4th Mammalian Embryo Genomics meeting

October 9 to 11, 2013, Hilton Quebec City

Hosted by the EmbryoGENE Network / Université Laval

EmbryoGENE
Genomics Epigenomics
Nutrition Environment

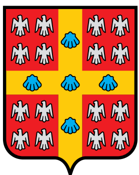
4th Mammalian Embryo Genomics meeting
October 9 to 11, 2013, Hilton Quebec City

Hosted by the EmbryoGENE Network / Université Laval

Book of Abstracts

Organizing Committee:

Marc-André Sirard
Claude Robert
Michael Dyck
Julie Nieminen


UNIVERSITÉ LAVAL

NSERC CRSNG

Table of contents

Preface 1

Scientific Program 2-6

- Wednesday October 9 2-3
- Thursday October 10 4-5
- Friday October 11 6

List of Abstracts 7-10

Invited Speaker Abstracts 11-25

Short Communications Abstracts 26-34

Poster Abstracts 35-73

List of Participants 74-77

Invited Speaker Bios 78-82

Embry

Genomics Epigenomics
Nutrition Environment

4th Mammalian Embryo Genomics meeting

Welcome to all,

I am very pleased to welcome you to the 4th Mammalian Embryo Genomics meeting hosted by the EmbryoGENE Network and Université Laval. The first edition of this international conference was held in Quebec City in 2002 and aimed at bringing together internationally recognised scientists in the genomics field, in an attempt to coordinate activities in the area of embryo genomics and create collaborations in this field of research. A little more than a decade and two other Mammalian Embryo Genomics meetings later (Paris, France and Bonn, Germany), we can claim: mission accomplished! Although researchers still face many challenges, the number of international collaborations is ever increasing and many groups, consortiums and networks were born from discussion held at these meetings.

These collaborations have no doubt contributed to the exciting technological advances and findings, which will be discussed this week. A special focus will be laid to large-scale genomics and epigenomics analyses, especially in light of the conclusion of the EmbryoGENE Network and its extensive database of transcriptomics and epigenetics data. Recent progress and questions in fields such as oocyte competence, early embryo development, maternal environment, assisted reproductive technologies, transcriptomics and epigenetics will also be addressed in the mammalian and domestic animal contexts.

We look forward to an exciting and interactive meeting.

Best wishes

Marc-André Sirard
Chair of the 4th Mammalian Embryo Genomics meeting
EmbryoGENE Network Co-Director

Wednesday October 9

7:30 – 8: 30	Breakfast and registration	2nd floor foyer
8:30 – 8:45	Welcoming address	Marc-André Sirard

Session 1 Oocyte Competence
Chair: Claude Robert

8:45 – 9:30	Changes in Large-Scale Chromatin Structure and Function During Oogenesis: A journey in Company of Follicular Cells	Alberto Luciano
9:30 – 10:15	Super Moms and Super Follicles: Granulosa Cell Function During Aging	Jaswant Singh
10:15 – 10:45	**Break**	**2nd floor foyer**

Session 2 Oocyte Competence and Embryo Development (short talks)
Chair: Kevin Sinclair

10:45 – 11:00	Relative Abundance of Extra-Cellular miRNAs in Bovine Follicular Fluid: Implication for Cell-Cell Communication During Oocyte Growth	Dawit Tesfaye
11:00 – 11:15	Global Transcriptome Analysis of Bovine Blastocysts Developed Under Alternative In Vivo/Vitro Culture Conditions During Specific Stages of Development	Ahmed Gad
11:15 – 11:30	ATOL and EOL Ontologies, Steps Towards Embryonic Phenotypes Shared Worldwide?	Isabelle Hue
11:30 – 11:45	Detection of Genes Associated with Developmental Competence of Bovine Oocytes, Quantification of Transcript Levels Before and After Oocyte Maturation	Jiri Kanka
11:45 – 13:30	**Lunch**	**Sainte-Foy (1st floor)**

All conferences will be held in the Beauport/Beaumont/Bélair Room on the 2nd floor of Hilton Quebec

Wednesday October 9

Session 3 Chair: Michael Dyck	Embryo Development	
13:30 – 14:15	Fine-Mapping of Genome Activation in the Bovine Embryo	Eckhard Wolf
14:15 – 15:00	Genomic Profiling to Improve Pig Embryogenesis	Randall Prather
15:00 – 15:45	Developmental Programming in the Peri-Implantation Embryo	Peter J. Hansen

15:45 – 16:15	Break	2nd floor foyer

Poster Session	
16:00 – 17:30	Saint-Louis (1st floor)

Cocktail	
17:00 – 19:00	Finger food and cash bar – Saint-Louis (1st floor)

All conferences will be held in the Beauport/Beaumont/Bélair Room on the 2nd floor of Hilton Quebec

Thursday October 10

7:30 – 8: 30	Breakfast and registration	2nd floor foyer
Session 4 **Chair: George Foxcroft**	**Maternal Environment**	
8:30 – 9:15	Towards Building the Cow Folliculome	Marc-André Sirard
9:15 – 10:00	Elevated NEFA Concentrations and Reduced Oocyte Quality: An Intersting Pathway to Subfertility	Jo Leroy
10:00 – 10:30	**Break**	**2nd floor foyer**
Session 5 **Chair: Poul Hyttel**	**Culture Conditions / ART**	
10:30 –11:15	Embryo Transcriptome Response to Environmental Factors: Implication for its Survival Under Suboptimal conditions	Karl Schellander
11:15 – 12:00	Reproductive Technologies & the Porcine Embryonic Transcriptome	Michael Dyck
12:00 – 13:30	**Lunch**	**Sainte-Foy (1st floor)**

All conferences will be held in the Beauport/Beaumont/Bélair Room on the 2nd floor of Hilton Quebec

Thursday October 10

Session 6 Maternal Environment, Culture conditions, ART, Epigenomics (short talks) Chair: François Richard		
13:30 – 13:45	FECUND EU – Project: Optimisation of Early reproductive Success in Dairy Cattle Through the Definition of New Trait and Improved Reproductive Biotechnology	John Williams
13:45 – 14:00	Influence of Metabolic Activity on Gene Expression and Phenotype of Holstein and Jersey Breeds Embryos	Luis Manuel Baldoceda Baldeon
14:00 – 14:15	Hyperglycaemia: A New Player in Oocyte Epigenetics	Hannah Brown
14:15 – 14:30	Optimization of DNA counterstaining for Methylation and Hydroxymethylation Immunostaining in Bovine Zygotes	Sonia Heras
14:30 – 15:00	Break	2nd floor foyer

Session 7 Chair: Marc-André Sirard	Ethics	
15:00 – 15:45	Of Wicked Ethics and Wrong Impressions: the Regulation of Animal Biotechnology in Canada	Lyne Létourneau
15:45 – 16:30	Mammalian Embryo Genomics – What Are the Ethical Issues?	Peter Sandøe

Banquet	
19:00 – 23:00	Les Plaines (23rd floor)

All conferences will be held in the Beauport/Beaumont/Bélair Room on the 2nd floor of Hilton Quebec

Embry

Genomics Epigenomics
Nutrition Environment

Scientific Program

4th Mammalian Embryo Genomics meeting

Friday October 11

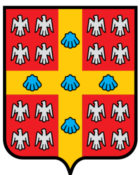
8:00 – 8: 30	Breakfast and registration	2nd floor foyer
Session 8	**Workshop**	
8:00 – 9:30	New Technologies, SOPs, Best Laboratory Practices, Bioinformatics	Marc-André Sirard
9:30 – 9:45	**Break**	**2nd floor foyer**
Session 9 **Chair: Marc-André Sirard**	**Epigenomics**	
9:45 – 10:30	Integrating the DNA Methylome and Transcriptomics to Better Define the Long Term Impacts of Assisted Reproductive Technologies	Claude Robert
10:30 – 11:15	Pluripotency: In Embryo and In Vitro	Véronique Duranthon
11:15 – 12:00	It Starts With the Egg: Epigenetic Effects of the Oocyte Maturation Environment	Trudee Fair

End of meeting

All conferences will be held in the Beauport/Beaumont/Bélair Room on the 2nd floor of Hilton Quebec

4th Mammalian Embryo Genomics meeting

List of Abstracts



Poster #	Name of Presenter	Title	Abstract page
1	Fazl A. Ashkar	Differential Gene Expression Profile of In Vitro Produced Bovine Blastocyst in the Presence and Absence of Thyroid Hormones	36
2	Luis Baldoceda	Vitamin K2 Supplementation Rescues Impaired Mitochondrial Activity to Improve Blastocyst Rate in In Vitro Cultured Bovine Embryos.	37
3	Luis Baldoceda	Influence of Cattle Breed on Gene Expression and Phenotype of Holstein and Jersey Embryos	32, 38
4	Hannah Brown	Hyperglycaemia: A New Player in Oocyte Epigenetics	33, 39
5	Julieta Caballero	Profiling of Long Non-Coding RNAs During Bovine Embryonic Development Reveals Their Enrichment in Pre-Embryonic Genome Activation Stages	40
6	Isabelle Dufort	Discovery, Identification and Sequence Analysis of RNAs Selected for Very Short or Long Poly A Tail in Immature Bovine Oocytes	41
7	Eric Fournier	Streamlining Microarray Analysis with the EDMA Analysis Pipeline	42
8	Ahmed Gad	Global Transcriptome Analysis of Bovine Blastocysts Developed Under Alternative Vivo/Vitro Culture Conditions During Specific Stages of Development	28, 43
9	Ahmed Gad	Laser Irradiation Affect Gene Expression and Maturation Rate of Buffalo Oocytes	44
10	Karen Goossens	The Effect of the Insulin Concentration on Developmental Competence and Gene Expression in Bovine Embryos Produced In Vitro	45
11	Annie Girard	Transcriptome Analysis of Bovine Granulosa Cells of Preovulatory Follicles at 30, 60, 90 and 120 Days Postpartum	46
12	Annie Girard	Transcriptomic Differences Between Growth Phases in Cattle Dominant Follicles	47
13	Jason Grant	An Update on ELMA – The EmbryoGENE Data Storage and Analysis Platform	48
14	Nicolas Guillemin	Cow Fertility Markers Identified by Genetical Genomics	49
15	Sonia Heras	Optimization of DNA Counterstaining for Methylation and Hydroxymethylation Immunostaining in Bovine Zygotes.	34, 50
16	Sayed Morteza Hosseini	Can we Manipulate the Time Window of Pluripotency Gene Expression?	51

