-expression was sufficient to stimulate promoter-reporter activity. Mutating the cis-elements alone or together blunted or blocked this effect. In homologous LβT2 gonadotrope-like cells, the promoter conferred increased reporter activity relative to the empty vector. This activity was blocked or attenuated by mutations in the SF-1 cis-elements or siRNA-mediated knockdown of endogenous SF-1 expression. Finaly, we generated gonadotrope-specific SF-1 (product of the Nr5a1 gene) knockout mice using the Cre-lox system. These mice exhibited profound hypogonadotropic hypogonadism, as reported in previous SF-1 knockout models. Remarkably, Ibbp mRNA expression was nearly absent in pituitaries of these mice. Collectively, the data show that SF-1 regulates Ibbp transcription via two cis-elements in the proximal promoter. These elements are conserved in the human IBBP promoter, suggesting that SF-1 may regulate gonadotrope-specific expression of the gene across species. Funding sources: supported by CIHR and the Solomon Studentship Award (Division of Endocrinology, McGill University Health Centre)

Conférencière invitée - Invited Speaker

Johanne Cameron

Johanne Cameron a grandi près de la ferme familiale de son grand-père Louis-Philippe McCarthy). Ce contact avec l'élevage l'a poussé vers l'agronomie où elle a terminé son Baccalauréat en 2001 (Université Laval). Suite à l'obtention de ce diplôme, elle a débuté une maîtrise sur l'utilisation de la photopériode avec le chercheur François Castonguay d'AAC. Depuis la réalisation de sa maîtrise en 2001, elle a travaillé sur de nombreux projets de recherche portant sur la photopériode, ce qui lui a permis de développer de nouvelles connaissances, de développer des outils pour les producteurs et surtout, de se spécialiser dans ce domaine. En juin 2003, elle entre dans les rangs du *Centre d'expertise en production ovine du Québec*, où elle occupe à temps plein le poste de Coordonnatrice de la vulgarisation, jusqu'à l'automne 2010. À l'automne 2010, elle quitte temporairement le CEPOQ pour mettre plus de temps au travail de la ferme. Toutefois, dès le printemps 2011, elle reprend un poste de chargée de projet au CEPOQ pour travailler sur différents projets de recherche et de développement pour la production ovine, à temps partiel. En juin 2018, Johanne a décidé de cesser de travailler dans la recherche pour concentrer son travail dans la ferme qui se développe constamment. Elle enseigne toujours la production ovine au Collège d'agriculture de St-Hyacinthe en hiver et agit à titre de conseillère auprès de certains producteurs ovins de sa région. Johanne est très impliquée dans l'industrie du mouton, dans l'agriculture et dans la communauté. Elle fait partie du conseil d'administration de l'Association canadienne des éleveurs de moutons depuis 2011 (SCEA) et elle est présidente de la Société des éleveurs de moutons de race pure du Québec depuis 2018 (SEMRPQ). Elle est également membre du Comité agricole pour la MRC de la Vallée du Richelieu, présidente du Concours des Jeunes agriculteurs d'élites du Canada, Section Québec et elle a siégée au conseil municipal de 2013 à 2017. En 2012, Johanne et Martin ont été co-récipiendaires du Concours des Jeunes agriculteurs d'Élite du Canada. C'était la première fois que des éleveurs de moutons remportaient ce concours au niveau national.

Johanne Cameron présentera un séminaire en production animale, le jeudi 14 octobre intitulé :«Utilisation d'un programme lumineux intensif pour produire à l'année chez les ovins - Un succès québécois»

Présentations orales - Session II / Oral presentations – Session II
14 octobre - October 14th
13h00 – 14h00

Présidente / Chair : Clémence Belleannée
Co-présidente / Co-Chair : Céline Augière et Laura Giradet

I. **The consequences of prolonged M-phases in mammalian oocytes and embryos (#15)**
Adélaïde Allais, PhD Student, Université de Montréal (Page 43)
13h00 – 13h15

II. **Involvement of Fragile-X-related proteins in the formation of the network of cumulus cell-oocyte transzonal projections (#22)**
Mathilde Marchais, PhD Student, Université Laval (Page 44)
13h15 – 13h30

III. **Whole genome DNA methylation biomarker screening for bull fertility (#25)**
Ying Zhang, PhD Student, Université Laval (Page 45)
13h30 – 13h45

IV. **Steroidogenic factor 1 (SF-1; Nr5a1) regulates primordial follicle assembly and activation (#65)**
Camilla Hughes, Postdoctoral Fellow, Université de Montréal (Page 46)
13h45 – 14h00

The consequences of prolonged M-phases in mammalian oocytes and embryos (#15)

Adélaïde Allais[1], Ido Feferkorn[2], Keren Rotshenker[2], Melissa Pansera[2], Gaudeline Rémillard-Labrosse[1], William Buckett[2], Greg FitzHarris[1, 3]

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Aneuploidy is frequent in human eggs and is known to reduce reproductive success. In somatic cells, extended mitosis and persistence of the mitotic spindle can cause premature separation of sister chromatids, a phenomenon known as 'cohesion fatigue', which could cause aneuploidy, and we reported at the last RQR that mouse embryos are also susceptible to cohesion fatigue. Here we set out to ask whether the Metaphase-II oocyte, where the spindle persists for several hours until fertilization, might be equally susceptible to CF. Human oocytes, plus young (2-3 months) and old (16 months) mice oocytes were collected before germinal vesicle breakdown and cultured until MII stage and fixed at different time prior first polar body extrusion. Immunofluorescence imaging was used in order to determine whether the meiotic structure is disturbed. Contrary to in preimplantation embryos, in oocytes the spindle length did not increase with time (in mouse 22.17µm after 4 hours vs. 20.73µm after 12 hours; in human 10.60µm after 7h and 10.10µm after 17h30). Imaging revealed an increase in the incidence of misaligned chromosome with aging in mouse oocyte (in young 0.2 misaligned chromosomes vs. in old 1.3 misaligned chromosomes). However, this increase in the frequency of misaligned chromosomes was not associated with the duration of MII (in young oocytes, 14% at 4 hours and 11% at 12 hours vs. in old oocyte, 25% at 4 hours and 23% at 12 hours) and was not associated with cohesion fatigue in mice or human oocytes (in old oocyte 15% of chromosome individualisation after 4 hours vs. 10% after 12 hours). These results suggest that despite spindle tension and the loss of cohesion associated with aging reported in previous studies, misaligned chromosomes are not individualized. We concluded that, contrary to preimplantation embryos, mammalian oocytes are not susceptible to CF. Supported by a CIHR Operating Grant and Fondation Jean-Louis Lévesque [to GF], doctoral fellowships from Université de Montréal, CRCHUM and CRRD [to AA]

Involvement of Fragile-X-related proteins in the formation of the network of cumulus cell-oocyte transzonal projections (#22)

Mathilde Marchais[1, 2, 3], **Isabelle Gilbert**[1, 2, 3], **Karen Nenonene**[1, 2, 3], **Alexandre Bastien**[4], **Robert Viger**[2, 3, 5], **Edouard Khandjian**[6], **Claude Robert**[1, 2, 3]

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Communication between the oocyte and somatic cells is essential for successful ovarian folliculogenesis. We previously have shown that messenger RNA expressed in the cumulus cells can transit across the transzonal projections (TZPs) to be transferred to the oocyte. We hypothesize that proteins from the Fragile X Related (FXRP) family are involved in establishing the TZP network and its associated mRNAs transport. The FXRP protein family is composed of FMRP, FXR1P and FXR2P. Whereas a reduced expression of FMRP is associated with a high prevalence of premature ovarian failure in women, FMRP null mice do not show a reproductive phenotype. Our preliminary data in mice shows a compensatory mechanism by FXR2P in absence of FMRP, with higher expression of FXR2P in the compartments and stages where FMRP would normally be abundant. This compensatory mechanism is also present in Fmr1-/-/Fxr2-/- females who exhibit shorter and more abundant transzonal projections than those from Fmr1+/+/Fxr2-/- and Fmr1-/-/Fxr2+/+, compared to wild-type counterparts. Although Fmr1+/-/Fxr2+/- and Fmr1+/-/Fxr2-/- mice display a reduced reproductive lifespan, their ovarian reserve at 8 weeks is not different from Fmr1+/+/Fxr2+/+ animals. Moreover, Fmr1-/-/Fxr2-/- males and females mice exhibit facial abnormalities and Fmr1-/-/Fxr2-/- and Fmr1-/-/Fxr2+/- show behavioral abnormalities. In males, allele inactivation of any of the genes leads to macroorchidism. The ovarian reserve and hormonal profiles from the different FXRP genotypes are currently being characterized. To date, our results strongly support the involvement of FXRP proteins in the formation of the TZP network which is essential to produce a good quality egg. Ultimately, the roles of FXRP proteins could highlight a new key physiological process for folliculogenesis and oogenesis. This project is supported by a grant from the Natural Sciences and Engineering Research Council of Canada to Claude Robert.

Whole genome DNA methylation biomarker screening for bull fertility (#25)

Ying Zhang[1], **Marc-André Sirard**[1]

[1]Université Laval, Département des sciences animales

Nowadays, many efforts are being made to improve dairy cattle fertility particularly on females. However, bull fertility had been ignored, new methods and tools should be developed for improving male fertility independently. This study adopted enzymatic methyl sequencing to identify candidate DNA methylation biomarkers in sperm correlated with bull fertility. We filtered and selected 12 bulls (High Bull Fertility = 6; Low Bull Fertility = 6) with extreme bull fertility phenotypes according to the Bull fertility index via 6000 Artificial Insemination data from a private database. Libraries were prepared with Enzymatic Methyl-Seq kit following manufacturer's instructions. After sequencing, reads were processed in a bioinformatics pipeline for base calling and methylation detection. A total of 450 DMCs (Differentially Methylated Cytosines) presented a methylation difference higher than 20% and a significant q-value ($q<0.01$) between groups. Meanwhile, a 500 bp sliding bin size and a 100 bp step had been used to search for DMRs (Differentially Methylated Regions). Based on the top 70 candidate bins, 16 DMRs were screened after comparing the high bull fertility and low bull fertility groups when using a cutoff of $q<0.01$; 5 of these DMRs contain significant DMCs. Furthermore, 22 DMGs (Differentially Methylated Genes) could be mapped into reported specific genes. Interestingly, some of those genes (LOC781841, SPATA1, and MGC134232) have been correlated with spermatogenesis. In conclusion, this study was able to identify potential regions with differentially DNA methylation that could be used to complement the existing genetic methods and provide a better tool to predict bull fertility.

Steroidogenic factor 1 (SF-1; Nr5a1) regulates primordial follicle assembly and activation (#65)

Camilla Hughes[1], Olivia Smith[1], Mylène Brunelle[2], Nicolas Gévry[2], Bruce Murphy[1]

[1]Université de Montréal, [2]Université de Sherbrooke

The ovarian reserve of follicles is a key determinant of fertility. Depletion of the orphan nuclear receptor steroidogenic factor 1 (SF-1; Nr5a1) in pregranulosa and granulosa cells of the ovary results in adult mice that are infertile, whereas depletion later in the follicular trajectory results in normal fertility or subfertility, indicating that SF-1 is required early in follicular development. To investigate the role of SF-1 in the establishment and activation of the ovarian reserve, we generated SF-1 conditional knockout (cKO; Nr5a1(fl/fl) Amhr2-Cre) mice. Ovaries were collected on embryonic day (ED) 17.5 and postnatal days 1 (PND1) and 4 (PND4), the period of primordial follicle formation and activation. In wildtype mice, SF-1 was abundant during this period in ovarian somatic cells. SF-1 cKO ovaries had decreased volume compared to ovaries from control littermates (CON; $P<0.05$) on ED17.5, PND1, and PND4. Moreover, cKO ovaries failed grow between PND1 and PND4 as CON counterparts did ($P<0.05$), suggesting inhibition of follicular growth. On both ED17.5 and PND1, the number of oocytes remaining in cysts tended to be lower in cKO than CON ovaries ($P<0.08$). On PND1 and PND4, there were fewer primordial follicles in cKO relative to CON ovaries ($P<0.05$) and on PND4 primary follicle numbers were reduced ($P<0.05$). Number of primordial follicles per oocyte and number of primary follicles per primordial follicle were reduced in cKO ovaries ($P<0.05$), indicating inhibition of follicular assembly and activation. Transcription factors (TFs) that regulate follicle assembly, oocyte growth, or granulosa cell differentiation were downregulated in cKO ovaries, including Nobox ($P<0.05$) and Figla ($P=0.06$) on ED17.5 and Sohlh1 and Foxl2 on both PND1 and 4 ($P<0.05$). KIT-KITL signaling is a driver of both primordial follicle assembly and activation. The Kitl transcript was reduced in cKO ovaries on ED17.5, PND1, and PND4 ($P<0.05$). Both Kit and an upstream KITL regulator, Mtor, were reduced by cKO on PND4 ($P<0.05$). In summary, SF-1 is a novel regulator of primordial follicle assembly and activation, with effects elicited upstream of key TFs and the MTOR-KITL-KIT signaling system. Supported by a CIHR grant to BDM. CHKH is funded by a Lalor Foundation Fellowship.

Session d'affiches I / Poster session I
14 octobre - October 14th
14h00 – 15h30

Quercetin mitigates H2O2-induced oxidative stress in bovine parthenogenetic embryos: in vitro cleavage assessment and reactive oxygen species quantification (#1), *Ernesto Orozco-Lucerno, PhD, Universidad Autonoma de Ciudad Juarez (page 64).*

Frailty considerations in pediatric lung transplantation (#6), *John Johnson, MSc Student, University of Alberta (page 68).*

Fibronectin Type III domain containing 5 (FNDC5) expression in bovine ovary and in vitro effect on bovine granulosa cell proliferation and steroidogenesis (#7), *Mathilde Daudon, PhD student, Université de Montréa, (page 69)*

Investigating the temporal control of mitotic exit in mammalian embryos (#9), *Henry Brennan-Craddock, MSc student, Université de Montréal (page 71)*

Développement d'une nouvelle méthode de caryotypage chez le porc (#10), *William Poisson, MSc student, Université Laval (page 72)*

The placenta for early identification of high-risk infants born at 29-36 weeks gestation (#16), *Jonathan Charron, MSc student, Université de Montréal (page 75)*

Single-cell look-seq to identify error prone transcriptional profiles in oocytes (#18), *Karolina Kravarikova, MSc student, Université de Montréal (page 76)*

The Forensic Science behind the Caribou (Rangifer) SNP Chip Validation (#19), *Mallorie Trottier-Lavoie, MSc student, Université Laval (page 77).*

Structure and assembly dynamics of kinetochore in oocyte meiosis-I (#20), *Lin Yin, PhD student, Université de Montréal (page 78)*

SFRP4 inhibe l'action des gonadotrophines dans les cellules de granulosa via un mécanisme GSK3β/CTNNB1 dépendant (#35), *Michael Bérubé, PhD student, Université de Montréal (page 84).*

La voie de signalisation Slit/Robo est un antagoniste de la signalisation LH dans les cellules de la granulosa murines (#36), *Florine Grudet, PhD student, Université de Montréal (page 85).*

Effect of elevated NEFAs exposed during in vitro maturation on the cocultured porcine granulosa cells (#37), *Meihong Shi, PhD student, Université Laval (page 86).*

Cross-Species analysis of Wnt pathway involvement during preimplantation development and lineage specification in the human and the mouse (#38), Katherine Vandal, *PhD student, Université de Montréal (page 87).*

Implication of Fragile X-Related Proteins and neurotrophic factors in establishing transzonal projections (#42), *Mélodie Desnoyers, MSc student, Université Laval (page 91).*

Rôle du récepteur nucléaire LRH-1 dans le contrôle du métabolisme lipidique lors de la formation du corps jaune (#48), *Florence Gagnon, MSc student, Université de Sherbrook (page 94).*

Are Hippo pathway effectors potential key players of dairy cattle cystic ovarian disease pathogenesis? (#52), *Esdras Corrêa dos Santos, PhD student, Université de Montréal (page 96).*

Dynamique du remodelage de la chromatine et co-occurence de motifs dans les cellules de la granulosa murine suite au signal ovulatoire (#53), *Fanny Morin, MSc student, Université de Montréal (page 97).*

Ablation in vivo du motif de liaison GATA dans le promoteur des gènes Star et Cyp19a1 (#56), *Julia Picard, MSc student, Université Laval (page 98).*

In vitro embryo production in Common Marmoset (#64), *Karina Gutierrez, Postdoctoral fellow, McGill University (page 103).*

The Role of Janus Kinase 3 (JAK3) in Later Stages of Follicular Development (#66), *Amir Zareifard, MSc student, Université de Montréal (page 104).*

3-D mitochondrial network organisation in porcine cumulus cells (#70), *Amel Lounas, PhD student, Université Laval (page 105).*

Peroxiredoxin 6 peroxidase and Ca2+-independent phospholipase A2 activities are essential for male mouse fertility (#71), *Edrian Gabrielle Bumanlag, MSc student, McGill University (page 106).*

ZEB1 inhibits Lhb transcription by blocking the stimulatory actions of GnRH and EGR1 (#73), *Hailey Schultz, PhD student, McGill University (page 108).*

The role of microRNA in the regulation of gap junction intercellular communication proteins (#74), *Cameron Confuorti, MSc student, INRS (page 109).*

Impact of the inhibition of the transcription factor FOXO3a during Toxoplasma gondii infection (#75), *Andrés Felipe Díez Mejía, PhD student, INRS (page 110).*

ATF3 regulates FSH synthesis in vitro but not in vivo (#78), *Carlos Agustin Isidro Alonso, Postdoctoral fellow, McGill University (page 111).*

Conférencier invité - Invited Speaker

Stéphane Fabre

Dr. Stéphane Fabre received his doctorate in Molecular Biology from the Clermont-Ferrand University in 1995, and was a postdoctoral fellow in human retrovirology at the Ecole Normale Supérieure from 1996–1999 in Lyon. He obtained a researcher permanent position in 1999 at the French National Research Institute for Agriculture and Environment (INRAE) in Nouzilly, working in molecular reproductive physiology on the ovarian function. In 2011, he moved to INRAE in Toulouse to focus his research more on molecular genetics. Thanks to whole genome approaches (SNP chip genotyping, GWAS, high throughput DNA and RNA sequencing), he tries to evidence causal mutations and cellular mechanisms controlling fertility associated traits in small ruminants (ovarian follicle reserve, ovulation rate, litter size, embryonic lethality, sperm parameters). He is author or co-author of 74 peer-reviewed research articles, reviews and book chapters. He also serves as a fellow of the scientific council of the INRAE Animal Genetics division and supervises a small ruminant experimental farm.

On Thursday October 14th, Stéphane Fabre will give a talk entitled: ***"Genetic regulation of ovulation rate and ovarian follicular development in sheep"***

Session d'affiches II / Poster session II
15 octobre - October 15th
9h00 – 10h30

Steroidogenesis and androgen/estrogen signaling pathways in in vitro matured testicular tissues of prepubertal mice (#3), *Laura Moutard, PhD student, Université de Rouen Normandie (page 65).*

Developmental outcomes and therapeutic benefits of ultraviolet radiation during pregnancy (#4), *Peter Anto Johnson, MSc student, University of Alberta (page 66).*

Integrated view of the maternal and placental contribution to preterm birth syndrome (#5), *Camille Couture, PhD student, Université de Montréal (page 67).*

Improved Breakome Analysis in Sperm and Spermatids (#8), *Rebecka Desmarais, MSc strudent, Université de Sherbrooke (page 70).*

Elucidating the Mechanism and Genetic Impact of Post-Meiotic DNA Double-Strand Breaks of the Male Haploid Gamete (#11), *Tiphanie Cavé, PhD student. Université de Sherbrooke (page 73).*

Evolutionary Role of the Post-Meiotic DNA Double-Strand Breaks: Lessons from Fission Yeast Model (#12), *Loïs Mourrain, PhD student, Université de Sherbrooke (page 74).*

CAMKI coopère avec les facteurs de transcription COUP-TFII et GATA4 dans les cellules de Leydig (#23), *Kenley Joule Pierre, PhD student, Université Laval (page 79).*

miRNA regulation of lineage specification in the human preimplantation embryo (#24), *Savana Biodic, PhD student, Université de Montréal (page 80).*

Allele-specific methylation editing of imprinting genes (#27), *Gilberto Duran Bishop, MSc student, Université de Montréal (page 81).*

Epididymal Basal Cells Express the Adult Stem Cell Marker LGR5 (#31), *Laurie Pinel, PhD student, INRS (page 82).*

Adapted i-BLESS Method to Detect Double-Strand Break Hotspots in Human Sperm Cell Breakomes (#32), *Chloé Lacombe-Burgoyne, MSc student, Université de Sherbrooke (page 83).*

Implication of mutated DNMT3A in the methylation landscape and the pathogenesis of Tatton-Brown-Rahman Syndrome (#39), *Alexandra Langford-Avelar, PhD student, Université de Montréal (page 88).*

Abnormal placental DNA methylation and gene expression associated with assisted reproduction: early detection and effect of folic acid supplementation (#40), *Rita Gloria Ihirwe, MSc student, McGill University (page 89).*

Evidence of inter but not transgenerational effect of folate deficiency and supplementation (#41), *Edgar Martínez Duncker Rebolledo, MSc student, McGill University (page 90).*

Elucidating MEF2-interacting partners and targets in Leydig cells (#43), *Karine De Mattos, PhD student, Université Laval (page 92).*

Étude des vésicules extracellulaires ciliaires dans le contrôle de la maturation spermatique (#44), *Ludovic Vinay, MSc student, Université Laval (page 93).*

Characterization of Heritable Epigenetic Dysregulations in Promoter Regions Following a Temporary Lack of DNMT1 in Mouse Embryonic Stem Cells (#50), *Anthony Lemieux, MSc student, Université de Montréal (page 95).*

Variations in mtDNA copy number in oocytes cultured in different in vitro maturation conditions (#58), *Camila Bruna De Lima, Postdoctoral fellow, Université Laval (page 99).*

Studying the dynamic of DNA methylation in rat male germ cells during gametogenesis by Methyl-Seq capture (#60), *Rhizlane El omri-Charai, PhD student, INRS (page 100).*

Obesity in Pregnancy Leads to Preeclampsia-Like Placental Features and Inflammatory Biomarkers (#62), *Pascale Charette, PhD, University of Toronto (page 101).*

Functional mutations in DNA Methyltransferase 3A (DNMT3A) leads to altered gene regulation in induced-pluripotent stem and neural progenitor cells (#63), *Karine Doiron, Postdoctoral Fellow, Université de Montréal (page 102).*

Regulation of lysophosphatidic acid signaling in human spermatozoa (#72), *Haoyu Liao, MSc student, McGill University (page 107).*

Conférencière invitée - Invited Speaker

Déborah Bourc'his

Deborah Bourc'his obtained her PhD at Paris Diderot University in 2000, on the topic of DNA methylation, its implication in an immunodeficiency syndrome and profile changes resulting from somatic cloning. She then did a postdoc at Columbia University in New York, where she identified a DNA methylation co-factor, DNMT3L, absolutely essential for mammalian fertility, due to its involvement in the establishment genomic imprinting and repression of transposable elements during gametogenesis. She established her research team in 2009 at the Institut Curie in Paris. Her team studies the epigenetic determinants of mammalian reproduction, mainly using the marine model, and through approaches of genomic mappers, functional genetics and high throughput screens.

On Friday, October 15th, Deborah Bourc'his will give a talk entitled: ***"The dichotomy of DNA methylation is spermatogenesis: protect or program"***.

Conférencière invitée - Invited Speaker

Lisa Greenhill

Dr. Lisa Greenhill currently serves as the Senior Director for Institutional Research and Diversity at the Association of American Veterinary Medical Colleges (AAVMC). Dr. Greenhill directs the Association's internal study of academic veterinary medicine through collaborative research, analysis and publication efforts. She also manages the *DVM: DiVersity Matters* initiative, which promotes increased representation of underrepresented persons in academic veterinary medicine, inclusive academic environments and the inclusion of diversity related professional competencies in the DVM curriculum. Dr. Greenhill previously served nearly three years with the AAVMC, during which time she managed legislative and regulatory policy issues including agriculture production, biomedical research, professional education, food safety and environmental health. She holds a Masters in Public Administration with a concentration in public policy from George Mason University in Fairfax, VA and a Doctorate in Education from Benedictine University in Lisle, IL. She is mom to an amazing daughter and a terrier mix.

Lisa Greenhill will give a lecture on Friday, October 15th entitled : **"*Be More than a DEI Supporter, Be an Ally***".

Présentations orales - Session III / Oral presentations – Session III
15 octobre - October 15th
13h30 – 15h00

Président / Chair : Serge McGraw
Co-président / Co-chair : Karina Gutierrez

I. **Primary cilia-dependent response of epididymis principal cells to fluid shear stress (#17)**
Sepideh Fakhari, PhD Student, Université Laval (Page 57)
13h30 – 13h45

II. **LATS1/2 regulate YAP/TAZ activity to control proper development of the testis cords (#33)**
Nour Abour Nader, PhD Student, Université de Montréal (Page 58)
13h45 – 14h00

III. **La protéine ciliaire Arl13b joue un rôle essentiel dans la physiologie des vas efferents (#54)**
Céline Augière, Postdoctoral Fellow, Université Laval (Page 59)
14h00 – 14h15

IV. **P2Y14 receptor in the human epididymis: potential role in vasectomy-induced inflammation (#55)**
Larissa Belardin, Postdoctoral Fellow, Université Laval (Page 60)
14h15 – 14h30

V. **Basal Cells Primary Cilia Regulate Regeneration of the Epididymal Epithelium through the Hedgehog signaling pathway (#57)**
Laura Giradet, PhD Student, Université Laval (Page 61)
14h30 – 14h45

VI. **L'expression du gène PLAU induite par une augmentation d'AMPc intracellulaire dans les cellules de Leydig est réprimée par la kinase AMPK (#61)**
Zoheir Demmouche, PhD Student, Université Laval (Page 62)
14h45 – 15h00

Primary cilia-dependent response of epididymis principal cells to fluid shear stress (#17)

Sepideh Fakhari[1], Clémence Belleannée[1], Laura Girardet[1], Marie-Pier Scott-Boyer[1], Ezequiel Calvo[1]

[1]Université Laval

Context: Primary cilia (PC) are sensory organelles protruding from the cell surface and whose functional defects are associated with ciliopathic disorders and male infertility issues. PC regulates the expression of target genes involved in cell polarity, differentiation, and proliferation in response to the shear stress exerted by bodily fluids on epithelial and endothelial cells. While we detected PC at the surface of non-differentiated and epididymal principal cells of the epididymis at prepubertal stages, their role as shear stress mechanosensors remains unexplored. By combining in silico fluidic modeling with in vitro biofluidic strategies, we assessed the molecular response of ciliated epididymal principal cells to luminal shear stress. Methodology: Immortalized distal caput epididymis principal cells (DC2) were cultured on fluidic microplates and subjected to static condition (no flow) vs. fluid shear stress of 1 dyn/cm2. To assess PC contribution in that response, Ciliobrevin D Cytoplasmic Dynein Inhibitor was applied on DC2 cells. Analyzes performed for the study are RNA-Sequencing for gene expression profiling, quantitative real-time PCR, pathway analysis by combining algorithms of ShinyGO, DAVID, STRING, Metascape, and MouseMine, and in silico modeling on COMSOL Multiphysics software. Results: According to in silico modeling shear stress from the epididymis ranges from 1.5 to 11 dyn/cm2 depending on physiological viscosity and luminal diameter reported in the literature. Under shear stress of 1dyn/cm2, the expression of 63 genes was significantly increased in DC2 cells compared to static condition (Fold change >2, FDR< 0.05). Some of these genes have been found associated with shear stress response from other model systems, including Sox9, Fosb, Btg2, Fos, Edn1, and Myc. Pathway analysis showed MAPK, TNF, and TGF-beta signaling pathways were the most responsive to shear stress. Expression levels of shear-stress responsive genes Serpine1, Ccn1, and Ccn2, measured by qRT-PCR, significantly changes following impairment of primary ciliogenesis, pointing to the role of PC organelles as a fluid-mechanosensor in the epididymis. Acknowledging that Serpine1 is involved in the control of epididymal functions important to sperm maturation, our findings shed light on a possible mechanical control of epididymal functions induced by testicular-derived fluid and/or the first wave of spermatozoa at puberty time.

LATS1/2 regulate YAP/TAZ activity to control proper development of the testis cords (#33)

Nour Abou Nader[1], Bérengère Deffrennes[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 J2S 7C6

Sertoli cells occupy a unique place in testis development, playing major roles in the process of sex determination and the ensuing differentiation of the testis cell lineages. It is therefore important to have a better understanding of the signaling pathways involved in the regulation of these processes. Hippo pathway is a signaling pathway with well-established roles in cell proliferation and differentiation mechanisms, however its role in the developing Sertoli cells still needs to be elucidated. To study the involvement of the Hippo pathway in the development of the testes, we generated a mouse model (Lats1flox/flox;Lats2flox/flox;Amh-Cre) in which Lats1 and Lats2 were conditionally deleted in Sertoli cells during development. Characterization of this model demonstrates a decrease in adult testicular weight in the mutant animals. Histopathologic evaluation reveals that LATS1/2 are necessary for the maintenance of spermatogenesis, and that their loss leads to the progressive degeneration of the testis cords. To determine if the phenotype observed was associated with the activation of the downstream effectors of Hippo signaling, YAP and TAZ, we generated a quadruple knockout mouse model in which Lats1, Lats2, Yap and Taz were conditionally deleted in Sertoli cells. Preliminary characterization of the quadruple KO demonstrates a partial rescue of the phenotype confirming that Hippo signaling activation is required to maintain the function of the Sertoli cells. Furthermore, overexpression of TAZ seems to have a larger impact than the overexpression of YAP on the phenotype observed in testes of Lats1flox/flox;Lats2flox/flox;Amh-Cre animals. Further investigations are needed to identify the downstream targets of YAP and TAZ and the mechanisms of action of the Hippo signaling pathway in Sertoli cells.

La protéine ciliaire Arl13b joue un rôle essentiel dans la physiologie des vas efferents (#54)

Céline Augière[1], Gabriel Campolina[1], Vito Mennella[2], Charles Joly Beauparent[1], Arnaud Droit[1], Ezequiel Calvo[1], Rex Hess[3], Clémence Belleannée[1]

[1]Université Laval-Chu de Québec, Québec (QC), Canada, [2]University of Southampton, UK, [3]University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

Introduction. Les vas efferents (VE) sont de petits tubules reliant le testicule à l'épididyme qui concentrent les spermatozoïdes grâce à des mécanismes de réabsorption de fluide. Les cellules épithéliales de ces tubules possèdent des cils motiles et primaires, structures connues dans d'autres organes pour jouer un rôle de senseur de l'environnement grâce à la transduction de voies de signalisation. Toutefois le rôle de la voie de signalisation Hedgehog, pourtant uniquement médiée via les cils chez les vertébrés, n'a jamais été décrit dans la physiologie des VE. Méthodes. Afin d'étudier les fonctions sensorielles des cils dans la physiologie des VE et la fertilité masculine, nous avons développé un modèle murin Villincre; Arl13bflox altérant la transduction de la voie Hedgehog. Une combinaison de différentes approches d'imagerie par microscopie électronique, haute résolution et de vidéomicroscopie ainsi qu'une analyse RNA seq ont été utilisées pour caractériser le rôle des cils dans les VE. Résultats. La perte de la protéine ciliaire Arl13b résulte en un agrandissement du diamètre des tubules des VE, une diminution de l'expression de gènes impliqués dans la réabsorption hydrique ainsi qu'une augmentation significative de la prolifération cellulaire illustrant un rôle multifactoriel de Arl13b. Nous montrons également que la structure et la fonction des cils est affectée suggérant un défaut de transport protéique le long du cil. Malgré que les souris cKO présentent des dysfonctions au niveau des tubules des VE, les mâles n'ont pas de problème majeur de fertilité. Bien que des défauts similaires des VE soient toujours associés avec un phénotype d'infertilité, nos résultats de RNA seq suggèrent que l'activation du système immunitaire local associés avec une infiltration de macrophages dans la lumière des tubules des VE pourraient jouer un mécanisme compensatoire intrinsèque. Conclusion. Nos résultats se limitant à l'étude fonctionnelle des cils, nous souhaitons, par la suite, discriminer le rôle des cils motiles versus les cils primaires pour mieux comprendre l'implication de chaque type cellulaire dans la physiologie des VE grâce à l'étude d'une nouvelle souris modèle inactivant spécifiquement les cils primaires. Notre projet permet de mettre en lumière le rôle des cils dans la physiologie des VE.

P2Y14 receptor in the human epididymis: potential role in vasectomy-induced inflammation (#55)

Larissa Belardin[1, 2], Christine Légaré[1, 2], Robert Sullivan[1, 2], Clémence Belleannée[1, 2], Sylvie Breton[1, 2]

[1]Centre Hospitalier Universitaire de Québec-Research Centre, [2]Université Laval

Vasectomy, a long-term male contraception method, is widely used worldwide. This procedure induces the accumulation of sperm in the epididymis, which may cause inflammation (epididymitis). Inflammation is triggered by danger-associated molecular pattern (DAMP) molecules, including UDP-glucose, which are released following tissue stress or injury and activate pro-inflammatory receptors. Activation of the P2Y14 receptor by UDP-glucose induces the production of chemokines by epithelial cells followed by recruitment of inflammatory immune cells in the lung, uterus, and kidney. Inhibition of P2Y14 in these tissues improved outcomes in murine models of inflammation. However, little is known about the expression of P2Y14 in the epididymis and its potential participation in epididymitis. Here, we aimed: (i) to characterize the expression of P2Y14 in the human excurrent duct; and (ii) to examine the effect of vasectomy on P2Y14 expression and production of pro-inflammatory chemokines in the epididymis. The epididymis samples are from the biobank of tissues of Centre Hospitalier Universitaire (CHU) de Quebec – Université Laval, Canada. Using in situ hybridization, RT-PCR, qPCR, immunohistochemistry, and western blotting, we detected P2RY14 mRNA and P2Y14 protein in all segments of the epididymis (caput, corpus, and cauda) and vas deferens, in control men with no vasectomy. Increased levels of P2RY14 mRNA were observed in the corpus and cauda of vasectomized men. Increased CXCL10 mRNA was detected in the cauda, while decreased CCL2 mRNA was observed in the corpus of vasectomized men compared to controls. No effect of vasectomy was observed on IL-8 and IL-1b mRNA levels. The increased level of P2RY14 and CXCL10 mRNA, that we observed after vasectomy, indicates that the UDP-glucose\P2Y14 axis may confer a pro-inflammatory susceptibility to the epididymis. In this context, the decrease in CCL2 mRNA might protect the epididymis against harmful inflammation following vasectomy. We can conclude that vasectomy is associated with an inflammatory response involving the P2Y14 receptor itself and chemokines known to participate in the innate and adaptive immune response. Targeting P2Y14 may provide a new avenue for the treatment of inflammatory disorders of the epididymis.