Poster #	Name of Presenter	Title	Abstract page
17	Sayed Morteza Hosseini	Upregulation of Myc Regulatory Network in the Transcriptome of Bovine Cloned Blastocysts	52
18	Isabelle Hue	ATOL and EOL Ontologies, Steps Towards Embryonic Phenotypes Shared Worldwide?	29, 53
19	Jiri Kanka	Detection of Genes Associated with Developmental Competence of Bovine Oocytes, Quantification of Transcript Levels Before and After Oocyte Maturation	30, 54
20	Daulat Raheem Khan	FSH Signalling: Transactivation of Epidermal Growth Factor (EGF) Pathway Mediates Acquisition of Oocyte Competence in Bovine Cumulus Cells	55
21	Kana Komatsu	Expression of Key Genes in a Trophoblastic Cell Line (Jag-1) Isolated From Porcine Day 14 Embryos	56
22	Rémi Labrecque	Bovine Oocyte Transcriptome in Relation to Chromatin Configuration of the Germinal Vesicle	57
23	Wenwen Li	Reference Gene Selection for Normalization of qPCR Data in Sexed Bovine Implantation Embryos Under Low and High Oxygen Tension	58
24	Angus Macaulay	Cumulus Cells Actively Transfer RNA to the Oocyte During Maturation	59
25	Lucie Nemcova	Gene Expression Profiles of Pig Cumulus-Oocyte Complexes Cultured In Vitro with Follicle Stimulating Hormone or Epidermal Growth Factor-Like Peptides	60
26	Anne-Laure Nivet	The Use of Granulosa Cells in Culture to Investigate Gene Pathways Discovered by Transcriptomic Analysis in Bovine	61
27	Gina Oliver	Methyl-Seq Analysis of d30 Pig Placentae Derived from Sows Feed Restricted During Lactation	62
28	Ernesto Orozco-Lucero	Rapidly Cleaving Bovine Two-Cell Embryos Have Better Developmental Potential and a Distinctive mRNA Pattern	63
29	Florence Pagé-Larivière	Spatiotemporal Expression of DNA Demethylation Enzymes and Histone Demethylases in Bovine Embryo	64
30	Sara Scantland	Cumulus Cell Transfer, Poly(A) mRNA Degradation and Phosphocreatine as Drivers of ATP Synthesis During Oocyte Maturation	65
31	Karl Schellander	Activity of NRF2 Mediated Oxidative Stress Response and Embryo Survival Under Oxidative Stress Condition	66
32	Habib A. Shojaei Saadi	EDMA: A Platform for Parallel Analysis of Bovine Embryo Genome-wide Methylome and Transcriptome	67

Poster #	Name of Presenter	Title	Abstract page
33	Marc-André Sirard	EmbryoGENE: A Transcriptomic Approach to Understanding Embryonic Stress During In Vitro Culture	68
34	Dawit Tesfaye	Relative Abundance of Extra-Cellular miRNAs in Bovine Follicular Fluid: Implication for Cell-Cell Communication During Oocyte Growth	27, 69
35	Dawit Tesfaye	Bovine Ovarian Hyperstimulation Induced Changes of Circulating Extra-Cellular miRNAs in Follicular Fluid and Blood Plasma	70
36	Svetlana Uzbekova	Global Transcriptome and Proteome Analysis of Bovine Cumulus Cells in Relation to Maturation and Developmental Competence of Enclosed Oocyte	71
37	John Williams	FECUND EU-project: Optimisation of Early Reproductive Success in Dairy Cattle Through the Definition of New Trait and Improved Reproductive Biotechnology	31, 72
38	Chi ZHOU	Gene Expression Profiling of In Vivo-Derived Female and Male Day 11 Porcine Embryos	73

Invited Speakers

Abstracts

Changes in Large-Scale Chromatin Structure and Function During Oogenesis: a Journey in Company with Follicular Cells

Alberto M. Luciano, Federica Franciosi, Cecilia Dieci and Valentina Lodde

Reproductive and Developmental Biology Laboratory, Department of Health, Animal Science and Food Safety, University of Milan, Milan, Italy

The mammalian oocyte nucleus or germinal vesicle (GV) exhibits characteristic chromatin configurations, which are subject to dynamic modifications through oogenesis. Aim of this review is to highlight how changes in chromatin configurations are related to both functional and structural modifications occurring in the oocyte nuclear and cytoplasmic compartments. During the long phase of meiotic arrest at the diplotene stage, the chromatin enclosed within the GV is subjected to several levels of regulation. Morphologically, the chromosomes lose their individuality and form a loose chromatin mass. The decondensed configuration of chromatin then undergoes profound rearrangements during the final stages of oocyte growth that are tightly associated with the acquisition of meiotic and developmental competence. Functionally, the discrete stages of chromatin condensation are characterized by different level of transcriptional activity, DNA methylation and covalent histone modifications. Interestingly, the program of chromatin rearrangement is not completely intrinsic to the oocyte, but follicular cells exert their regulatory actions through gap junction mediated communications and intracellular messenger dependent mechanism(s). With this in mind and since oocyte growth mostly relies on the bidirectional interaction with the follicular cells, a connection between cumulus cells gene expression profile and oocyte developmental competence, according to chromatin configuration is proposed. This analysis can help in identifying candidate genes involved in the process of oocyte developmental competence acquisition and in providing non-invasive biomarkers of oocyte health status that can have important implications in treating human infertility as well as managing breeding schemes in domestic mammals.

Supported by CIG-Marie Curie Actions FP7-People (Pro-Ovum) and by NSERC Strategic Network EmbryoGENE.

Super Moms and Super Follicles: Granulosa Cell Function During Aging

Jaswant Singh[1], Fernanda Dias[1], Muhammad Irfan-ur-Rehman Khan[1], Gregg P. Adams[1], Marc-André Sirard[2]

[1]Department of Veterinary Biomedical Science, University of Saskatchewan, Saskatoon SK Canada S7N 5B4
[2]Centre de Recherche en Biologie de la Reproduction, INAF, Université Laval, Québec, Canada G1V 0A6

The aim of this review is to present an overview of a series of studies in which the effects of follicular aging and maternal aging on the transcriptome of bovine granulosa cells and oocyte competence were examined. By design, we used a superstimulated bovine model and we discovered first that granulosa cells from FSH-stimulated follicles do not respond to LH properly when compared to untreated bovine dominant follicles. Four-day superstimulation also disturbed genes related to angiogenesis and activated oxidative stress response genes. Extending the duration of FSH stimulation from 4 to 7 days resulted in a 2.5-fold increase in the number of transferable embryos per cow (after in vitro culture). Compared to short FSH treatment, more of the up-regulated granulosa cell genes in cows given extended FSH treatment were markers of post-LH surge, and the cellular pathways were predictive of proximity to ovulation. Conversely, extended FSH starvation (>96hr) led to a greater proportion of lower quality oocytes, lower cleavage rates, and lower in vitro embryonic development. Transcriptomic comparison documented that granulosa cells that underwent FSH starvation did not respond properly to the LH surge. In a subsequent set of experiments, granulosa cell gene expression was compared between aged cows (>15 years) cows and their 4- to 9-year-old daughters. Maternal aging was associated with elevated circulating FSH concentrations, a reduced response to superstimulatory treatment, and markedly decreased early embryonic development. Cellular pathway analysis based on differences in gene expression in the granulosa cells from growing dominant follicles of old vs. young cows has provided rationale for the hypothesis that age-related depression in oocyte competence is the result of reduced intercellular communication, decreased control of cell cycle check points, higher levels of oxidative stress, and reduced ability to process lipids and synthesize steroids. Furthermore, differences in gene expression suggest that the granulosa from old cows is less responsive to gonadotropin-induced changes in the cytoskeleton and extracellular matrix. In conclusion, gene expression analyses during maternal and follicular aging has allowed us to predict the impact of granulosa cell health/dysfunction on the developmental ability of the oocyte during first 7 days of embryonic life.

Fine-Mapping of Genome Activation in the Bovine Embryo

Alexander Graf[1], Stefan Krebs[1], Valeri Zakhartchenko[2], Helmut Blum[1], Eckhard Wolf[1,2].

[1]Laboratory for Functional Genome Analysis, Gene Center, Ludwig-Maximilians-Universität, Munich, Germany;
[2]Chair for Molecular Animal Breeding and Biotechnology, Ludwig-Maximilians-Universität, Munich, Germany.

In order to provide a full view of the transcriptome changes during the earliest stages of bovine development we sequenced the total RNA content of bovine oocytes, 4-cell, 8-cell and 16-cell embryos and the inner cell mass and trophoblast envelope of expanded blastocysts on the Illumina Genome Analyzer IIx. For each experiment pools of in vitro matured oocytes from the German Simmental breed were fertilized using sperm of a single bull and 10 embryos per developmental stage were collected to generate total RNA pools used for sequencing. Synthesis of cDNA was initiated directly in the cell lysate in order to avoid any losses during RNA preparation and was random primed in order to capture all RNA species. Amplified cDNA and sequencing libraries were prepared using kits from Nugen (Ovation RNA-Seq v2, Nugen, San Carlos, CA). Three biological replicates were generated with sperm from the same bull of the genetically distant Brahman breed.

The genome activation was detected by three complementary approaches. First, the emergence of new transcripts after fertilization was used. Further, the cross-breeding design allowed tracking of single sequencing reads back to the maternal or paternal genome, thus allowing the identification of genome activation by the appearance of breed-specific paternal SNPs after fertilization. Finally, for transcripts stored in the oocyte, the onset of replacement synthesis in the zygotic genome was analyzed by the appearance of unspliced transcripts (intronic reads) occurring during active transcription. These three approaches identified 269, 1150 and 8011 genes which were activated up to the blastocyst stage, respectively.

The numbers of genes which were activated at the 4-cell, 8-cell, and 16-cell stage corresponded to 337, 4893, and 1604. In the inner cell mass and trophectoderm of blastocysts 1147 and 432 de novo transcribed genes were found. The length of de novo activated genes increased significantly after the 8-cell stage.

The analysis confirmed previous findings that the minor genome activation occurs around the 4-cell and the major activation around the 8-cell stage. In summary, our dataset provides a comprehensive description bovine embryonic genome activation.

Genomic Profiling to Improve Embryogenesis in the Pig

Randall S. Prather, Kiho Lee, Lee D. Spate, Bethany K. Redel, Kristin M. Whitworth, Jeffrey J. Whyte

Division of Animal Science, University of Missouri, Columbia, MO U.S.A.

Over the past decade the technology to characterize transcription during embryogenesis has progressed from estimating a single transcript to a reliable description of the entire transcriptome. Northern blots were followed by sequencing ESTs, quantitative real time PCR, cDNA arrays, custom oligo arrays, and more recently, deep sequencing. The amount of information that can now be generated is overwhelming. The bigger challenge is how to glean information from these vast data sets that can be used to understand development and to improve methods for creating and culturing embryos in vitro, and for reducing reproductive loss. The use of ESTs permitted the identification of SPP1 as an oviductal component that could reduce polyspermy. Microarrays identified LDL and NMDA as components to replace BSA in embryo culture media. Deep sequencing implicated arginine, glycine, CSF2, and folate as components that should be added to our current culture system, and identified a characteristic of embryo metabolism that is similar to cancer and stem cells (similar to what Otto Warburg described in the 1920s). Mining of an oocyte data set has identified additional pathways that can be exploited to improve oocyte maturation and early embryo development. Transcriptional profiling has recently been used to identify genes expressed in donor cells that are associated with high rates of development after somatic cell nuclear transfer. These characterizations will be useful for identifying, or creating conditions for donor cells that will be more likely to result in normal development of cloned embryos. The low hanging fruit has been harvested, and now more sophisticated methods are being employed to advance our understanding of embryogenesis.

Developmental Programming in the Peri-Implantation Embryo

Peter J. Hansen

Department of Animal Sciences, University of Florida.

Events in the preimplantation period can have long-term consequences that affect embryo competence to establish and maintain pregnancy and which can extend into fetal and postnatal life. Colony stimulating factor 2, also termed granulocyte-macrophage colony stimulating factor, is a maternally-derived cytokine produced by the oviduct and endometrium that can act on the preimplantation bovine embryo during the morula to blastocyst transition to enhance competence of the embryo to establish and maintain pregnancy after transfer into females. Actions of CSF2 on the embryo include changes in gene expression, inhibition of apoptosis responsiveness, and an increase in numbers of cells in the inner cell mass (ICM). To evaluate consequences of CSF2 action on development at later stages of pregnancy, it was tested whether treatment with CSF2 from Day 5-7 alters extra-embryonic membranes (EEM) at Day 15. In vitro produced embryos were treated with either 0 or 10 ng/ml bovine CSF2 from Day 5 to 7. Expanded blastocysts were transferred into synchronized recipients. On Day 15, conceptuses were recovered by flushing the uterus and gender determined by PCR (n=4 males and 4 females per treatment). There was a gender x treatment interaction for conceptus length ($P<0.003$) and IFNT in uterine flushing ($P<0.05$) (as determined by antiviral activity). CSF2 increased length and IFNT in males and decreased length and IFNT in females. Analysis of the transcriptome of a subset (n=2-4 per subgroup) of extra-embryonic membranes (EEM) using the Agilent EmbryoGENE bovine microarray also indicated that gender affected the response to CSF2. In particular, CSF2 caused differential regulation of 94 genes in males and 56 genes in females. Only 7 differentially-regulated genes were regulated in both genders and, when shared, regulation by CSF2 was always in the opposite direction. The EmbryoGENE DNA Methylation Array was used to assess CSF2 effects at 418,805 positions across the genome in a subset of EEM. CSF2 caused hypermethylation for 9,842 probes in males. Differential methylation was not uniformly distributed but rather there were regions of hyper- and hypomethylation that varied with gender. Results indicate at changes in developmental programming of the bovine embryo caused by CSF2 occur in a gender-specific manner. This result suggests a possible mechanism by which environmental effects on the female affect male embryos differently than female embryos. (Support: AFRI Competitive Grant no. 2011-67015-30688 from USDA NIFA, Southeast Milk Checkoff Program and EmbryoGENE).

Toward Building the Cow Folliculome

Marc-André Sirard[1], Anne-Laure Nivet[1], Gabriel Douville[1], Isabelle Gilbert[1], Vittorio Gollini[2], Guiseppe Stradiolo[2], Jaswant Singh[3], Fernanda Dias[3], Muhammad Irfan-ur-Rehman Khan[3], Christopher Price[4], Annie Gagnon[1], François Richard[1], Annie Girard[1], Jean-Paul Laforest[5]

[1]Centre de Recherche en Biologie de la Reproduction, INAF, Université Laval, Québec, Canada
[2]Department of Agricultural and Environmental Sciences, University of Udine, Udine, Italy
[3]Department of Veterinary Biomedical Science, University of Saskatchewan, Saskatoon SK Canada
4Centre de recherche en reproduction animale, Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada
[5] Département des Sciences Animales, Université Laval, Québec, Canada

One of the goals of the EmbryoGENE network was to gather information on the conditions leading to competent oocytes. Using a combination of transcriptomic analyses we are building the foundation of the folliculome, which will take the form of a virtual follicle with gene expression profiling data spanning small to ovulatory or atretic follicles. The different models currently being established not only provide information on the follicular conditions leading to good outcome but also all the intermediary steps, including evolution towards atresia. The physiology of very few species has been covered to the extent of our database, which is the only one for mono-ovulatory species. The first interesting observation extracted from our data is related to the plateau phase of follicular development, which is not a linear intermediate between growth and ovulation but rather an important modification step of tissue ontogenesis during which growth switches to differentiation or atresia. The markers of cell division, matrix rearrangement, mesenchymal differentiation, oxidation, steroidogenesis and ovulatory changes identified through our efforts confirm some of the known changes but several others are now hinting to a more complex picture of this dynamic tissue. In addition to biomarkers, we now have insight into the multiple pathways involved during the last few days before ovulation. Our new ability to validate these networks in vitro using primary granulosa cells culture also contributes to the construction of a follicular blueprint. The amazing list of gene responding to FSH alone is a good start but a complete meta-analysis is likely to provide the foundation of the bovine folliculome.

Reduced Oocyte and Embryo Quality in Elevated Non-Esterified Fatty Acid Concentrations: An Interesting Pathway to Subfertility?

Veerle Van Hoeck, Peter E.J. Bols and Jo L.M.R. Leroy

Gamete Research Centre, Department of Veterinary Sciences, University of Antwerp, Belgium

Elevated serum non-esterified fatty acids (NEFAs) concentrations, associated with negative energy balance, obesity and type II diabetes, alter the follicular and oviductal micro-environment. We hypothesized that elevated NEFA concentrations during oocyte maturation or during early embryo development affect development and physiology of zygotes formed.

In a first series of experiments, oocytes were matured in elevated NEFA conditions for 24h and routinely grown to blastocysts. Maturation under elevated NEFA resulted in significantly lower blastocyst cell number, increased apoptotic cell ratio and altered mRNA abundance of *DNMT3A*, *IGF2R* and *SLC2A1,* genes related to REDOX maintenance and fatty acid synthesis. Blastocysts displayed reduced oxygen, pyruvate and glucose consumption, up-regulated lactate consumption and higher amino acid metabolism. Inhibiting the fatty acid β-oxidation during maturation in elevated NEFA restored developmental competence. The mitochondrial membrane potential tended to be higher in zygotes derived from NEFA-exposed oocytes. Lower glutathione content and a reduced number of lipid droplets per cell were observed in stearic acid exposed oocytes and resultant morulae (respectively). Transcriptomic analyses in day 7.5 blastocysts revealed that major pathways affected are related to lipid and carbohydrate metabolism and cell death.

In a second series of experiments, routinely matured and fertilized zygotes were cultivated for 6.5 days in medium containing elevated NEFA concentrations. Stearic acid was the most toxic one while adding oleic acid buffered the negative effect on development. Elevated NEFA concentrations during culture influenced gene expression patterns in blastocysts through pathways mainly related to oxidative metabolism and ceramide formation.

These data indicate that exposure of maturing oocytes or early developing embryo to elevated NEFA concentrations has a significant negative impact on fertility through a reduced developmental capacity and through compromised early embryo physiology.

Embryo Transcriptome Response to Environmental Factors: Implication for its Survival Under Suboptimal Conditions

Karl Schellander, Dessie Salilew Wondim, Dawit Tesfaye

Institute of Animal Science, Dept. of Animal Breeding and Husbandry, University of Bonn, Germany

Development and survival of early stage bovine embryos is supported by maternal transcripts and proteins accumulated during the oocyte growth and maturation. However, after embryonic genome activation, the embryo decides its destiny by its own transcripts and protein activated and accumulated over time. Indeed, when embryos are exposed to suboptimal culture environment at any stage of developmentt, the embryo respond to the environmental insults either by inducing the expression of array of genes or by switching off the transcriptional activity of transcripts associated with stress factors. On this regard, using state-of-the-art nonsurgical endoscopic flushing and transfer of early stage bovine embryos, we have recently shown alteration in transcriptome profiles of the blastocysts depending on the stage and the duration of exposure to in vitro or in vivo culture environment. For instance, the gene expression of blastocysts derived from embryos that were cultured in in vivo until 4-cell stage and then developed in vitro until day 7 exhibited an elevated activity of NRF2 mediated oxidative stress response pathway. The altered expression of NRF2-mediated oxidative response pathway was found to be associated with alteration in the expression of genes involved in metabolism, excretion and translocation of lipid and activation of antioxidant proteins. Further analysis of the NRF2 including its inhibitor KEAP1 and downstream genes during early stages of bovine embryos exposed to high or low oxidative stress condition revealed differential activity of NRF2 pathway depending on the time of embryonic genome activation. Since the embryo depends on its maternal store of NRF2 and antioxidants transcripts, there was no difference in NRF2 activity between the embryos from high or low oxidative stress environment until 8-cell stage. After this stage of development survived embryos showed elevated activity of NRF2 and antioxidants when exposed to oxidative stress condition. This may suggest that the survival and developmental competence of embryos under suboptimal culture condition depends on its ability to activate transcripts required to tackle with the stress factors resulting in accumulation of reactive oxygen species (ROS), which negatively affect the mitochondrial activity and the physiology of the embryo. Therefore, the present review will focus on embryo´s response to unfavorable environmental factors during the course of their development in terms of transcript activity and the contribution of that response for the survival of the embryo.

Reproductive Technologies and the Porcine Embryonic Transcriptome

Michael K. Dyck, Chi Zhou, Stephen Tsoi, Jason Grant, Walter T. Dixon, George R. Foxcroft

Swine Reproduction and Development Program, Swine Research & Technology Centre, University of Alberta, Edmonton, Alberta, CANADA, T6G 2P5

The domestic pig is not only an economically important livestock species, but also an increasingly recognized biomedical animal model due to its physiological similarities with humans. As a result, there is strong interest in the factors that affect the efficient production of viable porcine embryos and offspring using either *in vivo* or *in vitro* production methods. The application of assisted reproductive technologies (ART) has the potential to greatly increase reproductive efficiency when applied in livestock. These technologies include, but are not limited to, artificial insemination (AI), fixed-time AI, embryo transfer, cryopreservation of sperm/oocytes/embryos, *in vitro* maturation/fertilization and somatic cell nuclear transfer (cloning). However, the application of ARTs has been found to be much less efficient in the pig than in many other mammalian species such as cattle. Until recently, the underlying causes of these inefficiencies have been difficult to study, but advances in molecular biology techniques for studying gene expression have resulted in the availability of a variety of options for gene expression profiling such as DNA microarrays and next generation sequencing technologies. Capitalizing on these technologies we have collaborated with the livestock and biotechnology industries to develop a porcine embryo specific gene expression microarray (EMPV1; Tsoi *et al.,* 2012 BMC Genomics 13:370). Using the EMPV1 microarray, the effects of various ARTs on the porcine embryonic transcriptome has been determined and their impact on the related biological pathways and functions have been evaluated. The implications of these results on the efficiency of ARTs in swine will be discussed, as well as the potential consequences of applying these technologies on the developing embryo and any resulting offspring.

Research supported by the Natural Sciences and Engineering Research Council, EmbryoGENE Strategic Research Network.

Of Wicked Ethics and Wrong Impressions: the Regulation of Animal Biotechnology in Canada

Lyne Létourneau & Olga Carolina Cardenas Gomez

Centre de Recherche en Biologie de la Reproduction, Département des sciences animales, Université Laval, Québec, Canada

For legal scholars working within an interdisciplinary perspective, animal biotechnology provides an interesting case in point about policymaking. Mixing a share of scientific uncertainty, value conflicts, multiple framings, corporate power and (public) mistrust, it has all the makings of a great thriller... with the exception of timing. In Canada, there is a strong sentiment that policymakers dragged their feet. For instance, we have frequently heard scientists complaining that "Canada still has no legislation in place to deal with animal biotechnology and future applications from animal genomics and epigenomics." In actual fact, Canada is known to be a "late adopter," meaning that it is generally considered to be slow in adopting new policies while other countries are doing so more readily. Yet, there is more to the story. On the one hand, Canada's perceived lack of promptness is inextricably linked with the "ethics" surrounding the whole issue of agriculture biotechnology. Not only are ethical issues particularly challenging to regulate as they amount to "wicked problems" of public policy requiring policymakers to do things differently (which the latter are not always equipped, or disposed to do), but these issues also never quite completely fade away. On the other hand, there is an enduring misperception – almost misapprehension - as regards the Canadian regulatory system. Indeed, the complexity of the system supports the belief that nothing has been done, whereas all the workings are set into place. In this presentation, we will discuss both of these elements with a view to setting the record straight and showing that, ultimately, it all boils down to expectations.

Mammalian Embryo Genomics – What Are the Ethical Issues?

Peter Sandøe, Poul Hyttel & Jesper Lassen

University of Copenhagen

Novel research on genomics and epigenomics (mechanisms regulating the expression of the genome) relating to farm animals is currently generating fundamental insights into biological mechanisms. However, when considerable resources are allocated to this research, it is likely that practical applications will emerge, and will be utilized by breeding companies and other commercial stakeholders. Such future practical applications may give rise to ethical issues; and these issues should be discussed in due time, before the research is transformed into applied technology.