Basal Cells Primary Cilia Regulate Regeneration of the Epididymal Epithelium through the Hedgehog signaling pathway (#57)

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

[1]Université Laval, [2]INRS - Armand Frappier

Introduction. The primary cilium (PC) is a signaling antennae essential for the development and homeostatic control of organs through the transduction of the Hedgehog pathway (Hh). Their dysfunction is correlated with several clinical consequences, including male infertility. While PCs are found on epididymal basal cells from adult tissues, their role in reproductive functions remains unknown. Results. We developed a conditionally deleted mouse model (cKO) for the ciliary GTPase Arl13b in basal cells. Following the validation of this model, we showed a decrease in the length of the PCs as well as an alteration of the Hh pathway by qPCR and quantitative microscopy in eight weeks old mice. Although cKO mice do not present fertility issue at this age, our results indicate a deregulation of epididymal homeostasis: the level of expression of several markers of basal cells (Krt5, Krt14, p63) decrease significantly, whereas those of principal cells (AQP9, CFTR) increase. Furthermore, the lumen area of the tubules and epithelial thickness fluctuated throughout the epididymis of cKO mice compared to controls without evidence of changes of proliferation (PCNA, Ki67) or apoptosis (Casp3) in the epithelium. To establish the capacity of regeneration in the epithelium, we performed efferent duct ligation (EDL), a technique known to induce a wave of apoptosis and proliferation within 48h in the proximal epididymis. We established that the initial segment and the caput of epididymis in cKO mice where less prone to regenerate after injury (increased apoptosis and decreased proliferation in cKO compared to control, n=3). Conclusions. Our results suggest that the invalidation of the ciliary Arl13b gene leads to deregulation of the Hh pathway accompanied by an alteration in the homeostatic maintenance of the tissue. The fact that the epididymis is less prone to regenerate after tissue damage suggests that the transduction of the Hh signaling through basal cell PC may control their stemness properties. Ultimately, complete invalidation of PC organelles will open new avenue regarding their contribution to male infertility issues.

L'expression du gène PLAU induite par une augmentation d'AMPc intracellulaire dans les cellules de Leydig est réprimée par la kinase AMPK (#61)

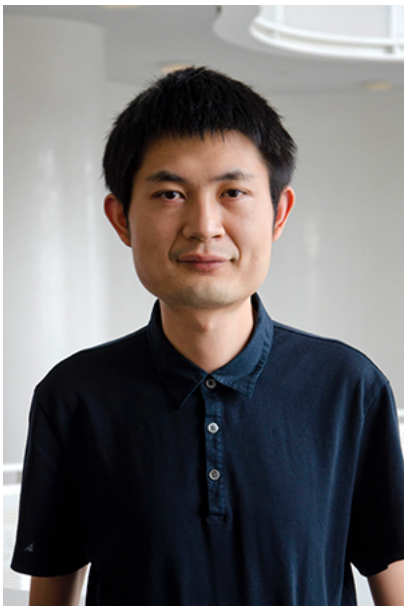
Zoheir B. Demmouche[1], **Houssein S. Abdou**[1], **Jacques J. Tremblay1**[, 2]

[1]Reproduction, Santé de la mère et de l'enfant, Centre de Recherche du Centre Hospitalier Universitaire de Québec-Université Laval, Québec, Canada., [2]Centre de recherche en Reproduction, développement et santé intergénérationnelle, Département d'obstétrique, gynécologie et reproduction, Faculté de médecine, Université Laval, Québec, Canada

Dans les cellules de Leydig, la LH induit une augmentation d'AMPc intracellulaire augmentant l'expression de gènes impliqués dans la stéroïdogenèse. La dégradation subséquente de cet AMPc en AMP conduit à l'activation de la kinase AMPK. Cette dernière exerce un rétrocontrôle négatif sur la stéroïdogenèse en phosphorylant des protéines présentement inconnues, réprimant ainsi l'expression de gènes de la stéroïdogenèse. Notre objectif est d'identifier de nouveaux gènes dont l'expression est modulée lorsque l'AMPK est activée. Pour y parvenir, une analyse plus poussée de données de microarray déjà disponibles au laboratoire a été réalisée comparant le transcriptome des cellules de Leydig MA-10 où la stéroïdogenèse est induite normalement ou en présence d'un activateur de l'AMPK. Cette analyse a permis de mettre en évidence un nouveau gène candidat, le gène PLAU, en plus de gènes précédemment caractérisés dont Star et Nur77 validant l'approche. PLAU code pour la sérine protéase uPA impliquée dans la synthèse du cholestérol par les macrophages. Dans certaines cellules, uPA est également impliqué dans l'activation de la tyrosine kinase EGFR, qui joue un rôle dans la stéroïdogenèse dans les cellules de Leydig. La variation dans l'expression de ce gène après activation de l'AMPK a été confirmée par qPCR. Un fragment du promoteur du gène PLAU a été isolé, ainsi que des délétants de ce promoteur afin de réaliser des transfections pour identifier les éléments de réponses ciblés par l'AMPK. Différents éléments de réponses riches en GC ont été mis en évidence. Des co-transfections avec différents facteurs de transcription sont en cours afin de déterminer s'ils sont impliqués dans l'expression du gène PLAU, et si l'activation d'AMPK diminue l'expression de ce gène via les facteurs de transcription mis en évidence. AMPK étant la première kinase identifiée réprimant la stéroïdogenèse, manipuler son activité et ses cibles ouvrira de nouvelles voies thérapeutiques pour les pathologies hormono-dépendantes. Subvention des IRSC (PJT-148738).

Conférencier invité - Invited Speaker

Bin Gu



Dr. Gu completed his Ph.D. degree in Cell Biology at Zhejiang University in China. He then completed his Postdoc training in Dr. Janet Rossant's Lab at SickKids Hospital in Toronto. There he developed a few genome editing technologies to generate mouse models with large insertion and chromosome structural defects. Bin joined Michigan State University as a assistant professor in 2020 and his lab use genome editing technology to develop challenging mouse models and study human diseases.

On Friday, October 15th, Bin Gu will give a talk entitled: "**2-Cell Based Genome Editing: From Embryonic Development to Disease Modeling**".

Quercetin mitigates H2O2-induced oxidative stress in bovine parthenogenetic embryos: in vitro cleavage assessment and reactive oxygen species quantification (#1)

Ernesto Orozco-Lucero[1], Melissa Molina-Garcia[1], Marbella Chavez-Solano[2], Jose M. Carrera-Chavez[1], Christian Y. Felix-Delgado[1], Armando Varela-Ramirez[3], Diana M. Beristain-Ruiz[1], Alejandro Martinez-Martinez[2], Andres Quezada-Casasola[1]

[1]Universidad Autonoma de Ciudad Juarez-Departamento de Ciencias Veterinarias, [2]Universidad Autonoma de Ciudad Juarez-Departamento de Ciencias Quimico Biologicas, [3]The University of Texas at El Paso-Department of Biological Sciences-The Border Biomedical Research Center

Antioxidants can diminish oxidative stress, improving embryonic development. Here, we evaluated the effects of an oxidative stress inducer, hydrogen peroxide (H2O2), and the antioxidant quercetin (QUE; plant-derived flavonoid) supplementation, by assessing in vitro cleavage development and reactive oxygen species (ROS) levels in bovine parthenotes. The parthenotes were either untreated, exposed to H2O2 (85 µM), QUE (2 µM), or double-exposed. Development was assessed at 96 hours post-parthenogenetic activation. The rates were estimated for: Cleavage, 2-cell (2C), 4-cell (4C), early 8-cell (e8C: 5-8 cells), late 8-cell (L8C: 10-16 cells), and 5-16 cell (5-16C; e8C+L8C) stages. All cleaved parthenotes were stained with 2',7'-dichlorofluorescin diacetate to quantify ROS. The possible association between development and ROS levels was evaluated by correlation analysis. The H2O2-exposed parthenotes showed a significantly lower e8C-developmental rate than untreated ($P<0.05$). Similarly, H2O2-exposed parthenotes revealed less L8C-developmental rate than QUE-treated ($P<0.05$). Also, in H2O2-treated parthenotes, the 5-16C-rate was less than untreated ($P=0.0557$) and QUE-treated ($P=0.0502$). Concerning ROS, the H2O2-treated parthenotes exhibited higher quantities than QUE-treated 2C-parthenotes ($P<0.05$). Furthermore, ROS levels were higher in H2O2-exposed than in untreated ($P<0.001$), QUE-treated, and double-exposed in e8C-parthenotes ($P<0.0001$). Moreover, The H2O2-exposed parthenotes exhibited higher ROS levels, as compared with untreated ($P<0.001$), QUE-treated, and double-exposed ($P<0.0001$) at the 5-16C stage. Finally, the trends in the correlation analysis suggested inverse association between development and ROS levels. In conclusion, parthenogenetic cleavage and ROS levels appear inversely correlated. Quercetin inhibits H2O2-inflicted oxidative stress by mitigating ROS levels and increases in vitro cleavage in bovine parthenotes of five to 16 cells.

Steroidogenesis and androgen/estrogen signaling pathways in in vitro matured testicular tissues of prepubertal mice (#3)

Laura Moutard[1], Justine Saulnier[1], Aurélie Rives-Feraille[1], Ludovic Dumont[1], Nathalie Rives[1], Christine Rondanino[1]

[1]EA 4308 Gametogenesis and Gamete Quality (GQG), Rouen Normandy University

Chemotherapy has a recognized toxicity on germline stem cells that could lead to infertility when administered before puberty. In order to preserve and restore the fertility of patients, prepubertal testicular biopsies need to be frozen/thawed and later matured to produce spermatozoa for assisted reproductive technology. In vitro maturation, developed in mice to obtain spermatozoa from fresh or thawed prepubertal testicular tissue, has a poor yield despite the supplementation of culture media with retinol. Since steroid hormones play an essential role in spermatogenesis and gamete quality, it appears necessary to ensure that their synthesis and mechanisms of action are not altered in in vitro cultured tissues. The aim of this project is therefore to study steroidogenesis and the androgen and estrogen signaling pathways during in vitro maturation of prepubertal mouse testicular tissues in order to increase the spermatogenic yield. The mRNA expression of the actors of steroidogenesis and androgen/estrogen signaling was measured by RT-qPCR during mouse postnatal development and in in vitro cultured testicular tissues. 3β-HSD and INSL3 immunostaining was performed to investigate the presence of Leydig cells and their maturity. The first results of this study show that the actors involved in the synthesis and mechanisms of action of steroid hormones, such as encoding the LH receptor, Cyp11a1, Cyp17a1, Hsd17b3, Hsd3b1 and Cyp19a1 encoding steroidogenic enzymes, Esr1, Esr2 and Gper1 encoding the estrogen receptor and androgen/estrogen target genes (Rhox5, Septin12, Faah), have a decreased mRNA expression in vitro. Addition of hCG, a homolog of LH, to the medium induces an increase in Lhcgr expression, enhances the expression of several steroidogenesis genes (Cyp11a1, Cyp17a1, Cyp19a1, Eppin). Leydig cells are functional in vitro since they respond to hCG stimulation. Furthermore, Leydig cells expressing 3β-HSD were detected after 30 days of organotypic culture but seem to be partially mature with a weak expression of INSL3, a biomarker of Leydig cell functionality. Quantification of the percentage of mature Leydig cells and of the protein levels of the actors of androgen/estrogen signaling pathways will have to be performed.

Developmental outcomes and therapeutic benefits of ultraviolet radiation during pregnancy (#4)

Peter Anto Johnson[1], **John Christy Johnson**[1]

[1]University of Alberta

Objectives/Hypothesis: Sunlight exposure during pregnancy may be implicated in the physiological fetal development. Although several studies suggest the involvement of ultraviolet radiation-mediated vitamin D synthesis, current understandings of sunlight exposure effects during pregnancy remain incomplete. We aimed to (i) summarize the existing body of research on the influence of sunlight exposure on birth and long-term health outcomes and (ii) determine its implications for therapeutics and public health policy. Methods: We conducted a scoping review following PRISMA-ScR guidelines followed by a qualitative narrative synthesis. Databases including PubMed/MEDLINE, EMBASE and Google Scholar were screened, and no time, setting, or language restrictions were imposed on the search strategy. Primary research articles such as case studies, systematic reviews and meta-analyses, were included. Experimental and animal studies were excluded. Results: A total of 14 studies were included after screening and exclusion. Of the studies identified on birth outcomes, the majority (5/8) demonstrated an association between sunlight exposure and reduced adverse birth outcomes (e.g., low birth weight, preterm births, small for gestational age, etc.), 2/8 studies showed no association, and 1/8 suggested a negative association between sunlight exposure and reduction of these adverse birth outcomes. Of the studies examining long-term health outcomes, sunlight exposure during pregnancy was shown to promote skeletal growth and development (2/6), and reduce the incidence of multiple sclerosis (2/6), asthma (2/6) and pneumonia (1/6). However, several of these studies used different methodologies and populations making it difficult to compare and integrate findings. Based on these results, we examined: the importance of exposure at different stages of pregnancy, proposed mechanisms by which sunlight exposure could lead to optimal outcomes, epidemiological differences influencing the findings, and necessary practical considerations prior to the implementation of public health policy recommendations. Conclusion/Significance: While these associations are promising and suggest sunlight exposure intervention may have therapeutic benefits during stages of pregnancy, randomized controlled trials are warranted to support these recommendations.

Integrated view of the maternal and placental contribution to preterm birth syndrome (#5)

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Pregnancy includes complex cascades of events which need to be properly orchestrated for optimal health of the baby. Unfortunately, complications are often encountered such as preterm birth (PTB). Affecting 8-10% of pregnancies PTB is the main cause of infant mortality. In surviving newborns, PTB has important negative health impacts, such as neurodevelopmental disorders and cardiovascular diseases. Inflammation, in both maternal circulation and at the maternal-fetal interface, is being studied as a potential therapeutic strategy for PTB. Inflammation is a key regulator of both physiological and premature induction of labor, however a strong heterogeneity related to PTB which has prevented advancements in identifying immune and inflammatory changes involved in PTB.In order to better understand the mechanisms underlying PTB, we aimed to acquire an integrated view of the PTB syndrome via studies of immune and inflammatory profiles in women with term and preterm deliveries. We investigated the inflammatory profiles of the placenta, fetal membranes and maternal circulation to understand their contribution to PTB. We have preliminary data, obtained through ELISA and flow activated cell sorting, which suggest that the placenta holds a dramatically different inflammatory profile when compared to the maternal circulation, including increased IL-6 expression. Furthermore, we notice clear changes associated to PTB but not to labor itself. It also appears that fetal membranes do not contribute to the inflammatory milieu found in PTB. In light of these new findings and to further address the gaps in our understanding of the differences in inflammatory profiles in uncomplicated pregnancies and PTB, we aim to link our current findings to whole genome sequencing of the placenta and to infants' development. Additional correlation to gestational age as well as immunohistochemistry analyses will further strengthen our integrated understanding of PTB and facilitate the identification of women who could benefit from targeted anti-inflammatory therapeutic interventions.

Frailty considerations in pediatric lung transplantation (#6)

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Introduction: There has been an explosion in 'frailty' data with various scores in the transplant world. Frailty after lung transplantation is associated with impaired health-related quality of life and mortality [1]. Although this is typically mentioned in the adult population, there has been extensive publication around frailty in children with liver disease [2]. This project explores how 'frailty' impacts post-transplant outcomes in the pediatric lung by examining lung function testing post transplant and rejection episodes. Methods: We are undertaking a retrospective study of patient charts and case records of pediatric patients selected for lung transplantation. Frailty scores were assessed using the 5 classic Fried Frailty Criteria – slowness, weakness, exhaustion, diminished physical activity, and shrinkage. Test scores were translated to age- and sex-dependent z scores. Once frailty is quantified, post transplant lung spirometry testing was used to assess whether there were any significant correlations. Anticipated results: Based on preliminary work [3], we anticipate a trend whereby increased scores for frailty are linked with poorer prognostic lung transplant outcomes including statistical reductions in forced expiratory volume (FEV1) and forced vital capacity (FVC) over time. We also predict higher incidence of rejection episodes for patients that have higher frailty scores. Significance: This project is the first of its kind to shed some light on frailty, a complex clinical syndrome associated with impaired suboptimal health outcomes, in the pediatric candidates considered for lung transplantation. Further research will be necessary to identify potential interventions and ways to combat risk factors for poor prognostic outcomes in this population. References:[1] Venado A, Kolaitis NA, Huang CY, et al. Frailty after lung transplantation is associated with impaired health-related quality of life and mortality. Thorax. 2020 Aug;75(8):669-678. doi: 10.1136/thoraxjnl-2019-213988. Epub 2020 May 6. PMID: 32376733; PMCID: PMC8023537. [2] Lurz E, Quammie C, Englesbe M, et al. Frailty in Children with Liver Disease: A Prospective Multicenter Study. J Pediatr. 2018 Mar;194:109-115.e4. doi: 10.1016/j.jpeds.2017.10.066. PMID: 29478492. [3] Tomic R, Perottino G.M., Collins M., et al. Frailty Measurements are Poor Predictor of Lung Transplantation Outcomes, J Heart and Lung Transplant. 2021 Apr;40(4):S361-362. doi: 10.1016/j.healun.2021.01.1018.