In this presentation we will discuss ethical issues related to three areas where research in genomics and epigenomics is likely to be applied: 1) Increasing the efficiency of farm animal production; 2) Translation and refinement of assisted reproductive technologies (ARTs) into the human field; and 3) Use of animals as models for the study of human diseases. In the first area there are already ongoing discussions of animal welfare and the ethics of food production. As can be seen in existing debates about genetic modification and cloning of food animals, a key question here will be whether the results feed into an accepted technology, such as conventional breeding, or link up with the mentioned controversial technologies. In the second area there are concerns about potential risks to human health, but at the same time a wide acceptance of initiatives aimed at helping people dealing with reproductive problems. Finally, there is currently relatively wide acceptance of using animals as disease models, provided that a reasonable balance between welfare costs to animals and potential benefits to humans is maintained

Integrating the DNA Methylome and Transcriptomics to Better Define the Long Term Impacts of Assisted Reproductive Technologies

Claude Robert

Centre de Recherche en Biologie de la Reproduction, INAF, Université Laval, Québec, Canada

Nowadays, application of assisted reproductive technologies (ART) is common practice both for human and livestock. In fact, the commercialization of ART services is greatly expanding and in some cases the pace at which specific ART technologies is developed does not fulfil the current demand. A prime example of this derives from the current use of genomics (DNA genotyping) to provide the animals' genetic merit, thereby increasing the rate of genetic improvement. Indeed, by identifying elite animals more efficiently we increase genetic pressure and by determining genetic merit at birth or even before through embryo biopsy, we reduce generation intervals. The pressure is now to select gametes bearing the best haplotypes produced from the youngest possible animals. However, concerns regarding the impacts of ARTs cannot be disregarded. They arose more than 20 years ago when in vitro produced embryos were associated with poorer cryotolerance and a higher frequency in abnormal offspring. The production of somatic nuclear transfer clones in the mid 2000's provided support to these potential impacts of ART. Since very little is known about the mechanisms supporting early embryonic development, many research teams performed comparative transcriptomic surveys in an attempt to highlight sensitive cellular pathways. These resulted in a better understanding of embryogenesis but also provided many more questions. It was noticed that embryos react to their surrounding environment and have means to compensate and even survive in harsh conditions. It is currently very difficult to determine which gene expression deviations are in fact transitory means of compensation from the ones carrying long term consequences. To answer this difficult question, we hypothesized that long term potentiation of a stress needs to be carried by the epigenome through the establishment of stable marks. We focussed on DNA methylation as it represents a logical candidate for its involvement in long term establishment of cell lineages. The DNA methylation platform enables the addition of an extra layer of information over the transcriptome and additional questions to be asked. So far, it was found that sorting the information using both dataset can highlight deviations with close relationship with known epigenetic mechanisms. We believe superimposing both types of information is necessary to differentiate between short term transitory cellular compensation from gene expression deviations with long term phenotypic impacts.

Pluripotency in Embryo and In Vitro

Barbara Schmaltz-Panneau[1,2], Luc Jouneau[1,2], Nathalie Daniel[1,2], Pierre Osteil[3,4,5,6], Yann Tapponnier[3,4,5], Marielle Afanassieff[3,4,5,6], Pierre Savatier[3,4,5,6],Delphine Dubé[1,2], Anne-Clémence Veillard[1,2], Alice Jouneau[1,2], Nathalie Beaujean[1,2], Véronique Duranthon[1,2]

1 INRA, UMR1198, Biologie du Développement et Reproduction, F-78350 Jouy-en-Josas, France
2 ENVA, UMR 1198 F-94704 Maisons-Alfort, France
3 INSERM, U846, F-69500 Bron, France
4 Stem Cell and Brain Institute, F-69500 Bron, France
5 Université de Lyon, F-69100 Villeurbanne, France
6 INRA, USC1361, F-69500 Bron, France

During embryonic development, two distinct cell populations appear at the blastocyst stage: the differentiated trophectoderm lineage and the inner cell mass (ICM) whose pluripotent cells are able to colonize all the lineages of the fetus. Later on, the still pluripotent epiblast and the hypoblast segregate from the ICM. In the mouse, two kinds of pluripotent stem cells can be derived in vitro from the embryo : naïve pluripotent ESC cells (obtained from the ICM) and primed pluripotent EpiSC cells (derived from the epiblast). Our analyses evidence a role for DNA methylation in the epigenetic barrier between these two pluripotent states. More recently, induced pluripotent stem cells (iPS) have been obtained by reprogramming somatic cells in vitro. In most domestic animal species, research are still necessary to obtain naïve pluripotent stem cells from embryos and true iPS from differentiated cells. To characterize pluripotent cells in the rabbit, we first analyzed the transcriptome of the "in embryo" pluripotent cells compared to that of their differentiated counterparts. Then, in an attempt to assess the "quality" of rabbit "ES" and iPS cells, we characterized their transcriptome fingerprint and compared it to that of rabbit ICM and epiblasts. Our data evidence major differences between rabbit "in embryo" and "in vitro" derived pluripotent cells. These differences will be discussed in regards to naïve and primed pluripotency.

It Starts with the Egg: Epigenetic Effects of the Oocyte Growth & Maturation Environment

Alan O'Doherty, Lynne O'Shea, Edward Daly & Trudee Fair

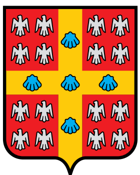
School of Agriculture & Food Science, University College Dublin, Belfield, Dublin 4, Ireland.

Mammalian female gametes are stored in the ovaries as inactive oocytes surrounded by a single layer of flattened granulosa cells until activated to grow. During the lifespan of the female, endogenous and exogenous factors, such as husbandry practices, production demands and disease can induce biochemical changes in the ovarian environment. These changes may have a deleterious effect on ovarian oocyte quality and subsequent embryo development. Although there is no consensus regarding the period of follicular oocyte development that is particularly sensitive to insult, an adverse ovarian environment at the time of key molecular and morphological events that occur during oocyte and follicle growth, such as activation of the oocyte transcriptome, establishment of maternal imprints and resumption of meiotic maturation may have drastic consequences for the oocyte. Using the cow as our model, we have employed a number of techniques including, candidate gene pyrosequencing, immunocytochemistry and whole methylome analysis, to determine if the endogenous metabolic environment during the oocyte growth phase or the oocyte maturation environment induce epigenetic changes in the bovine oocyte. Epigenetic effects such as aberrant methylation at imprinted loci and genome wide variation in methylation status were not only observed in immature and mature oocytes, but were also present during the implantation stage of embryo development. Additionally, altered gene expression, compromised DNA integrity and chromosome mis-alignments were also observed. These findings will be presented and discussed.

This work was funded by SFI grant number 07/SRC/B1156.

Short Communications

Abstracts



Relative Abundance of Extra-Cellular miRNAs in Bovine Follicular Fluid: Implication for Cell-Cell Communication During Oocyte Growth

Sohel MMH, Seifi Noferesti S, Salilew-Wondim D, Hoelker M, Rings F, Tholen E, Udin J, Looft C, Schellander K, Tesfaye D

Animal Breeding and Husbandry Group, Institute of Animal Science, University of Bonn, Germany

Here we aimed to investigate the expression pattern of the circulating extra-cellular miRNAs in exosome and non-exosomal fraction of follicular fluid consisted of fully grown or growing oocytes and to validate exosome mediated cell-cell communication between follicular cells. For this, follicles of 5-8 mm diameter (n=120) were isolated and individual COCs were subjected to brilliant cresyl blue (BCB) staining and classified as BCB+ (fully grown, n=60) and BCB- (growing, n=60) groups. The corresponding follicular fluid, granulosa cells and theca cells were used for further molecular analysis. MiRNAs isolated from exosomal and nonexosomal portion of follicular fluid from the two categories was used for cDNA synthesis and subsequent analysis using a human miRNA PCR array (with 745 miRNA). Results revealed that 25 miRNAs (16 up and 9 down regulated) in exosomes and 30 miRNAs (21 up and 9 down regulated) in non-exosomal portion of follicular fluid were differentially expressed (fold change ≥ 2 and $p < 0.05$) between growing vs. fully grown oocyte group. Among these miRNAs, miR-654-5p and miR-640 were found to be enriched in exosomal portion of follicular fluid containing growing oocytes, while miR-526b* and miR-373 were highly abundant in exosomal portion of follicular fluid containing fully grown oocytes. In-silico analysis of miRNAs enriched in follicular fluid containing growing oocytes revealed that genes are involved in different signaling pathways like ubiquitin mediated proteolysis, focal adhesion, oocyte meiosis, MAPK signaling pathways to be potential targets, which are crucial for follicular development. Co-culture of granulosa cells with PKH67 dye labeled exosomes showed successful uptake of exosomes by those cells and subsequently elevated the endogenous miRNAs level in 2.5-5.5 fold. In conclusion, the present study highlighted the oocyte growth status dependent differences of circulating miRNA profiles in follicular fluid and exosome mediated uptake of those molecules by surrounding cells in follicular microenvironment.

Global Transcriptome Analysis of Bovine Blastocysts Developed Under Alternative Vivo/Vitro Culture Conditions During Specifics Stages of Development

Gad A[1,4], Besenfelder U[2], Havlicek V[2], Hölker M[1], Rings F[1], Dufort I[3], Sirard MA[3], Schellander K[1], Tesfaye D[1]

[1]Institute of Animal Science, University of Bonn, Bonn, Germany
[2]Institute of Animal Breeding and Genetics, University of Veterinary Medicine, Vienna, Austria
[3]Centre de recherche en biologie de la reproduction, Université Laval, Québec, Canada
[4]Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt.

During the period from maturation until blastocyst formation, several critical events occur in embryos which are regulated by a harmonized expression of genes. However, the exact influence of in vitro culture conditions during each of these critical events/steps is still unknown. Therefore, we aimed to examine the effect of alternative vivo/vitro culture conditions during main developmental stages on the transcriptome profile of bovine blastocysts. We have used state-of-the-art nonsurgical endoscopic flushing and transfer of early stage embryos to the bovine oviducts to produce two different sets of blastocysts (6groups each) under alternative vivo/vitro culture conditions. For the first set, oocytes/embryos were produced in vitro and transferred to synchronized recipients at different time points (matured oocyte, fertilized oocyte, zygote,4-cell,16-cell and morula stage) then flushed out at day7 blastocyst stage. For the second set, embryos were produced in vivo, flushed out at different time points (2-,4-,8-,16,32-cell and morula stage) and cultured in vitro until day7 blastocyst stage. Complete in vitro (IVP) and in vivo blastocysts were used as controls. Gene expression pattern between each blastocyst group and in vivo control group were compared using EmbryoGENE's bovine microarray over six replicates of each group. Results showed that oocyte origin critically determined the developmental rates and the ability of embryo to react with changing culture conditions. Transcriptome analysis indicated three time points: fertilization, embryonic genome activation (EGA) and blastocyst formation as the most critical stages affected by changing culture conditions from in vivo to in vitro or vice versa. Molecular mechanisms and pathways that are influenced by altered culture conditions were defined. These results indicate the critical stages of early bovine embryo which are sensitive to environmental factors during the course of development. This in turn will help to design new strategies to modify culture environment in stage specific manner for better developmental potential.

ATOL and EOL Ontologies, Steps Towards Embryonic Phenotypes Shared Worldwide?