Fibronectin Type III domain containing 5 (FNDC5) expression in bovine ovary and in vitro effect on bovine granulosa cell proliferation and steroidogenesis (#7)

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In Canada and worldwide, the fertility of dairy cows has declined significantly since the 1950s. It is estimated that only 30% of cows in production have reproductive capacity to maintain herds. It is widely accepted that at the beginning of lactation the dairy cow is in negative energy balance (NEB) that decreases reproductive performance, and she therefore mobilizes adipose tissue for energy and milk production. Adipose tissue is a source of hormones (adipokines) and rapid adipose mobilization changes the circulating adipokine landscape. A recently characterized family of adipokine-myokines is the Fibronectin Type III domain containing (FNDC) proteins. The most studied is FNDC5 and its cleaved and secreted fragment called irisin. Two potential receptors for irisin, ITGAV and ITGB1 have been described in peripheral tissues. The effect of FNDC5 and irisin on metabolism during the lactation and on the functioning of the ovary in cows remains unknown. We hypothesized that FNDC5 and irisin are linked to NEB in dairy cows and could affect fertility by acting on ovarian steroidogenesis. Thus, here we investigated firstly the expression of FNDC5, ITGAV and ITGB1 in bovine follicles, secondly the effect of various adipokines (leptin, adiponectin, resistin, TNFα, visfatin and chemerin) on granulosa cell (GC) FNDC5 expression and finally the role of human recombinant irisin on GC proliferation and steroidogenesis in vitro. By RT-qPCR we showed that FNDC5 (p-value=0.0265), ITGAV (p-value=0.0002) and ITGB1 (p-value=0.0123) was more expressed in GC from small follicles than in GC from large follicles (p=0.0265) and that visfatin increases FNDC5 expression (p-value=0.0060). Addition of human recombinant irisin to cultured GC increased cell proliferation in a dose dependent manner at 1, 10 and 100 ng/ml (p<0,001) (10ng/mL is the concentration found in plasma). At this latter concentration, irisin decreased progesterone secretion at basal state and in response to FSH and/or IGF1 (p<0.0001). In contrast, irisin increased oestradiol secretion (p<0.0001). Taken together, these data show that FNDC5 is expressed in bovine ovary and modulates granulosa cell steroidogenesis.

Improved Breakome Analysis in Sperm and Spermatids (#8)

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De novo mutations (DNM) are associated with many neurological disorders in children and are transmitted to offspring four times more frequently by fathers than mothers. However, the origin of this bias is unclear. Recently, a transient surge of post-meiotic DNA double-strand breaks (DSB) associated with chromatin remodeling in male gametes has been shown. Considering the haploid nature of spermatids and the instability of DSB repair in this context, this could represent a major source of male-driven DNMs. Results from a preliminary mapping of DSB hotspots (breakome) indicated that, when considering genes only, hotspots occur more frequently in neurodevelopmental genes. We hypothesize that transient post-meiotic DSBs in spermatids are the main source of DNMs and any rise in DSBs would statistically favor the transmission of DNMs to offspring especially in neurodevelopmental genes. The objective of this study is therefore to validate the preliminary results and optimize the DSBs mapping method while extending the results to spermatozoa. The DSB mapping method consists in sorting four populations of spermatids by flow cytometry, nick and gap repair of extracted DNA, DSB capture, DNA libraries preparation, and whole-genome sequencing. Improvements to the method include the use of an additional dye (DAPI) for cell sorting, allowing a specific selection of haploid cells and preventing contamination. The method allows for specific DSB capture so that good quality libraries of break sites were obtained using a method more suited for our type of samples and sent for sequencing at Génome Québec. The libraries should generate robust sequencing data providing us with crucial information regarding the evolution of the hotspot distribution during spermiogenesis. Optimization of many steps of the protocol allowed increase of both yield and richness of libraries for hotspot sequencing. The DSB mapping will allow the validation of hotspots distribution in the genome of mice spermatids and whether DSB in sperm arise from persistence of the transient DSBs in spermatids. This method could be used in a clinical context to evaluate the instability at these loci and the risk of transmitting DNMs to offspring, especially for neurodevelopmental disorders. Funded by Canadian Institutes of Health Research (#PJT-159719).

Investigating the temporal control of mitotic exit in mammalian embryos (#9)

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During the first divisions of the mammalian embryo it is essential that segregation of the chromosomes in anaphase and division of the cytoplasm in cytokinesis occur in sequence to produce two diploid daughter cells. In somatic cells these events are coordinated through proteosome-mediated destruction of cyclin B1 and securin. Currently, It is not understood how the timing of anaphase and cytokinesis onset is related to cyclin B1 destruction in the embryo and if the temporal relationship between these events changes throughout early development. To investigate how furrow ingression and anaphase onset are coupled during destruction of cyclin B1 we use live cell confocal imaging, GFP tagged cyclin B1 and fluorescent histone marker H2B:RFP to observe cyclin destruction, anaphase and cytokinesis onset through each division of the preimplantation embryo. My data so far suggests that unlike somatic cells where the metaphase-anaphase transition occurs after the majority of cyclin B1 is destroyed, anaphase onset in the 2-cell embryo takes place before the majority of cyclin B1 has been degraded. Cytokinesis occurs shortly after anaphase, also well before full cyclin B1 destruction. We plan to observe this relationship at the 4 cell, 8 cell and 16 cell mitoses to understand if changing cell size has any impact on this sequence of events. Alongside this, we will use chemical interventions to disrupt Cdk1-Cyclin B1 activity to observe if certain thresholds of Cdk1 activity mediate anaphase and furrow ingression as seen in previous somatic cell studies. A greater understanding of how cytokinesis is temporally regulated in embryos and development may help to understand why common mis segregation errors, such as binucleation and micronucleation, are seen in the fertility clinic.

Développement d'une nouvelle méthode de caryotypage chez le porc (#10),

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La production porcine est hautement compétitive et la durabilité des entreprises est directement liée à leur productivité et rentabilité. La prolificité des animaux est un facteur majeur alors qu'une taille de portée sous la moyenne entraîne des pertes économiques importantes. Il est connu que les anomalies chromosomiques affectent la fertilité des porcs atteints. À titre d'exemple, près de la moitié des verrats ayant des problèmes de fertilité en serait atteinte et le type d'anomalie le plus fréquent causerait une réduction moyenne de la taille de portée de près de 40 %. Il devient donc important de développer des outils de détection à faibles coûts et faciles d'exécution et d'implanter par la suite l'analyse chromosomique systématique des reproducteurs pour lutter contre cette entrave à la productivité. Il existe à ce jour des techniques pour identifier les chromosomes et leurs possibles réorganisations, mais ces dernières comportent des lacunes. En ce sens, le but du projet est de développer un outil de reconnaissance basé sur l'intelligence artificielle qui permettra d'évaluer et de confirmer l'intégrité des chromosomes porcins. De ce fait, cet outil permettra d'accéder à une autonomie, une rapidité et un faible coût de diagnostic, tout en étant convivial et précis. Jusqu'à présent, une technique de coloration des chromosomes performante et répétable a été développée. Cette méthode se base sur les principes d'hybridation in situ en fluorescence et permet le marquage de 96 régions spécifiques grâce à des oligonucléotides marqués. Plusieurs porcs, dont certains porteurs d'anomalies, sont actuellement soumis à l'analyse chromosomique avec cette méthode afin de développer le logiciel de reconnaissance. À terme, cette technologie sera transférée au Centre d'insémination porcine du Québec qui offrira ce service.

Elucidating the Mechanism and Genetic Impact of Post-Meiotic DNA Double-Strand Breaks of the Male Haploid Gamete (#11)

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Paternal bias in the inheritance of de novo mutations (DNMs) have been linked to neurodevelopmental disorders such as those of the autism spectrum. This bias has been associated with the increasing number of germ cells division as male ages. However, the contribution of the chromatin remodeling steps in spermatids to DNMs has so far been elusive. We hypothesized that the transient formation of DNA Double-Strand Breaks (DSBs) observed in the haploid context of spermatids would represent a major source of genetic instability mostly responsible for the observed male mutation bias.Interestingly, meiosis in the fission yeast (Schizosaccharomyces pombe) shares similarities with that of the male mammals and is a potential model to investigate the genetic impact of DSBs. We have selected the S. pombe Pat1-114 strain as it allows for synchronization of meiosis and post-meiotic steps. Indeed, a transient post-meiotic surge in DNA double-strand breaks of enzymatic origin was confirmed using qTUNEL, a method specifically developed in our lab. We therefore established the highly conserved nature of the post-meiotic DNA DSBs as it is observed during yeast sporulation.The endonuclease Pnu1 was identified by in-gel nuclease assay and mass spectrometry as the likely candidate enzyme involved in the transient post-meiotic DNA DSB formation. Generation of Pnu1 deletion mutant prevented the post-meiotic DNA fragmentation demonstrating a key role in the process. Interestingly, immunofluorescence analyses showed that Endonuclease G, the mammalian functional homolog of Pnu1, is expressed at chromatin remodeling steps in the nuclei of mouse spermatids.Mapping of DNA DSBs during sporulation steps in both wild-type and Pnu1Δ strains will now allow us to highlight the potential contribution of post-meiotic DNA DSBs to mutagenesis and adaptation in fission yeast, and direct further analyses in mammalian spermatids. These results will be further discussed at the meeting. New insights regarding the paternal origin of genetic disorders could emerge from this work.
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Evolutionary Role of the Post-Meiotic DNA Double-Strand Breaks: Lessons from Fission Yeast Model (#12)

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De novo mutations (DNMs) could be viewed either as opportunities or risks for offspring. The unpredictability of DNMs does not allow for the same risk management as inherited diseases. However, unpredictability stops where statistical analysis starts. Indeed, the paternal germline is responsible for 80% of DNMs. DNMs result from complex chromosomic rearrangements and repair errors, most often produced from DNA double strand breaks (DSBs). Previous experiments from our laboratory have unraveled a cascade of events creating transient post-meiotic DSBs during spermiogenesis. The haploid character of post-meiotic cells prevents error-free repair that is normally provide by homologous recombination. In this context, DSBs will likely be repaired using more error-prone mechanisms causing possible mutations. Therefore, understanding DSBs formation in post-meiotic cells could help circumvent deleterious mutations leading to child diseases. Through proteomic analyses in fission yeast, our group identified a homolog of mammalian endonuclease G, that could be involved in DSB formation at post-meiotic steps. Although typically linked to apoptosis, its involvement in post-meiotic DSBs was formally demonstrated in fission yeast Schizosaccharomyces pombe. As observe in mammals, S. pombe undergoes a similar transient post-meiotic DNA fragmentation event. In a mutant model lacking the yeast EndoG homolog (Pnu1), a sharp decrease in DNA fragmentation is observed. The challenge is then to determine if DNMs load may be modulated by Pnu1 expression. To answer this question, wild-type and ΔPnu1 mutant strains will be submitted to multiple meiosis cycles, with regular single-cell bottleneck, producing so-called accumulation lines. DNMs are then measured through two different approaches: adaptation and genetic drift. While adaptation will be established by antibiotic resistance acquisition, genetic drift will be monitored by selected reporter genes sequencing and within repeated sequences to assess potential elongation or contraction (satellite sequences). By comparing wild-type and ΔPnu1 strains, we should establish for the first time the impact of post-meiotic DSBs on both evolution and adaptation. This investigation may provide key elements to better understand the genetic consequences of the conserved post-meiotic DSBs in mammalian spermiogenesis.Funded by Canadian Institutes of Health Research (#PJT-159719).

The placenta for early identification of high-risk infants born at 29-36 weeks gestation (#16)

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OBJECTIVES: Preterm birth (PTB) is a major complication of pregnancy associated with risk of developmental delay. However, children born preterm represent a heterogenous group making risk stratification challenging. It is therefore highly relevant to flag infants who will benefit the most from closer developmental follow-up. The placenta reflects the in-utero environment, and any changes in its function can impact development. Our objective was to investigate the association between placental markers and neuromotor development during the first year of life in preterm infants born at 29-36 weeks' gestational age (GA). METHODS: This prospective observational cohort study recruited 200 infants born between 29-36 weeks' GA. Placenta/fetal membranes samples were obtained. Histological analysis was used to classify the placentas as: no abnormality, structural or inflammatory defects. Analyses of Hofbauer cells polarisation, oxidative stress and villi vascularization were also performed. Maternal demographics and obstetrical data were obtained from the medical chart. Infant neuromotor development was evaluated at term equivalent age (TEA), 3.5 and 8 months of corrected age (CA) using standardized tests by an assessor blinded to placental results. RESULTS: Preliminary analysis of the first 50 placentas revealed that inflammatory lesions were predominantly observed in placentas from late preterm (>34-36 weeks' GA) delivery. Structural defects were observed across all GA. No specific histological lesion type was associated with growth restriction or abnormal neurological status at TEA. Interestingly, within pregnancies with inflammatory lesions, infants were more likely to have abnormal neuromotor evaluation at TEA or 3.5 months. Fetal membranes from early preterm delivery show more CD68+ cells than late preterm delivery. No difference was seen in 3-NT intensity in placentas from different GA. Significantly more blood vessels were observed in placentas from preterm infants that were born at later GA. CONCLUSION: Placental markers are associated with risk of neuromotor abnormalities in infancy. This indicates that the placenta could prove useful in refining risk stratification in preterm infants. Our ongoing work will include in-depth analysis of the placentas and correlation with obstetrical causes of prematurity as well as infant's neurodevelopmental follow-up at later ages.

Single-cell look-seq to identify error prone transcriptional profiles in oocytes (#18)

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Chromosomal segregation errors during early development lead to inheritance of incorrect numbers of chromosomes, known as aneuploidy, which causes infertility and birth defects. A recent study from our lab shows that lagging chromosomes, a specific type of segregation error in oocytes, can be classified into two main types – one of which causes aneuploidy and one which does not. Why these errors happen only in some oocytes is not known. Single cell RNA-Seq is a technique that allows us to observe and quantify changes in the RNA profile of individual cells. Previous research studying transcriptomic changes in oocytes of different age or maturation stages demonstrates that single-cell RNA-Seq is a valuable tool to perform comparative analyses on samples of limited quantities like oocytes of mice and human, however no research have been yet conducted observing RNA expression changes in oocytes with chromosomal segregation errors. In our study we are combining two single-cell approaches - single cell live oocyte microscopy, and single-cell RNA-sequencing – an combinational approach dubbed look-seq. Our aim is determine whether chromosomal segregation errors such as lagging chromosomes that can be seen in live microscopy, are associated with particular transcriptional profiles, and whether particular chromosome-segregation-related factors are misregulated in oocytes that exhibit errors. We have optimised the imaging and expect our first sequencing data to be ready at the time of the RQR meeting. Understanding the causes of infertility will depend on identifying the specific molecules misregulated in oocytes with age.

The Forensic Science behind the Caribou (Rangifer) SNP Chip Validation (#19)

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The Ministère des Forêts, de la Faune et des Parcs du Québec (MFFP) develops and implements tools to better monitor, manage and protect wildlife species. Genomic has recently been added to this toolbox through a genotyping SNP chip. In addition to monitoring evolutionary potential by metrics, such as genomic diversity, population connectivity and inbreeding levels, a genotyping chip will also serve as a wildlife forensic tool by determining the population of origin for anti-poaching law enforcement.Our aim was to document the specifications of the new caribou SNP chip by addressing its sensitivity, robustness, repeatability, specificity, and ability to distinguish mixed samples. We first focused on sample preparation. DNA was extracted from hair follicles, muscle biopsies, ear punches, tendons, blood swabs, and fecal pellets. Samples generated different DNA quality and quantity outputs. Various extraction methods have been tested to obtain high molecular weight DNA.Results showed that the SNP chip is robust, highly sensitive and reliable at 10X below recommended DNA input. DNA quality had little impact. DNA source was not an issue even for fecal pellets. Inter-species hybridization showed an important drop in call rates and extent of heterozygosity. Preliminary analyses indicated that mixed samples could be problematic depending on the proportion of each individual in the sample. These validation steps are crucial to develop a powerful tool that will fulfill various uses, especially in wildlife forensics.