Hue I *[1] , Bugeon J[2], Dameron O[3], Fatet A[4], Hurtaud C[5], Joret L[2], Meunier-Salaün MC[5], Nédellec C[6], Reichstadt M[7], Vernet J[7], Le Bail PY[2]

[1] INRA, UMR 1198 BDR, 78352 Jouy-en-Josas, France;
[2] INRA, UR 1037 LPGP, Campus de Beaulieu, 35042 Rennes, France;
[3] INSERM U 936, Université de Rennes I, 35000 Rennes, France
[4] INRA, UR 0085 PRC, 37380 Nouzilly, France
[5] INRA-AgroCampus UMR 1348 PEGASE, 35590 St-Gilles, France
[6] INRA, MIG, 78352 Jouy-en-Josas, France
[7] INRA UMRH 1213, 63122 Saint Genès Champanelle, France

The development and the use of ontologies contribute to a better organization and use of the huge amount of knowledge available in biology, but could even contribute better when used worldwide to share common bases when publishing morphological data or molecular phenotypes (Gene Ontology, for example). We have built ATOL (http://www.atol-ontology.com) for \"Animal Trait Ontology for Livestock\" that is devoted to the definition and organization of phenotypic traits of farm animals including fish, birds and mammals. Given that phenotypes result from the action of both the genotype and the environment, a precise description of the animal rearing environments is critical. We thus created EOL for \"Environment Ontology for Livestock\", to describe the livestock systems and the rearing conditions in a generic manner. We could however extend that for in vitro and in vivo produced embryos to define the culture media, the origins of the gametes or the statuses of the dams that carry the embryos (breed, parity, energy balance, farming conditions). The combination of embryonic traits (in ATOL) and rearing conditions (in EOL) would enable a standardized and precise annotation of phenotypic databases linked to explicit metadata. Such ontologies also appear critical resources for automated semantic analyses that retrieve accurate information from relevant scientific or technical documents. Moreover, integrative and systemic approaches based on modelling would gain from a formal representation of knowledge that could then be automatically processed. The context and the content of both ontologies, as well as their putative application to embryo production and development, will be presented.

Detection of Genes Associated with Developmental Competence of Bovine Oocytes, Quantification of Transcript Levels Before and After Oocyte Maturation

Pesanova D[1], Nemcova L[1], Vodickova-Kepkova K[1], Vodicka P[1,3], Jeseta M[2]; Machatkova M[2], Kanka J[1]

[1]Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Czech Republic
[2]Veterinary Research Institute, Brno, Czech Republic
[3]Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, USA

Developmental competence of oocytes is acquired progressively during folliculogenesis and is linked to follicular size. It has been documented that oocytes originated from larger follicles show greater ability to develop to the blastocyst stage. The differences in cytoplasmic factors like mRNA transcripts could explain differences in oocyte developmental potential. In order to characterize differences in gene expression profiles of oocytes either from medium (MF, 6–10mm) or small (SF, 2–5mm) follicles at germinal vesicle (GV) and MII stage, we employed Bovine oligonucleotide microarrays (Missouri Consortium, http://www.ag.arizona.edu/microarray/BOM.html). Following normalization of the microarray data, analysis revealed changes in the level of 61 transcripts (≥ 1.4 fold) between the two groups, corresponding to 50 upregulated and 11 downregulated transcripts in MF oocytes compared to SF oocytes. The gene expression data were classified according to gene ontology, the majority of these genes was associated with regulation of transcription, translation, cell cycle, and mitochondrial activity. Subsets of 15 differentially expressed genes were validated by quantitative real-time RT-PCR. Before maturation, significant differences ($p < 0.01$) were revealed at the level of TAF1A, MTRF1L and ATP5C1 between MF and SF oocytes, the differences in other genes (NGDN, PRPF18 and ATP5F1) were on borderline of statistical difference. After maturation, real time RT-PCR quantification revealed different regulation of individual transcripts. The level of transcripts remained stable for ATP5F1 and BRD7 in MF and SF oocytes; in the case of TAF1A, the level of transcript remained stable only in SF oocytes. In all other investigated genes the quantification revealed a substantial drop in the level of individual transcripts in MF and SF oocytes after maturation. These results suggest that developmental competency may be quantitative trait, which is dependent on small changes in the RNA transcription profiles of many genes. Supported by grant NAZV QI91A018 and RVO: 67985904

Theme: Oocyte competence

FECUND EU-Project: Optimisation of Early Reproductive Success in Dairy Cattle through the Definition of New Trait and Improved Reproductive Biotechnology

Iamartino D[1], Lonergan P[2], Ramelli P[1], Forde N[2], Fair T[2], Besenfelder U[3], Blanco A[4] and Williams J[1]

[1] Fondazione Parco Tecnologico Padano, Via Einstein, 26900, Lodi, Italy;
[2] University College of Dublin, Belfield 4, Dublin, Ireland;
[3] University of Wien, Veterinaerplatz 1, 1210 Vienna, Austria;
[4] University College of Dublin, Conway Institute, Flow Cytometry Core, Belfield 4, Dublin, Ireland.

The FECUND project, funded by the European Union, is investigating the problems of reproductive success in cattle using interdisciplinary approaches of biology, physiology, -omics and bioinformatics to integrate in vivo and in vitro studies. The project will address factors affecting early reproductive success and use the results to facilitate genomic selection for improved fertility, and to improve artificial reproductive biotechnology. FECUND is studying the mechanisms underlying differences in reproductive success between heifers and cows, and will define the genetic vs management factors involved. The central theme is to discover the reasons for fertilization failure and events prior to implantation resulting in embryonic loss. Focussed “animal models” will address the factors impacting on the establishment of a pregnancy, in order to assess whether dairy cow infertility is primarily a genetic or metabolic problem. The FECUND Energy Balance model compares lactating Holstein cows vs non-lactating cows and heifers, all with similar production and fertility EBVs. Twenty five pregnant cows and 6 heifers were managed under strictly controlled and standardised conditions, and at calving 13 of the cows entered a normal lactation while 12 were dried off. Heifers and cows were synchronised, between 35-45 days post partum for cows, using a GnRH/CIDR/PG protocol with GnRH administered 36 h after CIDR removal and embryos were transferred 7 days later. Blood samples were collected at days 0, 3, 10, 13, 16,19 and analysed for immune parameters. All animals were slaughtered at day 19 when 5 (38%) of the lactating cows were found to be pregnant, 8 (66%) dry cows and 4 (66%) heifers. Reproductive organs, follicular and oviduct fluid, uterus and embryos were collected and stored. Samples are being analysed to reveal molecular and physiological variation among the three groups: lactating vs dry cows and heifers.

Influence of Cattle Breed on Gene Expression and Phenotype of Holstein and Jersey Embryos

Baldoceda L[1], Vigneault C[2], Gilbert I[1], Gagné D[1], Blondin P[2] and Robert C[1]

[1]Centre de Recherche en Biologie de la Reproduction, Département des Sciences Animales, Université Laval, Québec, Canada
[2] L'Alliance Boviteq Inc., Saint-Hyacinthe, Québec, Canada

The reason for the decreased pregnancy rates of frozen Jersey (JE) embryos compared with Holstein (HO) embryos has not been determined. This problematic situation is related to the over-accumulation of lipids in the embryo, which causes cell damage during cryopreservation protocols. Several reports have shown the higher content of lipid droplets in blastomeres of in vitro produced bovine embryos appears to be the consequence of lipid uptake from serum supplementation as a consequence of impaired mitochondrial function which results in a darker cytoplasm. Based on these findings, we hypothesized that the breed specific phenotype results from lipid metabolism as JE diverges from HO due to differential mitochondrial activity and related gene expression. First, we described the phenotype of both breeds embryos under in vivo and in vitro conditions. These observations were associated with intracellular lipid droplet quantification and mitochondrial potential activity under both conditions. We observed high lipid content and a different lipid profile in JE embryos that was related with lower mitochondrial activity in each different environment when compared with HO embryos. We also investigated artificially increasing the mitochondrial activity of the embryos using additives in the culture media to reduce lipid content. Gene expression related to lipid metabolism had differential profile between breeds in different conditions. These results not only confirm the relationship between mitochondrial patterns and lipid content between JE and HO embryos, but also show that the breed impacts mitochondrial activity, lipid profile and gene expression.

Hyperglycaemia: A New Player in Oocyte Epigenetics

Brown HM, Frank LA and Thompson JG

The Robinson Institute, Discipline of Obstetrics and Gynaecology, University of Adelaide, Adelaide, SA, AUSTRALIA

Peri-conceptual maternal hyperglycaemia is an increasing global health issue. Poor glucose control at the earliest stages of conception and development is associated with greatly increased risks for the mother and her offspring. Our earlier published work showed that peri-conception hyperglycaemia increases glucose metabolism within oocytes and early embryos through the hexosamine biosynthesis pathway. This increases production of UDP-N-acetylglucosamine, which in turn provides the substrate for a specific protein modification, O-β-linked glycosylation that modifies function, akin to other protein transformations such as phosphorylation and acetylation. This is mediated by two enzymes, O-linked glycosyltransferase (OGT, which causes the protein glycosylation) and N-acetylglucosaminidase (OGA, which removes the glycosylation). Two new landmark papers demonstrate epigenetic regulation and modification involving O-β-linked glycosylation and the TET family. First, TET3 can enhance the activity of the OGT glycosylating enzyme to increase glycosylation of HCF1, a component of the H3K4me3 methylating complex, in turn, promoting gene transcription. Secondly, the TET family mediate glycosylation of the histone core protein; TET2/3 facilitates OGT-dependent histone O-linked glycosylation by binding to chromatin and appears to target all histones. This led us to assess these systems in the mouse cumulus-oocyte complex, where we demonstrated extensive co-precipitation of TET3 and OGT. In addition to this, we have recently been able to show for the first time that Histone 3 (H3) is epigenetically modified via glycosylation in the mouse ovary. Additionally, in vivo or in vitro treatment with hyperglycaemic mimetic, glucosamine altered the histone modification profile, particularly on H3, in a number of cell types, including the cumulus oocyte complex. We have therefore identified the TET family and glycosylation as a candidate direct link between hyperglycaemia and epigenetic programming of the oocyte and early embryo.

Optimization of DNA Counterstaining for Methylation and Hydroxymethylation Immunostaining in Bovine Zygotes

Heras S, Van Soom A

Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

DNA methylation differences in embryos are indicative for epigenetic changes imposed by culture conditions. Immunostaining is the preferred technique to assess methylation/hydroxymethylation status of zygotes because differences between paternal and maternal pronucleus can be assessed in single embryos. Antibodies anti-methylcytosine/hydroxymethylcytosine bind to ssDNA, therefore DNA denaturation is required. Most DNA-stains however, such as Hoechst, bind to dsDNA, and not to ssDNA. Ethidium homodimer 2 is a DNA dye able to bind ssDNA. The aim of this study was to optimize the DNA counterstaining for pronuclear stages in bovine. Bovine oocytes were matured in TCM 199 with 20 ng/ml EGF. At 12, 15, 18 and 22h post fertilization, presumptive zygotes were fixed. After fixation, zygotes were permeabilized and denatured with 2N (30, 45min or 1h) or 4N (5, 10, 20, 30min or 1h) HCl. Zygotes were blocked and incubated with primary antibodies (mouse anti-5-MeC, rabbit anti-5-HmC), secondary antibodies (goat anti-mouse FITC, goat anti-rabbit Texas Red), and finally counterstained with Hoechst or EthD-2. When EthD-2 was used, RNase A treatment was performed. 5-MeC/5-HmC fluorescence after denaturation with 2N HCl was only obtained after 1h of denaturation, and this signal was weak. With 4N HCl at least 30 min of denaturation were needed to obtain good resolution. For Hoechst, after 1h denaturation with 2N HCl the signal was faint or disappeared completely. After 4N HCL treatment for 5 and 10min the signal was present, whereas it was completely gone after 20 and 30min. For EthD-2, after denaturation with 4N HCl for 1h the signal of the dye was still present, although weak, but it allowed pronuclear delineation. In conclusion, for bovine zygotes, denaturation of at least 30min with 4N HCl is needed. In these conditions Hoechst cannot be used as DNA counterstain, but EthD-2 can. This work was funded by IWT grant 111438.

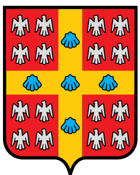
Embry

Genomics Epigenomics
Nutrition Environment

4th Mammalian Embryo Genomics meeting

Posters

Abstracts


UNIVERSITÉ
LAVAL

NSERC
CRSNG

Differential Gene Expression Profile of In vitro Produced Bovine Blastocyst in the Presence and Absence of Thyroid Hormones

Ashkar FA[1], Revay T[1], Dufort I[2], King WA[1]

[1] Department of Biomedical Sciences, University of Guelph, Guelph, ON, Canada
[2] Centre de Recherche en Biologie de la Reproduction, INAF, Département des Sciences Animales, Université Laval, Québec, Canada

In vitro embryo production (IVP) has been successfully used for infertility treatment, farm animal breeding and preservation of endangered species. Embryo culture media(IVC) and its composition play an important role in the developmental competency of in vitro produced embryos. To optimize IVP it is important to understand the physiology of the in vivo embryo medium. Previously, we confirmed the presence of thyroid hormones (THs) in the bovine reproductive tract and their beneficial effect on embryo quality in vitro. Also we confirmed the expression of TH receptors(TRs) in both mRNA level and protein level. The aim of this study was to further investigate the effect of THs in the transcriptome level of embryonic genome and differential gene expression when IVC media is fortified with THs. Supplementing the IVP media with 50ng/ml T3 and T4 significantly improved blastocyst rates, which was observed in both fast and slow cleaving embryos. Gene expression profile analysis in blastocyst stage showed that THs supplementation altered mRNA expression profile in blastocysts. 1,234 genes were expressed differentially in the treated embryos and these differences were statistically significant (>1.5 fold at $p < 0.05$); these findings were confirmed by qPCR. TH-related genes, including TRs mRNA and deiodinases (DIOs), were expressed in the gene array of both treated and control groups. Interestingly we found over expression of X-chromosome linked genes in the treated group suggesting delayed or escaped from X in activation. This study highlights the importance of THs in early embryo development (EED) and therefore the need to include these factors in culture media. Furthermore, this study has better characterized THs effects and identified possible regulatory roles of these hormones in EED. Further investigation into embryo competency and pregnancy outcome can provide more details

Acknowledgements: EmbryoGENE, CRC, NSERC, Internationalization of Research and Development Program of Agriculture and Agrifood Canada (AAFC).

Themes:

- Embryo development
- Culture conditions / Asssisted reproductive technologies

Vitamin K2 Supplementation Rescues Impaired Mitochondrial Activity to Improve Blastocyst Rate in In Vitro Cultured Bovine Embryos

Baldoceda L[1], Vigneault C[2], Blondin P[2] and Robert C[1].

[1]Centre de Recherche en Biologie de la Reproduction, Département des Sciences Animales, Université Laval, Québec, Canada.
[2] L'Alliance Boviteq Inc., Saint-Hyacinthe, Québec, Canada.

Proper Mitochondrial function is essential to early mammalian embryos because of their diverse cellular functions. In particular ATP production by the electron transport chain provides a resource of energy to biological processes in early embryo development. However, early embryos showed restricted mitochondrial activity during early developmental stages before the embryonic genome activation. Several reports indicate that in vitro culture procedures have negative effects on the phenotype as consequence of impaired mitochondrial function. Recently, Vitamin K2 (a membrane-bound electron carrier, similar to ubiquinone) was employed to rescue mitochondria from dysfunction and increase the efficiency of ATP production in eukaryotic cells. Therefore, the aim of the present study was to investigate the effects of supplementation of vitamin K2 in embryo culture media at 96 hours post in vitro fertilization (n=448 oocytes) compared with control group (n=239 oocytes). We observed that the vitamin K2–treated group had a significantly ($P<0.05$) higher blastocyst rate (+ 8.6%), expanded blastocyst rate (+7.8%) as well as better morphological quality compared to the control group. Blastocyst rate was related to higher mitochondrial activity in vitamin K2–treated embryos by a higher intensity of Mitotracker Red staining ($P<0.05$) versus control group. In conclusion, our data prove that supplementation of vitamin K2 during IVC of bovine embryos increases blastocyst rates and embryo quality. Future studies will focus on gene expression to identify targets implicated in impaired mitochondrial activity in in vitro bovine embryo production.

Theme :

- Embryo development
- Culture conditions / Assisted reproductive technologies

Influence of Cattle Breed on Gene Expression and Phenotype of Holstein and Jersey Embryos

Baldoceda L[1], Vigneault C[2], Gilbert I[1], Gagné D[1], Blondin P[2] and Robert C[1].

[1] Centre de Recherche en Biologie de la Reproduction, Département des Sciences Animales, Université Laval, Québec, Canada
[2] L'Alliance Boviteq Inc., Saint-Hyacinthe, Québec, Canada

The reason for the decreased pregnancy rates of frozen Jersey (JE) embryos compared with Holstein (HO) embryos has not been determined. This problematic situation is related to the over-accumulation of lipids in the embryo, which causes cell damage during cryopreservation protocols. Several reports have shown the higher content of lipid droplets in blastomeres of in vitro produced bovine embryos appears to be the consequence of lipid uptake from serum supplementation as a consequence of impaired mitochondrial function which results in a darker cytoplasm. Based on these findings, we hypothesized that the breed specific phenotype results from lipid metabolism as JE diverges from HO due to differential mitochondrial activity and related gene expression. First, we described the phenotype of both breeds embryos under in vivo and in vitro conditions. These observations were associated with intracellular lipid droplet quantification and mitochondrial potential activity under both conditions. We observed high lipid content and a different lipid profile in JE embryos that was related with lower mitochondrial activity in each different environment when compared with HO embryos. We also investigated artificially increasing the mitochondrial activity of the embryos using additives in the culture media to reduce lipid content. Gene expression related to lipid metabolism had differential profile between breeds in different conditions. These results not only confirm the relationship between mitochondrial patterns and lipid content between JE and HO embryos, but also show that the breed impacts mitochondrial activity, lipid profile and gene expression.

Hyperglycaemia: A New Player in Oocyte Epigenetics

Brown HM, Frank LA and Thompson JG

The Robinson Institute, Discipline of Obstetrics and Gynaecology, University of Adelaide, Adelaide, SA, AUSTRALIA

Peri-conceptual maternal hyperglycaemia is an increasing global health issue. Poor glucose control at the earliest stages of conception and development is associated with greatly increased risks for the mother and her offspring. Our earlier published work showed that peri-conception hyperglycaemia increases glucose metabolism within oocytes and early embryos through the hexosamine biosynthesis pathway. This increases production of UDP-N-acetylglucosamine, which in turn provides the substrate for a specific protein modification, O-β-linked glycosylation that modifies function, akin to other protein transformations such as phosphorylation and acetylation. This is mediated by two enzymes, O-linked glycosyltransferase (OGT, which causes the protein glycosylation) and N-acetylglucosaminidase (OGA, which removes the glycosylation). Two new landmark papers demonstrate epigenetic regulation and modification involving O-β-linked glycosylation and the TET family. First, TET3 can enhance the activity of the OGT glycosylating enzyme to increase glycosylation of HCF1, a component of the H3K4me3 methylating complex, in turn, promoting gene transcription. Secondly, the TET family mediate glycosylation of the histone core protein; TET2/3 facilitates OGT-dependent histone O-linked glycosylation by binding to chromatin and appears to target all histones. This led us to assess these systems in the mouse cumulus-oocyte complex, where we demonstrated extensive co-precipitation of TET3 and OGT. In addition to this, we have recently been able to show for the first time that Histone 3 (H3) is epigenetically modified via glycosylation in the mouse ovary. Additionally, in vivo or in vitro treatment with hyperglycaemic mimetic, glucosamine altered the histone modification profile, particularly on H3, in a number of cell types, including the cumulus oocyte complex. We have therefore identified the TET family and glycosylation as a candidate direct link between hyperglycaemia and epigenetic programming of the oocyte and early embryo.

Profiling of Long Non-Coding RNAs During Bovine Embryonic Development Reveals Their Enrichment in Pre-Embryonic Genome Activation Stages

Caballero J; Gilbert I; Fournier E; Scantland S; Robert C.

Centre de recherche en biologie de la reproduction, Institut sur la nutrition et les aliments fonctionnels (INAF), Département des sciences animales, Université Laval

Long non-coding RNAs (lncRNAs) have long been thought to be devoid of function and the result of background transcription. However, over 18,000 lncRNA transcripts has been reported and many of them has demonstrated to play a role in the regulation of many important mechanisms, such as maintenance of pluripotency, modification of epigenetic marks, regulation of mRNA decay and activation of transcription. Considering that in bovine the embryonic genome activates 3-4 cell cycles post-fertilization, we hypothesize that lncRNAs are involved in maintaining and managing embryonic integrity during this period. We generated 30 rRNA-depleted RNAsequencing libraries prepared from oocytes at GV and MII stages and embryos at 1-, 2-, 4-, early 8-, late 8-, morula, early and expanded blastocyst. All libraries were spiked with ERCC spike-in references for downstream normalization. Sequencing yielded a total of 812 M paired-end Illumina HiSeq reads. Alignment to the bovine genome produced 200,725 transcripts in 119,060 loci. From them, 8,120 did not overlap with any known gene. Preliminary analysis of the transcripts allowed us to identify 140 novel lncRNAs that were spliced. These transcripts showed a steady decline in relative abundance from the 4-cells stage onward and a sharp decline in diversity from the 8-cells stage onward. The expression level of some selected candidates was corroborated by qPCR. Subcellular fractioning of GV and MII oocytes as well as early and late 8-cell embryos enabled surveying the polyribosome associated RNAs. Several hundred lncRNAs were found associated to this fraction. Moreover, in situ hybridization experiments evidenced cytoplasmic localization of some of the candidates in GV oocytes, Taken together, our results show that lncRNAs are present in the cytoplasmic region of bovine oocytes and embryos, more importantly in early stages sustained by maternal RNA stores. Evidence of function will be determined by targeted knock-down using DsiRNA microinjected in oocytes.

Theme :

- Embryo development

Discovery, Identification and Sequence Analysis of RNAs Selected for Very Short or Long Poly A Tail in Immature Bovine Oocytes

Gohin M; Fournier E; Dufort I; Sirard MA.

Centre de Recherche en Biologie de la Reproduction, Faculté des Sciences de l'Agriculture et de l'Alimentation, Département des Sciences Animales, INAF, Université Laval, Québec, Canada

One of the major challenge to apply genomic's to oocyte physiology is the fact that a given RNA presence in oocyte does not mean translation into protein, preventing making conclusion from RNAseq and array data. Oocyte maturation and early embryo development rely on maternal storage of specific RNAs with a short poly(A) tail which must be elongated for translation. To resolve the role of key gene during that period, we aimed to characterize both extremes of mRNA: deadenylated RNA and long polyA tails mRNA population in immature bovine oocytes. Using magnetic beads coupled to oligo-dT, we isolated deadenylated (A-, 20 to 50 adenosines) from polyadenylated (A+, up to 200 adenosines) RNAs. After transcriptomic analysis, we observed that A+ candidates are associated with short-term processes required for immediate cell survival (translation or protein transport) or meiotic resumption, while several A- candidates are involved in processes (chromatin modification, gene transcription and post-transcriptional modifications) that will be extremely important in the development of early embryo . In addition to a list of candidate probably translated early or late, sequence analysis of them revealed that Cytoplasmic Polyadenylation Element (CPE) and U3GU3 were enriched in A- sequences. Moreover, a Motif Associated with Polyadenylation Signals (MAPS, U5CU2) appeared to be enriched in 3'UnTranslated Regions (3'UTR) with CPE or U3GU3 sequences in bovine but also in zebrafish and Xenopus tropicalis. To further validate our methodology, we measured specific tail length of known candidates (AURKA, PTTG1, H2A1) but also determined the poly(A) tail length of other candidate RNAs (H3F3A, H1FOO, DAZAP2, ATF1, ATF2, KAT5, DAZL, ELAVL2). In this study, we reported a methodology to isolate deadenylated from polyadenylated RNAs in samples with small total RNA quantities such as mammals. Moreover, we identified deadenylated RNAs in bovine oocytes that may be stored for the long-term process of early embryo development and described a conserved motif enriched in the 3'UTR of deadenylated RNAs.