Structure and assembly dynamics of kinetochore in oocyte meiosis-I (#20)

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Oocyte meiosis-I is a highly idiosyncratic cell division in which chromosomes are frequently mis-segregated leading to aneuploidy. The central player in this process is macromolecular structure termed kinetochore, which binds to chromosomal DNA and spindle microtubules to mediate chromosome alignment and segregation. Detailed understanding of how the kinetochore is assembled and functions in mammalian oocytes is lacking and will be essential in unravelling the causes of chromosome segregation error that leads to oocyte aneuploidy and aging-associated infertility. Here, we have analysed expression of kinetochore components at different stages during CD1 mouse oocyte meiotic maturation. We use chemical inhibitiors ZM447439 and Roscovitine to examine the role of AurK and CDK1 pathways, respectively, in the process of kinetochore assembly and disassembly. Examinations of CENP-A, CENP-C and CENP-T protein expression revealed that they all localized at the kinetochore throughout meiosis-I. The staining intensities remained stable throughout meiosis-I and declined upon meiosis-I completion. In contrast to the other components, Ndc80 was not initially present at GV-stage centromeres but became recruited upon resumption of meiosis-I and peaked at 4h post IBMX release, and then decreased subsequently by the time of meiosis-I completion. Inhibition of AurK activity before germinal vesicle breakdown (GVBD) could significantly suppress Ndc80 assembly, whereas suppression of CDK1 activity showed no impact on the abundance of Ndc80, suggesting a key role for AurK activity in kinetochore assembly in mouse oocytes. Neither AurK nor CDK1 activity was necessary for kinetochore maintenance once it had been assembled. To investigate whether aging affects the organization of kinetochore, we compared the abundance of core kinetochore proteins between young and aged oocytes in the late meiotic stage. The results showed the assembly potential of kinetochore significantly attenuated with female age. Consistent with this result, we also observed remarkably less binding k-fibes upon the kinetochore in aged group. These findings imply that kinetochore assembly is unusual in mouse oocytes in which Aurk, not CDK1, appears to play an important role in the organizational process of kinetochore, and kinetochore structure and function undergo aging-associated decline throughout oocytes reproductive life.

CAMKI coopère avec les facteurs de transcription COUP-TFII et GATA4 dans les cellules de Leydig (#23)

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Les cellules de Leydig sont des cellules spécialisées responsables de la production de la testostérone et d'INSL3. Ces deux hormones sont essentielles à la différenciation sexuelle masculine et la santé reproductive de l'homme tout au long de la vie. Chez un adulte mature, la production de la testostérone est un processus très bien régulé qui débute par la sécrétion de la LH hypophysaire dans la circulation sanguine. Les récepteurs de la LH qui se trouvent sur les cellules de Leydig dans les testicules sont activés ce qui induit une cascade de signalisation intracellulaire et l'activation de protéines kinases qui phosphorylent des facteurs de transcription. Notre laboratoire a établi un rôle clé pour la CAMKI dans les cellules de Leydig. Notre hypothèse est que les effets stimulateurs de la CAMKI s'effectuent, au moins en partie par la phosphorylation directe de certains facteurs de transcription incluant COUP-TFII et GATA4 et que cette phosphorylation augmenterait leur potentiel d'activation de l'expression génique. Des transfections transitoires ont été réalisées dans la lignée de cellules de Leydig MA-10 et dans des cellules fibroblastes CV-1 en utilisant le promoteur du gène Star fusionné au rapporteur luciférase. Le promoteur Star a été choisi puisque son expression est fortement stimulée par la LH ce qui requiert une CAMKI active. En outre, le promoteur Star est aussi activé par ces facteurs de transcription. Le promoteur Star a été mis en présence des facteurs COUP-TFII et GATA4 avec ou sans une CAMKI constitutivement active. Nos résultats montrent que la CAMKI augmente le potentiel d'activation de COUP-TFII autant dans les cellules MA-10 que dans les CV-1. Pour GATA4, aucun effet de la CAMKI n'est observé dans les cellules MA-10 mais une stimulation est présente dans les cellules CV-1. Ces données suggèrent l'existence de mécanismes d'action différents de la CAMKI en fonction du contexte cellulaire. Des études sont en cours afin de déterminer si la CAMKI phosphoryle directement les facteurs COUP-TFII et GATA4. Nos données démontrent l'importance de la CAMKI dans l'activation des facteurs COUP-TFII et GATA4 dans la régulation de l'expression de gènes stéroïdogéniques.

miRNA regulation of lineage specification in the human preimplantation embryo (#24)

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INTRODUCTION: Preimplantation embryonic development is a critical period to ensure proper development and the success of assisted reproductive technologies. During this time, totipotent embryonic cells specify into the first distinct cell populations; the trophectoderm (TE) and inner cell mass (ICM), the latter further differentiating into the epiblast (EPI) and primitive endoderm (PE). In mice, the leading model for ICM-TE specification involves differential activation of Hippo signalling. However, our lab's recent work with human embryos suggests that this mechanism may involve a molecular component other than transcription. In murine embryos and human embryonic stem cells (ESCs), it has been shown that miRNAs, which are known to regulate transcription, play a role in TE specification. In addition, specific miRNAs have been identified between ESCs (representative of ICM) and trophoblast stem cells (representative of TE). As such, we hypothesize that miRNAs are important upstream drivers of lineage specification and aim to define their regulatory role in human embryos. METHODS: Human cryopreserved embryos were secured from CReATe Fertility. Embryos from embryonic day (E)3-E7 (n= 8-12 embryos/day) were collected, TE and ICM populations were isolated and dissociated into single-cells, sequenced using smallseq and analyzed using our inhouse pipeline. We classified the cells into their respective lineages and quantified the total number of molecules per miRNA each cell expressed. RESULTS: At E5, coinciding with lineage specification, 439 miRNAs were significantly differentially expressed between the ICM (containing the EPI and PE) and TE. Examining the expression dynamics of these miRNAs before and after segregation, 25 miRNAs of interest were identified. KEGG analysis determined that the gene targets of these key miRNAs are components of the Hippo signalling pathway. In addition, we observed significant differences between the TE and ICM lineages in the gene expression of miRNA processing proteins DROSHA, DGCR8 and Dicer1. CONCLUSION: This preliminary data suggests differences exist in miRNA biogenesis between TE and ICM lineages. Further, our data suggests that miRNAs may drive lineage specification in the embryo and that this is, at least in part, due to Hippo signalling. Additional functional studies are required to further elucidate this potential regulatory model of lineage specification.

Allele-specific methylation editing of imprinting genes (#27)

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Following fertilization, a major reprogramming wave removes most DNA methylation signatures across the genome to instigate the embryonic developmental program. Through mostly unknown mechanisms, parent-of-origin-specific methylation marks on one allele of imprinted genes (e.g., Igf2: Insulin-Like Growth Factor 2/H19 locus) are able to escape the embryonic reprogramming wave and retain their profiles via maintenance by the DNA methyltransferase 1 (DNMT1) family proteins. Failing to maintain proper genomic imprinting patterns during embryonic reprogramming leads to neurodevelopmental disorders characterized by developmental delay. It is still unclear how embryonic cells are able to re-establish DNA methylation profiles across most of the genome following a temporary lack of DNA methylation maintenance, while other regions such as imprinted loci become permanently dysregulated. Published and current data from the McGraw lab show that, for Igf2/H19 and Peg13 imprinting loci, a transient loss of DNA methylation maintenance in mouse embryonic stem cells leads to permanent loss of DNA methylation, decrease levels of repressive histone modification H3K9me3 associated with imprinting loci, increase levels of active mark H3K4me3 associated with actively transcribed genes, increase in H3K27ac associated with open chromatin, and increase in gene expression. What remains to be elucidated is whether we can permanently re-establish normal profiles of DNA methylation and subsequent histone modifications on imprinted (allele-specific) sequences following the loss of their DNA methylation imprinting status. To set the foundation for a clear understanding of the mechanisms behind re-establishing the loss of inherited methylation profiles, we hypothesize that we can re-establish specifically and permanently the lost DNA methylation imprints at specific loci by targeted epigenome editing in embryonic cells. This project will further the understanding of the fundamental complex biochemical mechanisms of epigenetic regulation that drive allele-specific gene expression in embryonic cells. It will reveal how such mechanisms are involved in imprinting maintenance during early embryogenesis and will elucidate possible causes leading to imprinting developmental disorders.

Epididymal Basal Cells Express the Adult Stem Cell Marker LGR5 (#31)

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The epididymal epithelium is formed by various cell types including principal and basal cells. Basal cells share common properties of adult stem cells as they can form organoids, self-renew and differentiate into principal cells in vitro. The characteristics of basal cells support the notion that these serve as a stem cell population residing at the base of the epididymal epithelium. However, there is currently no marker to specifically identify epididymal stem cells. The present objective is to identify a specific marker of this population. Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5) is a seven-transmembrane G-coupled receptor. It shares homology with two members of the LGR family, LGR4 and LGR6; all three proteins are receptors for R-Spondin, which modulate Wnt signaling. LGR5 is a well-established marker of adult stem cells in a variety of tissues. LGR5, 4 and 6 transcripts were expressed throughout the epididymis during postnatal development. LGR5 and LGR6 mRNA levels decrease during postnatal development while LGR4 mRNA levels remained constant. LGR5 was immunolocalized to undifferentiated columnar cells of the epididymis as early as PND7. However, as the epithelium differentiated, LGR5 became associated with basal cells. In the adult epididymis, LGR5 was localized primarily to basal cells, although weak staining was observed in narrow cells. Co-localization of LGR5 with the basal cell marker TP63 in the adult epididymis indicated the existence of at least 3 basal cell sub-types: LGR5+/TP63-, LGR5+/TP63+ and LGR5-/TP63+. LGR5 was overexpressed in isolated basal cells and was highly express in organoids formed by basal cells during the early proliferation phase of the culture as compared to organoids undergoing differentiation. Western-blots using epididymides of rats that were either sham-operated (controls), orchidectomized or orchidetomized and given testosterone implants demonstrated that LGR5 is regulated by androgens. Together these data demonstrate that LGR5 is expressed in a sub-population of basal cells of the epididymis and is a good candidate as an epididymal basal stem cell marker. Supported by CIHR, CIRD and the Canada Research Chairs Program.

Adapted i-BLESS Method to Detect Double-Strand Break Hotspots in Human Sperm Cell Breakomes (#32)

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In Canada, one in six couples will encounter fertility issues while trying to conceive. While there are many reasons for having difficulty conceiving, one of the hypotheses is the higher number of double-strand breaks (DSBs) in the sperm cell DNA. The male germ cells are seemingly responsible for a high number of de novo mutations. These mutations might be the result of residual breaks following chromatin remodeling during spermiogenesis. Preliminary findings also show that most DSB clusters tend to happen in neurodevelopmental genes. Consequently, the impact of DSBs in neurological disorders needs to be further studied.The current methods available for DSB detection are not specific nor sensitive enough. Therefore, the objective of this project is to develop a method allowing a specific and sensitive DSB capture that can be used in a clinical setting. The i-BLESS method was originally created by N. Crosetto and his team, for DSB detection in yeast. The i-BLESS method allows a capture of both mechanical and enzymatic pre-existing DSBs. This is done without creating more mechanical breaks through handling, by using agarose encapsulation. Our laboratory has adapted the i-BLESS method to allow the capture of DSBs in sperm cells. This modified version was tested on HeLa-I-SceI-3LI cells, where DSBs can be induced at specific loci from the I-SceI restriction enzyme digestion, in order to confirm its specificity.This optimized version has allowed to capture DNA from double-strand break sites with sufficient yield for library preparation and breakome sequencing. The sperm breakome analysis will allow us to correlate any increase or altered distribution of breaks associated with impaired fertility or neurodevelopmental disorders in offspring. These findings could be used as a prognosis tool in personalized medicine for at risk couples. Funded by Canadian Institutes of Health Research (#PJT-159719).

SFRP4 inhibe l'action des gonadotrophines dans les cellules de granulosa via un mécanisme GSK3β/CTNNB1 dépendant (#35)

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Bien que les rôles des gonadotrophines, soit l'hormone folliculostimulante (FSH) et l'hormone lutéinisante (LH), dans le développement folliculaire soient bien établis, leurs mécanismes de signalisation restent partiellement méconnus. La protéine Secreted frizzled-related protein 4 (SFRP4), inhibiteur dans le développement folliculaire et la fertilité chez la souris, pourrait réguler l'action des gonadotrophines par son rôle d'antagoniste de la signalisation WNT. Vu le rôle établi de la b-caténine (CTNNB1) dans la croissance folliculaire et de la stéroïdogenèse dans l'ovaire en agissant comme co-activateur transcriptionnel des gènes cibles de la FSH, notre hypothèse est que la SFRP4 agit en inhibant la CTNNB1. Pour élucider le mécanisme d'action de SFRP4 dans l'ovaire, des cellules de granulosa murines ont été cultivées et traitées avec de la SFRP4 recombinante exogène, suivi (ou non) de la FSH. L'effet des traitements sur les niveaux d'ARNm des gènes cibles de la FSH (Cyp19a1, Fshr et Lhcgr) a été évalué par RT-qPCR, et l'activité des voies de signalisation PKA (CREB), PI3K/AKT (AKT, mTOR, FOXO1) et WNT/CTNNB1 (GSK3β, CTNNB1) ont été évaluées par immunobuvardage. Des expériences supplémentaires ont été menées en utilisant des inhibiteurs pharmacologiques des voies WNT/CTNNB1, WNT/Ca2+ et de la polarité cellulaire planaire pour identifier l'implication de chaque voie dans la médiation des effets de SFRP4. Les résultats ont montré une diminution des niveaux d'ARNm de Cyp19a1, Fshr et Lhcgr après le traitement par SFRP4 ($p < 0,05$). De plus, le co-traitement avec SFRP4 et FSH a révélé que SFRP4 peut antagoniser l'induction médiée par la FSH de ses gènes cibles ($p < 0,05$). Des expériences avec des inhibiteurs ont révélé que la diminution médiée par SFRP4 des niveaux d'ARNm de Cyp19a1, Fshr et Lhcgr pouvait être inversée par l'ajout de SB216763, indiquant que les effets de SFRP4 sont dépendants de GSK3β. À l'inverse, les inhibiteurs de WNT/Ca2+ et de la voie de la polarité cellulaire planaire n'ont pas réussi à s'opposer à SFRP4. SFRP4 s'est également avéré capable de diminuer les niveaux de la forme active (non phosphorylée) de CTNNB1. Ensemble, ces résultats suggèrent que SFRP4 agit via un mécanisme dépendant de GSK3β/CTNNB1.

La voie de signalisation Slit/Robo est un antagoniste de la signalisation LH dans les cellules de la granulosa murines (#36)

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La voie de signalisation Slit/Robo est connue pour son rôle d'inhibiteur de la phosphorylation activatrice de la kinase AKT, impliquée dans la réponse aux gonadotrophines : l'hormone folliculo-stimulante (FSH) et l'hormone lutéinisante (LH). De plus, les souris haploinsuffisantes pour Robo1 et Robo2 sont hyperfertiles, cependant le mécanisme d'action n'est pas identifié. Cette étude consistait à caractériser l'expression des protéines Slit et Robo durant la folliculogenèse et déterminer les rôles de la signalisation Slit/Robo sur l'action des gonadotrophines. Afin d'étudier la régulation des ARNm des gènes Slit et Robo durant le cycle ovarien, des souris ont été traitées avec de la gonadotrophine chorionique équine (eCG) et de la gonadotrophine chorionique humaine (hCG) durant différents temps. À partir de cellules de la granulosa isolées, l'expression des gènes Slit1/2 et Robo1/2 a été détectée par RT-qPCR, avec une augmentation significative des ARNm de Robo2 et Slit2 4h après l'injection de hCG ($p<0,001$). Par immunohistochimie, la présence de SLIT2 et de ROBO1/2 dans l'ovaire a été confirmée. Pour déterminer les effets de SLIT2 sur la signalisation des gonadotrophines, des cellules de la granulosa isolées à partir de souris et mises en culture ont été exposées à un traitement avec des gonadotrophines et/ou du SLIT2 exogènes. Les analyses par immunobuvardage ont démontré que le traitement avec SLIT2 a entrainé une diminution de la phosphorylation d'AKT ($p<0,05$). Nous avons également généré un modèle Robo1-/-;Robo2flox/flox;Cyp19-cre (R1R2C) chez lequel Robo1 et -2 sont inactivés au niveau des cellules de la granulosa des follicules antraux. A 4h post-hCG, les animaux R1R2C présentent une augmentation des niveaux d'ARNm des gènes de réponse à la LH, Adamts1, Ereg, Ptgs2 et Tnfaip6 par rapport aux souris WT ($p<0,05$). Chez les souris R1R2C, à 12h post-hCG le niveau de progestérone sérique était supérieur à celui mesuré chez les souris WT ($p<0,05$).Les résultats mettent en évidence la présence des effecteurs de la voie Slit/Robo dans l'ovaire ainsi que l'implication de la voie dans la réponse à la LH. Mieux comprendre le mécanisme d'action de la voie Slit/Robo dans l'ovaire pourrait conduire à l'identification de nouvelles cibles thérapeutiques dans le cadre de l'infertilité.