Streamlining Microarray Analysis with the EDMA Analysis Pipeline

Fournier E, Robert C

Centre de Recherche en Biologie de la Reproduction, INAF, Université Laval, Québec, Canada

Analyzing microarray data can be a tiresome and time-consuming endeavour. Most microarray analysis packages have a steep learning curve, often requiring knowledge of scripting languages or command-line interfaces which are alien to most biologists. Furthermore, the sheer number of potential tools and analysis strategies is staggering to the uninitiated, who might understandably have little idea of how linear analysis, variance analysis and coexpression analysis differ, and whether or not such analyses are appropriate for their dataset.

To help alleviate these issues, we have developed the EDMA Analysis Pipeline, which allows users of the EmbryoGENE DNA Methylation Array to perform most standard analysis tasks with a minimum of work. The EDMA analysis pipeline produces quality control plots, performs data normalization, uses linear analysis of microarray data to detect differentially methylated loci, performs category enrichment analysis and produces circular plots integrating genome-wide epigenetic and transcriptomic data in a visually appealing way.

Future developments will feature the inclusion of pathway and coexpression analysis.

Global Transcriptome Analysis of Bovine Blastocysts Developed Under Alternative Vivo/Vitro Culture Conditions During Specific Stages of Development

Gad A[1,4], Besenfelder U[2], Havlicek V[2], Hölker M[1], Rings F[1], Dufort I[3], Sirard MA[3], Schellander K[1], Tesfaye D[1]

[1]Institute of Animal Science, University of Bonn, Bonn, Germany
[2]Institute of Animal Breeding and Genetics, University of Veterinary Medicine, Vienna, Austria
[3]Centre de recherche en biologie de la reproduction, Université Laval, Québec, Canada
[4]Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt.

During the period from maturation until blastocyst formation, several critical events occur in embryos which are regulated by a harmonized expression of genes. However, the exact influence of in vitro culture conditions during each of these critical events/steps is still unknown. Therefore, we aimed to examine the effect of alternative vivo/vitro culture conditions during main developmental stages on the transcriptome profile of bovine blastocysts. We have used state-of-the-art nonsurgical endoscopic flushing and transfer of early stage embryos to the bovine oviducts to produce two different sets of blastocysts (6groups each) under alternative vivo/vitro culture conditions. For the first set, oocytes/embryos were produced in vitro and transferred to synchronized recipients at different time points (matured oocyte, fertilized oocyte, zygote,4-cell,16-cell and morula stage) then flushed out at day7 blastocyst stage. For the second set, embryos were produced in vivo, flushed out at different time points (2-,4-,8-,16,32-cell and morula stage) and cultured in vitro until day7 blastocyst stage. Complete in vitro (IVP) and in vivo blastocysts were used as controls. Gene expression pattern between each blastocyst group and in vivo control group were compared using EmbryoGENE's bovine microarray over six replicates of each group. Results showed that oocyte origin critically determined the developmental rates and the ability of embryo to react with changing culture conditions. Transcriptome analysis indicated three time points: fertilization, embryonic genome activation (EGA) and blastocyst formation as the most critical stages affected by changing culture conditions from in vivo to in vitro or vice versa. Molecular mechanisms and pathways that are influenced by altered culture conditions were defined. These results indicate the critical stages of early bovine embryo which are sensitive to environmental factors during the course of development. This in turn will help to design new strategies to modify culture environment in stage specific manner for better developmental potential.

Laser Irradiation Affect Gene Expression and Maturation Rate of Buffalo Oocytes

Gad A[1,3]; El-Sayed A[1,3]; Abdel-Salam Z[2]; Khalifa M[3]; Harith MA[2]

[1]Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt
[2]National Institute of Laser Enhanced Science (NILES), Cairo University, Giza, Egypt
[3]Cairo University Research Park (CURP), Faculty of Agriculture, Giza, Egypt

Maturation of oocytes is one of the most important steps determining their developmental competence. In buffalo, maturation rate remain low compared to other species which negatively affect embryonic quality and developmental rate. Therefore, the objective of this study was to examine the impact of laser irradiation as a tool to improve quality and maturation rate of buffalo oocytes. Cumulus oocytes complexes (COCs) were aspirated from ovaries and grade A oocytes (n=450) were allocated into six different groups (3replicates each) with a total number of 75oocyte/group. A green light (532nm) from a Diode Pumped Solid State (DPSS) laser with a total out-put power of 1mW has been used to irradiate oocytes with irradiation spot area of 1.5 cm2. Oocyte groups were exposed to laser light for 0 (control), 2, 4, 6, 8 and 10 min with irradiance of 6.5x10-4 w/cm2. Afterwards, oocytes were matured in TCM-199 medium at 38.5oC and 5% CO2 in humidified air for 24h. Maturation rate was calculated based on extrusion of the first polar body and statistically analyzed by X2-test. Results showed an increased pattern of maturation rate with exposure time starting from 2 min until 8 min and decreased at 10min. Maturation rate was increased significantly in oocyte group irradiated for 8min compared to control group (59.3±2.2% vs 33.4±1.5% respectively, $p<0.05$). However, other groups showed no significant differences. Expression of 10 different marker genes (CCNB1, PTTG, ODC1, STAT3, POU5F1, BMP4, KRT8, ERK, DNAJC5 and SREBF2) were analyzed in 8min irradiated group using real-time PCR and were compared to control group. A significant increase in gene expression of all selected genes has been recorded in treated group compared to control one. These results indicate the possibility of adopting laser irradiation as an easy and straight forward technique for improving quality and in vitro maturation of buffalo oocytes.

Theme:

- Oocyte competence

The Effect of the Insulin Concentration on Developmental Competence and Gene Expression in Bovine Embryos Produced In Vitro

Goossens K[1], Wydooghe E[2], Thierens V[2], Peelman L[1], Van Soom A[2]

[1]Department of Nutrition, Genetics and Ethology, Ghent University, B-9820 Merelbeke, Belgium
[2]Department of Reproduction, Obstetrics and Herd Health, Ghent University, B-9820 Merelbeke, Belgium

Non-physiological, in vitro culture environments can lead to inappropriate epigenetic modifications during early embryo-genesis, affecting gene expression and causing abnormalities associated with ‘large offspring syndrome' (LOS). LOS is linked to serum addition to the in vitro culture medium, but the exact causes and molecular mechanisms have not been identified so far. Serum-containing media are more and more replaced by serum-free media. Recently, it was demonstrated by our group that replacing serum with albumin, insulin, transferrin and selenium (BSA+ITS) was successful for the culture of individual bovine embryos (Wydooghe et al., 2013). In this study we focused on the effect of insulin on the developmental competence and gene expression of bovine embryos produced in vitro. To this aim, embryos were cultured individually in BSA+ITS media with different insulin concentrations (0 – 0.005 – 1 – 5 – 25 – 75 and 125 µg/ml). Embryonic development was compared to the control group (5 µg/ml). The effects of the insulin concentration on the expression of INSR, GLUT-1, IGF-1R, IGF-2R, PDGFR and IRS were subsequently analyzed by RT-qPCR in expanded blastocysts. No difference was found in embryonic cleavage between the groups at 45 hpi. The blastocyst ratio however, decreased when low concentrations of insulin (0.005 µg/ml or 1 µg/ml) were added. These results suggest an optimal insulin concentration between 5 and 75 µg/ml, which is much higher than the physiological concentration (0.005 µg/ml). The different insulin concentrations had no significant effect on the expression of the analyzed genes ($P>0.05$). However, the addition of 1 µg/ml insulin resulted in tendency for a reduced IGF-2R expression ($P=0.054$) compared to the control group of 5 µg/ml. Loss of methylation of the imprinted gene IGF-2R was in the past linked to LOS (Young et al., 2001). Additional experiments are required to investigate the effect of insulin on the methylation status of IGF-2R.

Theme :

- Culture conditions / Asssisted reproductive technologies

Transcriptome Analysis of Bovine Granulosa Cells of Preovulatory Follicles at 30, 60, 90 and 120 Days Postpartum

Girard A[1], Golini VE[2], Stradaioli G[2], SirardMA[1]

[1]Département des Sciences Animales, Centre de Recherche en Biologie de la Reproduction, Université Laval, Sainte-Foy, Québec, G1K 7P4, Canada.

[2]Department of Agricultural and Environmental Sciences, University of Udine, via delle Scienze 206, 33100 Udine, Italy;

The nutritional requirements of high-producing dairy cow rapidly increase in the weeks post-partum due to increase milk production, and most cows enter in a state of Negative Energy Balance (NEB). Cows in this condition may preferentially divert nutrients away from reproduction, thereby experiencing a period of anovulatory anestrus, delayed ovulation of large follicles and a condition of impaired fertility. To better understand the changes occurring in these large follicles as a function of time post-partum granulosa cells of preovulatory follicles have been collected at different times: 30, 60, 90 and 120 days after calving from a minimum of 6 animals at each different time point. An analysis of the transcriptome was performed using a global bovine oligo-array microarray to map the differences in genes expression and cellular functions that occur in the follicular microenvironment during the progressive recovery from NEB condition in dairy cow, where 120 d was chosen as reference period, as cows are fully recovered from NEB. Data clustering indicate that the most different period from day 120 is day 60 with several hundred genes deregulated at that time. The principal functions affected are the cell proliferation which is reduced as the inflammation associated with the coming ovulation and the deregulation of steroidogenesis indicating immaturity in follicles from the 60 d period relative to day 120. One of the most interesting dowregulated pathways is the P38Map Kinase which is sensitive to the amount of retinoic acid or vitamin A, suggesting a possible treatment at this critical period.

Theme:

-Oocyte competence
-Maternal environment

Transcriptomic Differences Between Growth Phases in Cattle Dominant Follicles

Girard A, Douville G, Dufort I, Sirard MA

Département des sciences animales, INAF, Faculté des sciences de l'agriculture et de l'alimentation, Centre de la recherche en biologie de la reproduction, Université Laval, Quebec, Canada

The physiological state of the dominant follicle in monovulatory species is important, as it may be linked with the competence of the oocyte within. The objective of this study is to use whole genome microarrays to investigate the transcriptomic changes in granulosa cells of dominant follicles coming from different phases of follicular growth. Granulosa cells where collected from dairy cattle follicles with a diameter of more than 9 mm, and were classified in different phases of follicle growth based on flow cytometry profiles of DNA content marked by propidium iodide. The three phases are: growing (G), plateau (P) and atresia (A). A between group analysis (BGA) showed an apparent segregation of the three groups. The loop design was chosen for the hybridizations, and the various phases were hybridized against each others. Ingenuity Pathway Analysis (IPA) was used to identify the functions of the differentially expressed transcripts in the dataset and to seek interactions between them. Relative to the growth phase, the granulosa cells from the plateau phase showed an increase in the expression of STAR, which represent a limiting step in steroidogenesis. The C vs P contrast also presented a down regulation of metallothionins (MT1E, MT1A and MT2A) in the plateau granulosa cells. On the other hand, the contrast P vs A displayed an up-regulation of genes regulating response to oxidative stress (VNN1) and angiogenesis (NRP1 and ANGPT2) in the atretic granulosa cells. While the predicted activated function of the P cells compared to the C ones included steroids secretion and molecules transport, the predictions for A relative to P include an increase in apoptosis and cell death. Those observations are consistent with previous studies, and may help to understand the physiology of dominant follicle growth.