Effect of elevated NEFAs exposed during in vitro maturation on the cocultured porcine granulosa cells (#37)

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Elevated levels of non-esterified fatty acids (NEFAs) are known to impair the proliferation, apoptosis, and steroidogenesis of granulosa cells, the quality and development potential of oocytes, and may subsequently influence the metabolism and reproduction of offspring. Granulosa cells (GCs) are the follicular cells that are closely communicating with the oocyte, and the coculture of GCs during oocyte in vitro maturation (IVM) can decrease the detrimental effect of elevated NEFAs on blastocyst forming. However, the responses of GCs exposed to high levels of NEFAs when cocultured with cumulus-oocyte complexes (COCs), and how they attenuate the adverse effects of NEFAs on oocytes, are unclear. To better understand this protective effect, monolayers of porcine GCs cocultured with COCs during IVM in the presence or absence of elevated levels of NEFAs (468 mmol/L palmitic acid, 194 mmol/L stearic acid, and 534 mmol/L oleic acid) were collected to complete the transcriptomic analysis via microarray. IPA analysis was conducted to explore the responses of the GCs to the elevated levels of NEFAs. After limma algorithm analysis, 1,013 genes were differentially expressed between GCs cultured with and without elevated NEFAs. Among them, 438 genes were upregulated and 575 were downregulated. The differentially expressed genes were enriched in pathways related to metabolism and inflammation. The enriched tox lists were cell death and mitochondrial dysfunction. Besides, the upstream predicted regulations are involved in inhibiting the transition from granulosa to luteal cell, metabolism change, and anti-inflammation. In conclusion, apoptosis and mitochondrial dysfunction were induced in the coculture granulosa cells but the cocultured granulosa cells can attenuate the adverse effects of NEFAs on oocytes through regulation of metabolism, anti-inflammatory factors, and inhibition of the epithelial-mesenchymal transition.

Cross-Species analysis of Wnt pathway involvement during preimplantation development and lineage specification in the human and the mouse (#38)

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Introduction: The first week post-fertilization is critical to human development but is poorly characterized. Current knowledge pertaining to preimplantation development is primarily based on murine models. However, differences have been identified between the mouse and human and caution is warranted when translating findings. For example, although the involvement of Hippo signaling in inner cell mass (ICM)-trophectoderm (TE) segregation is clear in mice, it remains controversial in humans. Recent studies suggest that the Wnt pathway may be involved in human lineage segregation. In this study, we seek to better understand the role of the Wnt pathway during lineage segregation in both the human and the mouse preimplantation embryo. We hypothesize that 1) inhibition of the Wnt/β-catenin pathway is involved in ICM-TE segregation, and 2) the Wnt/Planar Cell Polarity pathway is required for blastocoel formation and TE specification. Methods/Results: Re-analysis of our human scRNA-seq dataset (E3-E7) shows a switch in the expression (on/off) of genes involved in Wnt signaling at late embryonic day (E) 5; corresponding to the segregation of epiblast (EPI), primitive endoderm (PE) and TE. Further, many of the genes involved in the Wnt/β-catenin pathway are down-regulated in TE cells compared to EPI and PE cells. Next, we performed a comparative analysis of human (OVO Fertility) and mouse preimplantation embryos treated with small molecule inhibitors (CHIR99021 and IWP2) to inhibit/activate the Wnt pathway. Embryos (N=2-10/group) were imaged by immunofluorescence using lineage markers (NANOG, GATA6, CDX2) and components of the Wnt pathway (B-catenin, pJNK). Inhibiting the Wnt pathway led to developmental arrest of human embryos around E4-E5 as expected, but did not prevent mouse development. Activating the Wnt pathway impacted CDX2 expression in both human and mouse embryos, suggesting a retainment of pluripotency and failure to form TE. Together our data suggests that Wnt signaling plays an important role in TE formation. Conclusion: Our preliminary results suggest species differences in the pathways driving ICM-TE segregation, emphasizing the need to human focused studies. Knowledge from these studies will shed light onto early human embryogenesis and may be translated to the field of Stem Cell Biology.

Implication of mutated DNMT3A in the methylation landscape and the pathogenesis of Tatton-Brown-Rahman Syndrome (#39)

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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 (NPC) to establish the first preclinical model of TBRS to specify the deleterious impacts of functional DNMT3A mutations on brain cell development. Using whole genome bisulfite sequencing and bioinformatics, we have uncovered that pathogenic heterozygous DNMT3A mutations lead to DNA methylation defects in both IPSC and NPC altering the normal programming of pluripotent stem cells into neural progenitors, interfering with developmental and neurodevelopmental events. Our bioinformatics analyzes allowed us to identify 66,523 differentially methylated regions (DMR; 100 base pair tile that do not overlap with sequencing coverage of 10X or more, containing at least 2 CpG with a difference in methylation of at least 20% and a q-value of less than 0.01) through the genome of our mutated IPSC samples compared to healthy controls. We also identified 165 844 DMRs when comparing our mutated NPC to the controls. The annotation of these regions using the HOMER tool allowed us to identify differentially methylated promoter of genes associated with development as well as neurodevelopmental. This project will uncover the functional impact of DNMT3A mutations on the epigenome during brain cell development can ultimately lead to disease pathogenesis in TBRS.

Abnormal placental DNA methylation and gene expression associated with assisted reproduction: early detection and effect of folic acid supplementation (#40)

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Background: In recent studies, assisted reproductive technologies (ARTs) have been associated with adverse perinatal outcomes and imprinting disorders related to abnormal embryonic and placental development. ARTs have been shown to alter major epigenetic reprogramming events related to DNA methylation during germ cell and early embryo development. Folate contributes methyl groups for proper DNA methylation establishment and maintenance during development. Aims: (1) To determine if DNA methylation perturbations induced by ART in pre-implantation embryos precede alterations in the expression of key genes involved in early placenta development, leading to abnormal placentation observed later in ART pregnancies. (2) To investigate the role played by folic acid (FA) supplementation in preventing these defects. Methods: Female mice were fed diets containing different levels of FA supplementation for 6 weeks prior to natural mating or ART (superovulation, in vitro fertilization, embryo culture and transfer) and continuing throughout gestation. Embryos and placentas were collected at midgestation, and RNA-sequencing performed on placentas from E10.5 female embryos. Among the genes affected by ART, 4 candidate genes involved in early placenta development (Phlda2, EphB2, L3mbtl1, cFos) were chosen for analysis of DNA methylation using bisulfite pyrosequencing and gene expression using droplet digital PCR (ddPCR). Results: We show that ART results in decreases in DNA methylation and increases in methylation variance in placentas from E10.5 and delayed female embryos at the Phlda2 imprinted control region. This result correlates with the decrease in expression reported by RNA-sequencing. Moderate FA supplementation partially improves methylation levels in E10.5 placentas while the high dose achieves the same effect in delayed placentas. We are currently performing ddPCR on the same cohort. We propose that these epigenetic alterations will help explain ART-induced adverse phenotypes observed later in gestation. (Supported by CIHR)

Evidence of inter but not transgenerational effect of folate deficiency and supplementation (#41)

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Evidence of inter but not transgenerational effect of folate deficiency and supplementation Edgar Martínez Duncker Rebolledo1,2, Donovan Chan1, Jacquetta Trasler1,2,3 1Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada2Department of Human Genetics, McGill University, Montreal, Quebec, Canada3Departments of Pediatrics Pharmacology and Therapeutics, McGill University, Montréal, Québec Canada Early germ cell development is a crucial time in which DNA methylation erasure and epigenetic reprogramming occurs. Environmental insults during this window affecting DNA methylation present a potential mechanism for the transmission of environmental exposures across multiple generations. A previous study has shown an intergenerational effect of folic acid deficiency (FD) and supplementation (FS) in lifetime exposed mice. Here we investigated whether DNA methylation of male germ cells is susceptible to environmental influences. Female mice were placed on a folic acid deficient (7FD) and supplemented diets (10FS and 20FS) prior to mating and throughout pregnancy. Resulting F1 litters were weaned on the respective diets. F2 and F3 male mice were obtained and placed on control diets. Using reduced representation bisulfite sequencing (RRBS) we assessed the genome-wide DNA methylation in F1 early spermatogonia, F1, F2 and F3 mature spermatozoa and investigated whether aberrant DNA methylation was transmitted across generations. In spermatogonia, we observed a total of 3015, 1208 and 5655 differentially methylated sites (DMCs) for the 7FD, 10FS and 20FS diets, respectively; a tendency for hypomethylation was observed. When looking at F1 spermatozoa there was a decrease in the DMCs (635, 493 and 760). Lastly, using Methyl Inheritance we sought to determine whether altered methylation was inherited from spermatogonia/F1 to the F3 generation, however, no DMCs were found to be significantly inherited. These results, using RRBS, suggest that altered DNA methylation in male germ cells is not transmitted across the generations. Further studies may reveal other epigenetic mechanisms responsible for transgenerational inheritance. Funding sources: Supported by CIHR

Implication of Fragile X-Related Proteins and neurotrophic factors in establishing transzonal projections (#42)

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In order to have a competent oocyte, a bidirectional dialogue between the oocyte and the somatic cells needs to be maintained throughout folliculogenesis. This takes place through paracrine secretions and the transfer of molecules by transzonal projections (TZP) from cumulus cells. This communication is of fundamental importance: on the one hand through its involvement in the maintenance of meiotic arrest by passing cAMP and cGMP, and, on the other hand, through the transfer of energy substrates. It has been shown that TZP can transfer not only small molecules (<1 kDa), but also large molecules, including mRNA. The TZP network has been established to have structural and functional similarities with synapses in the neural network. In the brain, Fragile X-Related (FXR) Proteins contribute to the network development through their ability to bind mRNA and are involved in translational control. In the ovary, the roles played by these proteins remain to be elucidated. We hypothesized that FXR proteins are involved in the establishment of the TZP network and that they contribute to the transfer of mRNAs essential for the development of the oocyte. The objectives are to study the effect of the addition of neurotrophic factors (NGF, BDNF, GDNF) on the development of the TZP network and to determine the impact of these on the expression of FXR proteins. In order to achieve this, follicular cultures will be carried out from mice of different genotype originating from the transgenic line Fmr1/Fxr2 KO with an encapsulation technique in a solution of fibrinogen/alginate. Phalloidin will be used to stain the actin filaments in the TZP network, and gene expression will be determined using quantitative RT-PCR. The project is in its early stages, but follicular cultures generated from bovine ovaries indicate that NGF and BDNF had beneficial effects on the TZP network and oocyte growth. This study will provide a better understanding of the modulatory role of neurotrophic factors and FXR proteins on the establishment of the TZP network. This project is funded by the NSERC Discovery Grants program.

Elucidating MEF2-interacting partners and targets in Leydig cells (#43)

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Localized in the testicular interstitial tissue, Leydig cells are the primary source of testosterone and insulin-like 3, hormones that play essential roles in male reproductive function and health. Although the signals and intracellular pathways controlling Leydig cell function have been well elucidated, the transcription factors downstream of these pathways remain to be characterized. We have detected members of the MEF2 family of transcription factors in the male gonad exclusively, more specifically in the somatic cells (Leydig and Sertoli) from early gonadal development throughout adult life, suggesting a role in testis development, somatic cell differentiation and male reproductive function. We also have identified reduced hormone production and deregulated expression of several genes when MEF2 factors are removed from Leydig cells in culture. Our hypothesis is that MEF2 factors (MEF2A-MEF2C-MEF2D) contribute to Leydig cell gene expression and function by cooperating with other transcription factors, forming a combinatorial code unique to Leydig cells. To characterize novel molecular targets of MEF2 in these cells, a ChIP-seq approach is being used. We have identified and thoroughly validated a suitable MEF2D antibody by gene specific ChIP and MEF2 knockdown/overexpression followed by Western blot and EMSA. Currently, we are constructing the DNA library for Next Generation Sequencing. We expect to identify genes to which MEF2 is directly recruited, and therefore, directly regulated by MEF2 factors, providing a complete picture of the global gene network regulated by MEF2 factors in Leydig cells. To identify MEF2-interacting partners we will use Turbo-ID approach, which allows the identification of protein-protein interactions through biotinylated proteins using streptavidin-coated beads and identification by LC-MS/MS. This approach will decipher the MEF2 interactome in Leydig cells, which is crucial to understanding the mechanism of MEF2 action in Leydig cells. This research will generate invaluable information on the regulation of Leydig cell function and may also provide novel insights into disorders of sexual development, infertility, and other Leydig cell-related dysfunctions in humans. Ultimately, we foresee that the knowledge gained during this project will contribute to the prevention, the development of molecular diagnostics, and the treatment of testicular disorders.

Étude des vésicules extracellulaires ciliaires dans le contrôle de la maturation spermatique (#44)

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Contexte. Les vésicules extracellulaires (VEs) regroupent différents types de particules membranaires libérées dans le milieu extracellulaire et circulant via les fluides de l'organisme. Grâce à leur capacité à transférer des molécules bioactives (sncRNA, protéines) d'une cellule émettrice à une cellule receveuse, les VEs contribuent de manière significative à la communication intercellulaire. Récemment, notre laboratoire a identifié une nouvelle population de VEs générées au niveau des cils primaires (CP), organelles cellulaires présents à la surface de cellules épithéliales des vas efferens. Ces derniers ainsi que l'épididyme constituent une zone de transit pour les spermatozoïdes qui y acquièrent motilité et pouvoir fécondant. Bien connu pour leur rôle de chimio-senseur ou de mécano-senseur dans différents tissus, la fonction des CP dans l'épididyme reste inexpliquée. Hypothèse. Le dysfonctionnement des CP notamment associé à de l'infertilité dans le cadre de maladies génétiques appelées ciliopathies, nous permet d'émettre l'hypothèse que les CP du système reproducteur mâle pourraient interagir avec les spermatozoïdes et contrôler des fonctions reproductives mâles via la libération de VEs.Objectifs. Afin d'évaluer le rôle des VEs ciliaires :1) Nous isolons et caractérisons les VEs dérivant des CP par cytométrie en flux à partir de fluide épididymaire de souris. Pour cela, nous utilisons une lignée de souris transgénique (Cetn2-GFP ; Arl13b-m-Cherry) dans laquelle le composant ciliaire Arl13b est couplé à la protéine fluorescente m-cherry. La composition moléculaire de ces vésicules est étudiée par séquençage d'ARN à haut débit et approche protéomique.2) Nous évaluons la contribution des vésicules extracellulaires ciliaires dans la maturation spermatique grâce à un modèle de souris invalidée génétiquement (VillinCre ; IFT88fl/fl), dans lequel la ciliogenèse et la libération de vésicules est bloquée au niveau des vas efferens. L'étude de fertilité des souris mâles, ainsi que l'analyse quantitative, moléculaire et fonctionnelle (motilité, reconnaissance ovocytaire) de spermatozoïdes isolés dans ce modèle est comparée avec celle provenant de souris contrôles. Contribution à l'avancement des connaissances. En plus de permettre une meilleure compréhension du rôle des CP et VEs ciliaires dans la maturation spermatique, cette recherche ouvre la voie à l'identification de nouvelles cibles potentielles pour le traitement et/ou le diagnostic non invasif de l'infertilité masculine.

Rôle du récepteur nucléaire LRH-1 dans le contrôle du métabolisme lipidique lors de la formation du corps jaune (#48)

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L'accumulation des lipides lors de la formation du corps jaune est importante pour la synthèse et la sécrétion rapide de la progestérone, indispensable pour l'implantation de l'embryon et le maintien de la grossesse. Toutefois, un excès lipidique systémique peut nuire à la fertilité féminine. En effet, plus de 40% des femmes éprouvant des dysfonctions ovariennes, de l'infertilité ou des fausses couches répétitives sont en surpoids ou obèses. De plus, le syndrome des ovaires polykystiques (SOPK), qui est la cause principale de l'infertilité féminine, est fréquemment associé avec de la dyslipidémie. Le récepteur nucléaire orphelin Liver receptor homolog 1 (LRH-1) jour un rôle clé dans le métabolisme lipidique en régulant plusieurs gènes impliqués dans la synthèse des sels biliaires, l'homéostasie du cholestérol et la synthèse des triglycérides. Des résultats antérieurs des laboratoires Gévry et Murphy ont démontré qu'au niveau des cellules de la granulosa, LRH-1 est essentiel au processus de lutéinisation, à la sécrétion de la progestérone et au maintien du corps jaune. Ce récepteur nucléaire est également surexprimé dans les cellules de la granulosa des femmes diagnostiquées avec un SOPK, cependant son rôle demeure inconnu. Notre hypothèse est que LRH-1 régule le métabolisme des lipides responsable de l'augmentation lipidique, observée lors de la formation du corps jaune, et qu'un excès lipidique pathologique bouleverserait le mécanisme d'action de LRH-1 et provoquerait ainsi les symptômes d'hypofertilité. Dans un modèle murin, l'abondance et la composition lipidique dans l'ovaire seront caractérisées à différents intervalles pendant le processus de la lutéinisation par lipidomique. Parallèlement, le réseau de gènes régulés par LRH-1 et impliqués dans le métabolisme lipidique sera déterminé par l'immunoprécipitation de la chromatine suivi d'un séquençage (ChIP-seq) et le séquençage du transcriptome (mRNA-seq) dans les cellules de la granulosa murines. Finalement, le rôle de LRH-1 dans ces processus sera approfondi par l'étude de ces mêmes mécanismes dans des modèles de souris obèses et/ou SOPK.

Characterization of Heritable Epigenetic Dysregulations in Promoter Regions Following a Temporary Lack of DNMT1 in Mouse Embryonic Stem Cells (#50)

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During the preimplantation period of mice embryos, DNA methylation (DNAmet) profiles are erased and reestablished genome-wide by a major reprogramming wave. Differentially methylated regions (DMRs) such as imprinted genes must escape reprogramming and maintain their precise DNAmet profiles by constant 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 (Dnmt1tet/tet), we previously showed that a temporary loss 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 reestablish their DNAmet profiles following Dnmt1 re-expression. Here we aim to define how a temporary lack of DNAmet maintenance remodels the chromatin landscape at genome-wide regulation regions such as promoters and how it modulates associated gene expression. Dnmt1 expression in Dnmt1tet/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 Chromatin Immunoprecipitation (ChIP-Seq) for H3K4me3, H3K4me1, H3K27me3 and H3K27ac histone marks, Reduced-Representation Bisulfite Sequencing (RRBS) and RNA-Seq. Our results showed that global gene expression isn't significantly altered by doxycycline treatment. However, a subset of the 18,166 defined promoters lost their methylation profiles after Dox treatment, altering gene regulation after recovery of DNMT1 activity. Also, some genes associated with promoters that have altered epigenome and DNAmet profiles showed no gene expression changes. This observation led us to hypothesized that in some cases gene regulation could not be affected in our ESCs but could be dysregulated in terminally differentiated tissues in which the genes must be expressed. We later want to analyze enhancers regions to identify 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.

Are Hippo pathway effectors potential key players of dairy cattle cystic ovarian disease pathogenesis? (#52)

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Among the causes of infertility in high-producing dairy cows, cystic ovarian disease (COD) represents a major problem and causes important economic losses to the dairy industry worldwide. Treatments to cope with COD present highly variable success and, for this reason, it is still critical to better identify, at the cellular level, the key components involved in the mechanism of cyst formation and persistence. Interestingly, the Hippo pathway effector yes-associated protein (YAP) has been described in humans and mice as a key player of anovulatory cystic disorders. Thus, we hypothesized that YAP deregulation in bovine granulosa cells can be also involved in the pathogenesis of COD in dairy cows. To start testing this hypothesis, we have generated a series of interesting results using samples from bovine ovaries collected from a local abattoir. First, we found that mRNA and protein levels for total YAP (and its transcriptional co-activator TAZ) are significantly higher ($P<0.05$) in granulosa cells (GCs) isolated from follicle cysts (follicular structures of at least 20 mm in diameter) in comparison to GCs isolated from healthy large follicles (>10mm). Taking into account that when accumulated in the nucleus, YAP/TAZ form complexes notably with TEAD family of transcriptional factors, we then decided to assess mRNA levels for the classic YAP/TAZ-TEADs transcriptional target genes CTGF, CYR61, BIRC5 and ANKRD1. The results indicated that the mRNA abundance for all these genes is increased in GCs from follicle cysts in comparison to healthy large follicles ($P< 0.05$). Ongoing IHC analyzes are being performed to determine the subcellular localization and phosphorylation pattern of YAP and TAZ (markers for Hippo effectors activity) in bovine follicle cysts in comparison to healthy large follicles. The next step of this study will then involve in vitro and in vivo approaches to determine if YAP/TAZ can be considered potential target proteins for the pharmacological treatment of COD. Together, these results will provide considerable insight of a completely novel signaling pathway as a critical player of the ovarian cystic disease pathogenesis in dairy cattle.

Dynamique du remodelage de la chromatine et co-occurence de motifs dans les cellules de la granulosa murine suite au signal ovulatoire (#53)

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Les cellules de la granulosa participent activement à folliculogenèse. Elles nécessitent, entre autres, l'action de l'hormone lutéinisante (LH) qui leur permet d'initier leur transition vers la phase de différentiation et devenir des cellules lutéales. Cette phase du cycle ovarien est finement régulée au niveau transcriptionnel, mais nous connaissons très peu les mécanismes moléculaires impliqués. Nous avons démontré que LRH-1 (liver receptor homolog-1), un récepteur nucléaire exclusif aux cellules de la granulosa, subit un grand changement dans sa liaison à l'échelle du génome suite à la LH. Régulant un programme d'expression génique spécifique, notamment, à cette phase. Nous avons également démontré qu'avant le signal ovulatoire et 4h post-LH, il y a d'importants changements au niveau de l'accessibilité à la chromatine. Notre hypothèse de recherche est que la LH induit un remodelage de la chromatine encore plus rapide que 4h post-LH permettant une réorganisation précoce du programme d'expression génique dans les cellules de la granulosa murine. L'étude de l'accessibilité à la chromatine, variant dans des courts laps de temps, et quel sont les motifs d'ADN co-occurrents régulés par un cis-régulateur qui sont disponibles durant ces temps précoces nous permettrons d'établir le processus de réorganisation de la chromatine pour enclencher la phase de différentiation. La technique de l'isolement des éléments régulateurs assisté par formaldéhyde suivi d'un séquençage (FAIRE-seq) nous permet de déterminer la modification de l'accessibilité des sites de la chromatine dans le temps. L'analyse du FAIRE-seq, couplé avec des données de transcriptomique mRNA-seq, nous permettra d'aller chercher des informations sur la co-occurrence des facteurs de transcription pouvant faciliter ces changements. Éventuellement, nous pourrons coupler nos données avec des bases de données de facteurs de transcription tel que LRH1. Nous aurons ainsi, des pistes pour déterminer les éléments régulateurs qui se mettent en place aux temps 1h et 2h post-LH. Ceci nous permettra d'avoir une vision globale des systèmes de régulation précoce important pour la transition vers la phase de différentiation que subissent les cellules de la granulosa.

Ablation in vivo du motif de liaison GATA dans le promoteur des gènes Star et Cyp19a1 (#56)

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Chez la souris, l'invalidation du gène codant pour le facteur de transcription GATA4 provoque un arrêt du développement de la crête urogénitale et sa différenciation ultime en testicule ou ovaire. Une insuffisance de GATA4 est aussi associée à une baisse des niveaux d'hormones sexuelles et à des problèmes de fertilité. Dans la population humaine, une dérégulation de l'expression de GATA4 a aussi été observée chez des individus présentant des différences de développement sexuel, des cas d'infertilité et des maladies hormonodépendantes dont l'endométriose et le syndrome des ovaires polykystiques. GATA4 exerce ses fonctions en modulant l'expression de plusieurs gènes cibles. Nous émettons l'hypothèse que GATA4 régule l'expression de gènes clés pour assurer le développement et le bon fonctionnement des gonades. Ce projet consiste à évaluer le rôle de GATA4 dans l'expression endogène des gènes steroidogenic acute regulatory protein (Star) et P450 aromatase (Cyp19a1), qui codent pour des enzymes essentielles pour la production des stéroïdes sexuelles. Pour ce faire, nous avons généré deux nouveaux modèles de souris dans lesquels le site de liaison GATA (qui lie le facteur GATA4) dans le promoteur endogène des gènes Star et Cyp19a1 a été muté ou délété par CRISPR/Cas9. Les gonades ont été prélevées à différents stades de développement (fœtal, puberté, adulte) afin de suivre l'expression des gènes Star et Cyp19a1 par qPCR et les niveaux des protéines par immunohistochimie et Western blot. Du sang a été collecté pour doser les hormones (testostérone, progestérone et estradiol). Les souris Star mutantes ne manifestent aucun phénotype externe apparent. Cependant, ces souris montrent une diminution significative de l'expression de Star dans les testicules et ovaires immatures et adultes. Les souris Cyp19a1 mutantes sont encore en cours de reproduction. Jusqu'à maintenant, les mâles ne présentent aucun phénotype visible. Toutefois, pour les quelques souris femelles adultes que nous avons évaluées, elles sont infertiles et leur tractus reproducteur est nettement sous-développé. Ces données préliminaires suggèrent que les facteurs GATA régulent directement l'expression des gènes Star et Cyp19a1. Ces études aideront à mieux comprendre le rôle de GATA4 dans les maladies associées à une sous-production d'hormones sexuelles chez les hommes et les femmes.

Variations in mtDNA copy number in oocytes cultured in different in vitro maturation conditions (#58)

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Mitochondrial DNA (mtDNA) plays an important role in regulating mitochondrial homeostasis, transcription and overall cell metabolism. Patterns of mtDNA vary among cell with different types and functions. In reproductive sciences, reports correlated low mtDNA copy number with poor oocyte quality and the occurrence of metabolic diseases in the offspring. It is also believed that mtDNA copy number changes according to the environment where oocytes are growing. In this context, here we investigated if the addition of different antioxidant molecules during in vitro maturation (IVM) could impact the number of mtDNA copies in bovine oocytes. For that, bovine ovaries were obtained from abattoir and oocytes of 3-5mm follicles were collected (GV; n=30). Next, IVM was performed in Control medium (n= 30) or in the presence of 2mM l-carnitine (Carn; n=30), 0.7uM melatonin (Mel; n=30) or a combination of both (C+M; n=30). Pools of 10 oocytes of each group were submitted to Proteinase K digestion 50mg/mL at 50°C for 20 min, followed by DNA extraction with QuickDNA Microprep Kit (Zymo Research) in 3 replicates. For Droplet Digital PCR, each 20 µL reaction contained 1× EvaGreen Supermix (Bio-Rad), 100nM primers for mitochondrial CYTB gene and DNA diluted to the equivalent of 1 oocyte. Samples were partitioned using a QX100 droplet generator (Bio-Rad) and emulsions were transferred to a 96-well plate. After ddPCR, fluorescence was measured using a QX100 droplet reader (Bio-Rad). The threshold for positive droplets was determined manually and the final output was in mtDNA copies/oocyte. Results were compared using ANOVA+Tukey's (=5%). The number of mtDNA copies significantly increased from GV-stage oocytes to mature Controls (GV=53,386 ± 5,519 vs. Control=71,844 ± 530). But when antioxidants were added to IVM culture media separately, this effect was not observed (Carn= 52,233 ± 4050 and Mel= 35,377 ± 6342). Surprisingly, the combination of both molecules, almost doubled the number of mtDNA copies (C+M= 82600 ± 3444). In conclusion, the environment surrounding the oocyte indeed impacts the number of mtDNA copies, but more tests are undergoing to understand how these variations will impact mitochondrial activity, and consequently oocytes' physiology and their potential to become viable embryos.

Studying the dynamic of DNA methylation in rat male germ cells during gametogenesis by Methyl-Seq capture (#60)

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In mammals, DNA methylation (5mC) reprogramming in male germ cells (GC) during gametogenesis can be under the influence of environmental toxicants, leading to abnormal germline programming and transgenerational epigenetic inheritance. However, we lack the comprehensive understanding of 5mC reprogramming during gametogenesis in rats, the preferred animal model in toxicology. We hypothesize that the establishment of 5mC is dynamic during perinatal life and spermiogenesis and aim to provide the first developmental 5mC map of the rat male germline.GC were purified by FACS, using transgenic rats expressing GFP exclusively in GC, combined with DNA staining to gate for ploidy and nuclear compaction. We obtained 9 populations of GC (Purity≥ 80% (n=4/stage)): 1- proliferative gonocytes from gestational day 16 (GD16); 2: gonocytes entering quiescent phase at GD18; 3: quiescent GD20 gonocytes; 4: postnatal day 5 spermatogonia (PND5); 5: spermatids (stages 1-8); 6: spermatids 9-12; 7: spermatids 13-14; 8: spermatids 15-17; 9: cauda epididymal sperm. 5mC was assessed using the Agilent rat MethylSeq kit. Data were analyzed using Bismark Bisulfite Read Mapper and methylation-extractor.Global methylation levels show that 5mC is minimal at GD18 and is gradually restored with 5mC patterns being almost similar between PND5 spermatogonia and spermatozoa. Using the SMART2 tool to identify stage specific differentially methylated regions (DMR), we analysed 103,720 regions (mean size of 472bp) and identified 68,407 DMRs. These include 11,105 DMRs at GD16, 54,265 common at GD16/20 and 2,224 at PND5, most being hypomethylated compared to later stages. Only 53 DMR were identified during spermiogenesis suggesting only little change in 5mC pattern during post-meiotic differentiation.We have established the first map of the rat male germline methylome at different stages of development, which will ultimately help identify epigenetic signatures of exposure to chemicals, and test their specific sensitivity at critical stages of maturation.

Obesity in Pregnancy Leads to Preeclampsia-Like Placental Features and Inflammatory Biomarkers (#62)

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Preeclampsia (PE) is notably heterogeneous in its clinical presentation as much as in its underlying placental dysfunction. Quantitative risk factors of PE, such as high BMI, are useful predictive tools, but should also be considered integral components of the pathophysiology behind hypertensive pregnancies. In a cohort of dominantly African-American pregnancies exhibiting early maternal age and unusually high BMI, we investigate relationships between clinical predictors, placental gene expression, serum biomarker levels and pregnancy outcomes of both mother and fetus. We first observed a strong, positive correlation between BMI and serum levels of inflammatory biomarkers tumor necrosis factor a (TNFa), leptin, c-reactive protein (CRP) and interferon gamma-inducible protein 10 (IP-10). These markers, secreted chiefly by adipose tissue and immune cells, are known to be present at higher levels in preeclamptic pregnancies. Interestingly, here, their correlation with BMI was strong regardless of diagnosis, suggesting that obesity causes systemic inflammation, not uniquely in preeclamptic pregnancies. By contrast, serum biomarkers such as sFLT1, sENG, PLGF and PL13, secreted by endothelium and/or trophoblast, showed expected differential levels between preeclamptic and non-preeclamptic pregnancies, a trend that was aggravated by high BMI. We conducted RNA sequencing of term placental samples, and identified, using unsupervised clustering, a group of seemingly extreme pregnancies in terms of aggravating maternal factors. Differential expression analysis and subsequent ontology interrogation revealed changes in cellular and extracellular structure; terms such as actin network organization, cell adhesion or extracellular matrix organization were enriched. These features, applied to syncytiotrophoblast structure, are indicative of poor integrity or turnover of the layer. Syncytiotrophoblast stress, such as this, is a known histopathological feature of preeclamptic placentas. Overall, this suggests that high BMI causes an inflammatory serum biomarker signature that mimics a preeclamptic signature. In pregnancies complicated by high BMI, placental function is impaired not by faulty placental development, but by systemic inflammation secondary to obesity. These findings highlight the profound effect of obesity on pregnancy health and the need, in future studies, to include BMI or a baseline inflammation profile in statistical models of pregnancy cohorts, to discriminate between obesity-driven and placenta-driven preeclampsia symptoms.

Functional in DNA Methyltransferase 3A (DNMT3A) leads to altered gene regulation in induced-pluripotent stem and neural progenitor cells (63)

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Activity from the DNMT3A enzyme is required during development for establishing DNA methylation, a stable epigenetic mark crucial for the regulation of gene expression. During brain development, DNMT3A is strongly expressed to drive and regulate proliferation and differentiation of neuronal populations. In mice, complete lack of DNMT3A enzyme is lethal, whereas Dnmt3a+/− animals are normal and fertile. In humans, DNMT3A-deficiency in embryonic stem cells leads to loss of DNA methylation in a lineage and tissue specific manner as cells differentiate. Rare heterozygous mutations in functional DNMT3A domains cause an overgrowth intellectual disability syndrome called Tatton-Brown-Rahman Syndrome (TBRS). Currently, we do not know how functional mutations in the human DNMT3A protein can impair neurodevelopment. We postulate that pathogenic heterozygous DNMT3A mutations will lead to DNA methylation defects that will alter gene expression profiles during neurogenesis. To investigate such events, we derived 2 different induced-pluripotent stem (iPS) cell lines, each carrying a single mutation in the functional methyltransferase domain of DNMT3A, and reprogramed these cells into neural progenitors. We generated RNA-seq profiles from iPS and neural progenitor cells and performed bioinformatics analyses. Our results show that alteration in DNMT3A function leads to abnormal expression in iPS cells (n=1333 genes) and neural progenitors (n=3936 genes) in comparison to controls. While the top enriched genes in iPS mutant cells are associated to development and neurodevelopment, the top enriched genes in mutant neural progenitors are associated to embryogenesis and cell fate decisions. These results show that heterozygous DNMT3A mutations in the methyltransferase domain lead to altered gene expression profiles in iPS cells that are exacerbated during differentiation into neural progenitors. Further analyses are needed to understand the mechanisms by which pathogenic DNMT3A alters gene regulation, and how this can affect lineage specification of neural progenitor cells.

In vitro embryo production in Common Marmoset (#64)

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Non-human primates are considered an optimal animal model for research because of similarities with humans that include their physiology, development, reproduction, and neuroanatomy. There is growing interest in the use of the common marmoset (Callithrix jacchus), a New World primate, in medical research because of its small size, reproductive efficiency, and ability to live in cohesive social groups. In vitro embryo production is a crucial step for the creation and optimization of new animal models. However, less invasive protocols for oocyte collection in marmosets and in vitro embryo culture conditions need to be improved, which was the main goal in this study. Here, we report successful rates of oocyte recovery by laparoscopic ovum pick-up (LOPU), and embryo development to the blastocyst stage after in vitro maturation (IVM), fertilization (IVF) and embryo culture (IVC). Prior to oocyte collection, females were treated with follicle stimulating hormone for 5 days. A total of 14 LOPUs were performed, which resulted in 667 structures collected, an average of 47.6 structures/female/LOPU. After selection, 433 cumulus-oocyte complexes (COCs) were considered morphologically normal and were placed in IVM for 24h in porcine oocyte maturation medium (POM) at 37.5°C with 5% CO2, 5% O2 and 90% N2. After IVM, 366 COCs were submitted to IVF for 18h by using 3.6 x 106 spermatozoa/ml prepared from fresh marmoset semen samples collected from fertile males and diluted in Toyoda, Yokoyama, Hoshi (TYH) medium. After IVF, cumulus cells were removed and 359 oocytes considered normal were placed in IVC for 4 days in Cleav medium (Origio). On day 4, cleaved embryos that had >4 cells were transferred to Blast medium (Origio) and were cultured for additional 8 days. The average rate of cleavage was 45.7% (164/359), of which 51.8% (85/164) developed to the blastocyst stage after 12 days of IVC. A proportion of the blastocysts (n=9) were fixed to evaluate the total number of cells, which had on average 186+41.7 cells. These preliminary findings indicate that LOPU, followed by IVM-IVF-IVC can be successfully applied for in vitro embryo production in marmosets, which may facilitate the creation of new animal models for research.

The Role of Janus Kinase 3 (JAK3) in Later Stages of Follicular Development (#66)

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Janus kinase 3 (JAK3) is a tyrosine kinase protein, functioning via the JAK/STAT pathway. JAK3 is differentially expressed in granulosa cells (GC) of bovine preovulatory follicles and is downregulated in ovulatory follicles by the endogenous luteinizing hormone (LH) and hCG injection. Following these observations, JAK3 signaling could modulate GC proliferation and follicular growth. Our objective was to analyze the effects of JAK3 inhibition/overexpression on the phosphorylation of target proteins including STAT proteins and newly identified JAK3 binding partners CDKN1B and MAPK8IP3. First, GC were obtained from small follicles (SF), dominant follicles (DF), ovulatory follicles (OF) for analyzing the in vivo regulation of JAK family members. Second, GC from slaughterhouse ovaries were cultured and treated with/without FSH and Janex-1, a JAK3 inhibitor along with the CRISPR/Cas9 knockout, then samples were analyzed by RT-qPCR and western blotting. RT-qPCR analyses using in vivo samples revealed differently regulation of JAK family members in different follicle stages and CL. In vitro experiments showed, Janex-1 treatment significantly decreased JAK3 mRNA expression in GC while FSH tended to increase JAK3 expression. Furthermore, steady-state mRNA expression for steroidogenic enzymes CYP19A1 and CYP11A1 and proliferation markers PCNA and CCND2 were upregulated in GC with FSH treatment and significantly decreased following Janex-1 treatment as compared to control. Western blot analysis showed that JAK3 overexpression increased STAT3 phosphorylation while Janex-1 treatment reduced STAT3 phosphorylation levels. However, FSH treatment partially rescued STAT3 phosphorylation in Janex-1-treated cells. These results suggest that JAK3 plays a key role in GC proliferation, follicular growth and steroidogenesis. This work was supported by a Discovery grant from the National Sciences and Engineering Research Council of Canada (RGPIN#04516 to KN). (Oral presentation of this project will be done by Amir Zareifard for 13th RQR Symposium)

3-D mitochondrial network organization in porcine cumulus cells (#70)

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Mitochondria are key organelles that regulate numerous cell processes such as ATP synthesis, steroid biosynthesis, and apoptosis. In recent years, several studies have demonstrated that mitochondrial function is widely regulated by its structure. Within the cell, the morphology of this organelle varied between individual structures and complex network structures. In this study, we were interested in the characterisation of mitochondrial network organisation of porcine cumulus cells in 2 and 3 dimensions. Active mitochondria were stained with TMRM (Tetramethylrhodamine, Methyl Ester, Perchlorate) and observed with confocal microscopy. Images showed different mitochondrial network in cumulus cells. They were classified according to different categories (fragmented, intermediated, elongated and highly elongated). Thus, the network phenotype was defined by quantifying each cell depending on organelles structure. The results showed that most cells were having elongated mitochondrial phenotype. Next, Scanning Electron Microscopy (SEM) analysis showed that different mitochondrial structures ranged from rounded, to small, intermediate, and elongated forms. In addition, 3-D visualisation by FIB-SEM microscopy showed a complex organisation of mitochondrial network in the form of different sizes of connected clusters as well as individual mitochondria. Thus, the biggest connected cluster measured about 2.43 µm³ in volume with 6 mitochondrial components. Our results support the presence of varied complexes mitochondrial morphologies in cumulus cells. Since mitochondrial dynamic is a key regulator of mitochondrial function, the characterisation of mitochondrial network organisation allows us to further study the regulation of mitochondrial responses in porcine cumulus cells.

Peroxiredoxin 6 peroxidase and Ca2+-independent phospholipase A2 activities are essential for male mouse fertility (#71)

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Human infertility is an important health problem and the underlying cause in half of cases can be traced to men. Oxidative stress (net increase of reactive oxygen species (ROS)) is present in ~30% of infertile men. Peroxiredoxin 6 (PRDX6) is the primary antioxidant enzyme that protects spermatozoa against oxidative stress. Infertile men's spermatozoa have low PRDX6 levels with impaired motility, high levels of lipid peroxidation and DNA damage. PRDX6 has a peroxidase and Ca2+-independent phospholipase A2 (iPLA2) activities that scavenge ROS and repair oxidized membranes respectively. Prdx6-/- male mice are infertile. Spermatozoa of PRDX6-/- mice have decreased litter sizes associated with higher levels of lipid peroxidation, DNA oxidation, and lower motility compared to wildtype controls. Tert-butyl hydroperoxide (t-BHP) treatment promotes an in vivo oxidative stress and impairs fertility in wildtype males and worsen the Prdx6-/- males' reproductive phenotype. Here, we determine whether the absence of PRDX6 peroxidase or iPLA2 activities impairs sperm motility, acquisition of fertilizing ability (capacitation), lipid peroxidation and nitro-tyrosine levels, and fertility in male mice.Spermatozoa from two-month-old male C57Bl6/J (wildtype), PRDX6-/-, C47S and D140 (peroxidase- and iPLA2 deficient) strains were incubated in PBS (2 hours; 37°C) to assess motility by CASA. Non- and capacitated spermatozoa (BWW medium+BSA/bicarbonate) were treated with progesterone to induce the acrosome reaction (AR). Percentages of AR were determined by Giemsa staining. Mice were treated with either saline or 60 mmol tBHP/100gbw for 9 days. Epididymal spermatozoa were collected 24 hours post-treatment. Control and treated mice were bred with age-matched wildtype females. Litter sizes and unsuccessful matings were recorded. 4-Lipoperoxidation (4-HNE) and nitro-tyrosine levels in spermatozoa were assessed by immunocytochemistry.Mutant strains' spermatozoa had high levels of 4-HNE and nitro-Y, impaired motility, capacitation and fertility than WT males. Treated wildtype mice had similar sperm 4HNE levels compared to untreated mutant males. Treated D140A, C47S and control PRDX6-/- mice had higher sperm nitro-tyrosine levels compared to control and t-BHP-treated wildtype mice. Treated PRDX6-/- mice had the highest sperm 4HNE and nitro-Y levels.In conclusion, PRDX6 peroxidase or iPLA2 activities are essential to prevent oxidative stress to ensure male fertility.Study supported by CIHR.

Regulation of lysophosphatidic acid signaling in human spermatozoa (#72)

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The lysophosphatidic acid (LPA) signaling is important to maintain germ cell viability during spermatogenesis. We previously demonstrated that the peroxiredoxin 6 (PRDX6) phospholipase A2 activity is important to maintain sperm viability, since its inhibition promotes sperm cell death. The PI3K/AKT pathway is involved in the maintenance of human sperm viability and it is modulated by PRDX6 iPLA2 activity. Exogenous addition of LPA to the incubation medium prevented the impairment of sperm viability due to inhibition of iPLA2. However, the presence of an active LPA signaling pathway and its regulation by kinases in mature spermatozoa is unknown. We hypothesize that the LPA signaling is active in spermatozoa and regulated by kinases. Our objectives are: 1) To determine the presence of LPA receptors (LPAR) in human spermatozoa and whether LPAR signaling activates the PI3K/AKT pathway; and 2) To study the regulation of the LPA signaling by kinases. Human spermatozoa were fractionated in cytosolic, Triton-soluble and -insoluble fractions to determine the presence of LPAR1 and LPAR6 and location of phospho-PI3K and phospho-AKT substrates in human spermatozoa by immunoblotting using specific antibodies. Spermatozoa were incubated for 3.5h at 37°C with or without Ki16425 (LPAR1-3 inhibitor), or with H89, chelerythrine, PD98059, PP2, and Tyrphostin A47 (PKA, PKC, MEK, non-receptor (NR-PTK) and receptor type tyrosine kinases (R-PTK), respectively) to determine phosphorylation of PI3K and AKT substrates.LPAR1 and LPAR6 were localized on the plasma membrane and the LPAR1 also found in the Triton-insoluble fraction of human spermatozoa. Phospho-PI3K and phospho-AKT substrates were found in cytosolic and Triton-insoluble fractions. Interestingly, most of the phospho-AKT substrates were found in the Triton-insoluble fraction. Phospho-PI3K and Phospho-AKT substrates were inhibited by Ki16425, chelerythrine and Tyrphostin A47.In conclusion, these results suggest the presence of an active LPA pathway that is regulated by PKC and receptor type-PTK, that activates the PI3K/AKT pathway is to prevent apoptotic-like changes and maintain sperm viability. These studies will help to decipher causes of sperm dysfunction associated with male infertility. Study supported by CIHR

ZEB1 inhibits Lhb transcription by blocking the stimulatory actions of GnRH and EGR1 (#73)

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Luteinizing hormone (LH), a pituitary heterodimeric glycoprotein, regulates gonadal function in both males and females. LH is composed of a common α-subunit and a hormone-specific LHβ-subunit (encoded by the Lhb gene). Hypothalamic gonadotropin-releasing hormone (GnRH) stimulates Lhb expression by inducing the expression of early growth response 1 (EGR1). EGR1 then partners with other transcription factors to drive Lhb transcription through cis-elements in the proximal promoter. Recently, it was reported that female mice lacking miRNA-200b and -429 exhibit a selective LH deficiency, leading to anovulation and infertility. This deficiency was attributed to the over-expression of zinc finger E-box binding homeobox 1 (ZEB1), a target of miR-200 family members. Consistent with this idea, siRNA-mediated knockdown of endogenous ZEB1 increased Lhb mRNA levels in gonadotrope-like LβT2 cells. While these data suggested that ZEB1 represses Lhb expression, its underlying mechanism of action was not elucidated. Here, we aimed to determine whether ZEB1 represses Lhb by antagonizing the stimulatory effects of GnRH and/or EGR1. First, we demonstrated that ZEB1 over-expression blocked GnRH-stimulated murine Lhb promoter-reporter activity in LβT2 cells. The GnRH effects on Lhb transcription can be recapitulated by EGR1 over-expression. EGR1 induction of murine Lhb promoter-reporter activity was similarly blocked by ZEB1 over-expression in both homologous (LβT2) and heterologous (HEK293) cells. ZEB1 generally binds to E-box cis-elements in target gene promoters. Three E-boxes in the murine Lhb promoter were previously shown to bind ZEB1 in gel shift assays. However, in our hands, ZEB1-mediated repression of murine Lhb promoter-reporters was maintained following mutagenesis or deletion of these E-boxes. ZEB1 and EGR1 physically interacted in co-immunoprecipitation assays, suggesting that ZEB1 may mediate its actions through protein-protein interaction rather than through DNA binding. However, ZEB1 over-expression did not repress GnRH- or EGR1-stimulated human LHB promoter-reporter activity. We therefore hypothesize that ZEB1 regulates murine Lhb transcription by binding to species specific cis-regulatory elements that have not yet been identified. We are currently comparing the human and murine promoters to home in on the critical sequences. Collectively, the available data suggest that ZEB1 inhibits LH production in mice through inhibition of GnRH/EGR1-induced Lhb subunit transcription.

The role of microRNA in the regulation of gap junction intercellular communication proteins (#74)

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Connexins (Cxs) are indispensable to mammary gland development through mediation of gap junction intercellular communication (GJIC) within the luminal and myoepithelial layers of the mammary gland epithelium. The stage-specific expression of Cxs is well described; however, mechanisms of regulation of Cxs, especially by miRNA, remains poorly characterized. miRNA are short, non-coding RNA that post-transcriptionally downregulate gene expression. This project aims to determine the role of miRNA in regulating Cxs stage-specific expression. We performed miRNA extraction and sequencing at 4 stages of mammary gland development, and validated our data using RTq-PCR. Our study identified 1009 miRNAs, 374 of which were shown to vary in copy amounts across groups. Further, the miRNA were categorized in silico into 6 clusters, shedding light on expression patterns observed throughout development. Database searching highlighted 33 miRNAs as potential candidates for Cxs regulation based on levels of miRNA and Cxs mRNA, and on an in silico analysis of their binding sequences. Future studies will aim to confirm their role in regulating Cxs expression. Interestingly, both miRNA and Cxs are dysregulated during breast cancer, favouring the tumour microenvironment and promoting its progression and metastasis. The project will thus add important insights on mammary gland development, but can also act as a prelude to further studies on one of the most serious diseases affecting women worldwide.

Impact of the inhibition of the transcription factor FOXO3a during Toxoplasma gondii infection (#75)

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The intracellular protozoan parasite Toxoplasma gondii (T. gondii) is the causative agent of toxoplasmosis, a serious public health concern worldwide. It is well-documented that T. gondii modulates the activity of several kinases and transcription factors to alter gene expression and subvert host cell functions. The transcription factor Forkhead box O3a (FOXO3a) has been associated with either protective or harmful host cell responses to viral and bacterial pathogens. However, the role of FOXO3a during T. gondii infection has not been investigated. FOXO3a controls a number of cellular processes that are dysregulated in T. gondii-infected cells. Interestingly, FOXO3a transcriptional activity is inhibited by AKT, a kinase that is phosphorylated by T. gondii. Hence, we postulate that AKT-dependent repression of FOXO3 affects down-stream transcriptional programs that contribute to host cell dysfunction and promote T. gondii survival. To test this hypothesis, we initially infected human foreskin fibroblasts (HFFs) with the RH (type I) T. gondii strain and monitored changes in the phosphorylation status of FOXO3a. Western blot experiments revealed that phosphorylation of FOXO3a at AKT-sensitive residues T32 and S253 (i.e. nuclear export signal) increases upon infection. As expected, treatment with the AKT inhibitor MK-2206 prevented AKT and FOXO3a phosphorylation by T. gondii. Consistent with this, immunofluorescence analyses of HFFs treated with MK-2206 confirmed that nuclear export of FOXO3a during T. gondii infection is AKT-dependent. In support of the notion that the transcriptional activity of FOXO3a is compromised upon infection, RT-qPCR and western blot data showed that the expression of FOXO3a transcriptional target genes involved in cell cycle, autophagy and redox responses is down-regulated in T. gondii-infected HFFs. Ongoing experiments in our laboratory that combine multi-omics approaches (i.e. RNA-seq, ChIP-seq) and forward/reverse genetics (i.e. FOXO3a-deficient and overexpressing cells) will provide further insight into the functional consequences of dysregulated FOXO3a transcriptional activity during T. gondii infection.

ATF3 regulates FSH synthesis in vitro but not in vivo (#78)

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Follicle-stimulating hormone (FSH), a dimeric glycoprotein produced by pituitary gonadotrope cells, promotes spermatogenesis in males and the growth of ovarian follicles in females. Hypothalamic gonadotropin-releasing hormone (GnRH) stimulates the transcription of the FSHβ subunit gene (Fshb), though the underlying mechanisms are poorly understood. To better understand how GnRH regulates FSH expression, we injected GnRH-deficient (hpg) mice once daily with GnRH, which stimulates FSH but not LH synthesis. After 10 days of treatment, we analyzed pituitary gene expression using bulk RNA-sequencing. Fshb, the GnRH receptor (Gnrhr), and activating transcription factor 3 (Atf3) were among the mostly highly upregulated genes. GnRH also stimulated Atf3 expression in the murine gonadotrope-like cell line, LβT2. ATF3 functions as a heterodimer with members of the ATF or the AP-1 (Fos and Jun) family to regulate gene transcription. To determine whether ATF3 can regulate Fshb transcription, we performed promoter-reporter assays in heterologous (HEK293) and homologous (LβT2) cells. Transient over-expression of ATF3 with ATF4 or c-Jun stimulated murine Fshb but not Lhb (LHβ subunit) promoter-reporter activity in both cell models. ATF3/ATF4 also potentiated the stimulatory effect of a constitutively active activin type I receptor (ALK4-T206D) on endogenous Fshb expression in LβT2 cells. We next generated gonadotrope-specific Atf3 knockout mice (cKO) to assess the protein's role in FSH production in vivo. FSH levels were equivalent between cKO and control littermates. LH levels were significantly reduced in cKO females on estrus morning. Nevertheless, cKO mice were fertile and did not show impairments in ovulation or sperm production. Pituitary expression of gonadotrope-specific genes (Fshb, Lhb, Cga, and Gnrhr) were also equivalent between genotypes. Collectively, the data indicate that ATF3 can selectively stimulate Fshb transcription in vitro but is not required for FSH production in vivo. It is possible that other ATF or AP-1 proteins compensate for ATF3's absence in cKO mice.

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