An Update on ELMA – The EmbryoGENE Data Storage and Analysis Platform

Grant JR[1,2], Fournier E[3], Arantes AS[2], Robert C[3], Sirard MA[3], Dixon WT[1], Dyck MK[1], Foxcroft GR[1], Stothard P[2]

[1] Swine Reproduction-Development Program, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada
[2] Livestock Gentec, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada
[3] Centre de recherche en biologie de la reproduction, Université Laval, Québec, Canada

ELMA (EmbryoGENE LIMS and Microarray Analysis) is a web-based application that has been created for the management and analysis of microarray data from EmbryoGENE projects. ELMA is a flexible and feature-rich web-based application developed using the Ruby on Rails framework. The LIMS component of ELMA stores several data types: protocols, platforms (array designs), samples (biological source material), arrays (intensity data for a single hybridization), and experiments (collection of arrays and analyses from a single study). Microarray analysis is performed using R and the Bioconductor package. Analysis results are integrated with gene cards, allowing quick visualization of expression data for all probes that target a particular gene. ELMA has over 30 active users and contains more than 200 experiments consisting of over 1600 arrays. Two major improvements have been incorporated into ELMA: updated annotation and the integration of an epigenetic platform. A custom microarray re-annotation pipeline was created to update the annotation of the swine and bovine embryo and splice transcriptome arrays, SEST and BEST, respectively. The annotation now provides each probe a Target Specificity Class (TSC) indicating the specificity and sensitivity of the probe. All old and new analyses in ELMA are automatically updated with the new annotation. The annotation pipeline can be rerun when new updates become available and can be easily adapted for any oligonucleotide array. The EmbryoGENE Network has recently developed a new bovine epigenetic microarray platform called EDMA (EmbryoGENE DNA Methylation Array) to study experimental differences in genomic methylation. ELMA data input and analysis workflows have been updated to work with the new epigenetic platform with the overall experience being very similar to working with expression data. These updates continue to make ELMA a valuable resource for all users of the EmbryoGENE arrays.

Theme:

- New technologies

Cow Fertility Markers Identified by Genetical Genomics

Guillemin N, Dufort I, Sirard MA

Centre de Recherche en Biologie de la Reproduction, Department of Animal Sciences, Laval University, Québec, Canada

Genetical genomics is a new methodology developed in 2001 in Arabidopsis thaliana, and then successfully applied in yeast and in other species. This methodology can finely dissect a QTL, identified by traditional genetics, to determine key genetic variations responsible of the installation and the control of a trait of interest.

The objective of this study was to identify, by genetical genomics, SNP genetic markers of cow fertility using the aromatase gene (CYP19A1) expression in granulosa cells as phenotype.

With a genomic approach (EmbryoGENE microarray and pathway analyze), we identified 350 genes related with the aromatase expression. We sequenced genomic DNA on a representative population of Holstein cows and constituted a genetic bank of 27 896 SNP variations on the 350 genes we selected.

On 83 cows, for which the CYP19A1 mRNA has been previously quantified by qPCR, we sequenced 15 selected SNPs, using the method of High Melting Resolution. The relationship between genetic variation and phenotypic variation was accessed by a linear regression method, which generate a prediction model of the phenotype from SNPs.

Analysis showed a significant relation between 6 SNPs on 5 genes (KRT8, CYP19A1, CREB1, LHCGR and ANXA1) and the CYP19A1mRNA abundance. Some genes are in known QTL related with fertility. The generated model can predict 43.6% of the CYP19A1 gene expression variability, and so constitute a pertinent and interesting model to make tests for the industry.

Jointly with the genetic analyze, the pathway analyze identified that some mechanisms such as oxidative stress, apoptosis, and immune response are important in the control of CYP19A1 gene expression

This study demonstrated the utility of the genetical genomic approach to finely dissect and analyze complex phenotypes, and bring new tools and perspectives for industrials in farm species for a lower cost and time than standard genetic approaches

Optimization of DNA Counterstaining for Methylation and Hydroxymethylation Immunostaining in Bovine Zygotes

Heras S, Van Soom A

Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

DNA methylation differences in embryos are indicative for epigenetic changes imposed by culture conditions. Immunostaining is the preferred technique to assess methylation/hydroxymethylation status of zygotes because differences between paternal and maternal pronucleus can be assessed in single embryos. Antibodies anti-methylcytosine/hydroxymethylcytosine bind to ssDNA, therefore DNA denaturation is required. Most DNA-stains however, such as Hoechst, bind to dsDNA, and not to ssDNA. Ethidium homodimer 2 is a DNA dye able to bind ssDNA. The aim of this study was to optimize the DNA counterstaining for pronuclear stages in bovine. Bovine oocytes were matured in TCM 199 with 20 ng/ml EGF. At 12, 15, 18 and 22h post fertilization, presumptive zygotes were fixed. After fixation, zygotes were permeabilized and denatured with 2N (30, 45min or 1h) or 4N (5, 10, 20, 30min or 1h) HCl. Zygotes were blocked and incubated with primary antibodies (mouse anti-5-MeC, rabbit anti-5-HmC), secondary antibodies (goat anti-mouse FITC, goat anti-rabbit Texas Red), and finally counterstained with Hoechst or EthD-2. When EthD-2 was used, RNase A treatment was performed. 5-MeC/5-HmC fluorescence after denaturation with 2N HCl was only obtained after 1h of denaturation, and this signal was weak. With 4N HCl at least 30 min of denaturation were needed to obtain good resolution. For Hoechst, after 1h denaturation with 2N HCl the signal was faint or disappeared completely. After 4N HCL treatment for 5 and 10min the signal was present, whereas it was completely gone after 20 and 30min. For EthD-2, after denaturation with 4N HCl for 1h the signal of the dye was still present, although weak, but it allowed pronuclear delineation. In conclusion, for bovine zygotes, denaturation of at least 30min with 4N HCl is needed. In these conditions Hoechst cannot be used as DNA counterstain, but EthD-2 can.

This work was funded by IWT grant 111438.

Can we Manipulate the Time Window of Pluripotency Gene Expression?

Hosseini SM, Hajian M, Jafarpour M, Abbasi H, Forouanfar M, Nasr-Esfahani MH

Department of Reproductive Biotechnology at Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran.

POU5F1 (previously known as Oct3 and Oct4) is a well-known key regulator of pluripotency and cell differentiation which belongs to POU transcription factor family and is encoded by a gene belonging to this group. POU5F1 is a crucial factor in the acquisition of developmental competence in oocyte and gain-of- function study showed that POU5F1 over expression during early, but not later, stage of reprogramming could improve cloned embryo development in mice. In bovine, POU5F1 gene expression triggered at the stage of zygote genome activation (ZGA) via drastic epigenetic modification on promoter region. Therefore, in this study using bovine fibroblasts stably transfected with POU5F1 promoter-driven EGFP, we investigated whether the time point of prluripotency gene expression, as an example of many genes start their expression at ZGA, can be manipulated by epigenetic modification using trichostatin A (TSA), the most widely used epigenetic drug in SCNT experiments acting via inhibition of histone-deacetylases. Obtained results indicated that in both treated and control groups the expression of POU5F1 expression was not detectable until day-3 when embryos acquired 8-16 cells. Importantly, POU5F1 expression was detected in both inner cell mass and trophectoderm of cloned blastocysts of both groups. However, cloned embryos treated with TSA had significantly higher i) POU5F1 expression, ii) yield and quality of blastocyst formation, and iii) inner cell mass/trophectodrm ratio compared to control clones. It was concluded that POU5F1 expression in cloned embryos is strictly controlled by the stage of embryo development and may not be altered by TSA-mediated changes in the levels of histone-acetylation of the zygote genome. From this study, further studies are needed to explore the transcriptomics pattern of gene expression in response to epigenetic modification before ZGA.

Keywords: POU5F1, zygote genome activation, pluripotency gene expression, epigenetic modification, bovine cloned embryos

Upregulation of Myc Regulatory Network in the Transcriptome of Bovine Cloned Blastocysts

Hosseini SM[1], Hajian M[1], Jafarpour F[1], Nasr-Esfahani MH[1], Dufort I[2], Sirard MA[2]*

[1]Department of Reproductive Biotechnology at Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran.
[2]Département des sciences animales, Pavillon INAF, Centre de recherche en biologie de la reproduction, Université Laval, Quebec, Canada

Reprogramming of a terminally differentiated somatic cell back to the pluripotency is now an established technique via an emerging list of approaches such as nuclear transfer (NT), cell fusion, transcription-factor transduction and chemical induction. In this context, oocyte-mediated nuclear reprogramming is inevitably considered as the standard due to its great similarities to natural method of reprogramming of fertilization. However, a comprehensive understanding of molecular underpinnings of cellular reprogramming is taking shape only recently using established biology systems that enable us to develop networks in which multiple critical regulatory factors act in combination. Using an embryo-specific transcriptomics platform covering over 45,000 sequences (EmbryoGENE), we investigated the gene expression profile of bovine cloned vs. in vitro fertilized (IVF) blastocysts. Obtained results indicated an extensive global reprogramming of the transferred fibroblast nuclei as the overall gene expression profile of NT blastocysts was closely resembled to naturally fertilized embryos. A subset of genes was misregulated which accounted for 1731 (~4%) genes differentially expressed (DEG) between cloned and IVF –derived blastocysts (P<0.05, FDR=25%, FC: 1.5). Among DEG, 654 (~ 1.5%) genes were down regulated and 1080 (~ 2.1%) genes were up regulated in cloned vs. IVF blastocysts. Up regulated genes were with a preponderance of constitutively expressed genes required for cellular movement, assembly, organization, and cell to cell signalling and interaction and those genes consistently down regulated were among those involved in cellular function and maintenance, and nervous system development and function. Importantly, data modeling using Ingenuity Pathway Analysis emerged three networks regulated by important genes involved in cancer incidence (MYC (up regulated), and P53 and TNF (both insignificantly down regulated)). These results could contribute in our better understanding of the oocyte mediating reprogramming errors overlooked and account for a new transcriptome feature of cloned blastocysts with important implication in etiology of cloning inefficiency.

Keywords: Bovine, Cloned blastocyst, transcriptome, Myc

ATOL and EOL Ontologies, Steps Towards Embryonic Phenotypes Shared Worldwide?

Hue I *[1] , Bugeon J[2], Dameron O[3], Fatet A[4], Hurtaud C[5], Joret L[2], Meunier-Salaün MC[5], Nédellec C[6], Reichstadt M[7], Vernet J[7], Le Bail PY[2].

[1] INRA, UMR 1198 BDR, 78352 Jouy-en-Josas, France;
[2] INRA, UR 1037 LPGP, Campus de Beaulieu, 35042 Rennes, France;
[3] INSERM U 936, Université de Rennes I, 35000 Rennes, France
[4] INRA, UR 0085 PRC, 37380 Nouzilly, France
[5] INRA-AgroCampus UMR 1348 PEGASE, 35590 St-Gilles, France
[6] INRA, MIG, 78352 Jouy-en-Josas, France
[7] INRA UMRH 1213, 63122 Saint Genès Champanelle, France

*Corresponding author: isabelle.hue@jouy.inra.fr

The development and the use of ontologies contribute to a better organization and use of the huge amount of knowledge available in biology, but could even contribute better when used worldwide to share common bases when publishing morphological data or molecular phenotypes (Gene Ontology, for example). We have built ATOL (http://www.atol-ontology.com) for \"Animal Trait Ontology for Livestock\" that is devoted to the definition and organization of phenotypic traits of farm animals including fish, birds and mammals. Given that phenotypes result from the action of both the genotype and the environment, a precise description of the animal rearing environments is critical. We thus created EOL for \"Environment Ontology for Livestock\", to describe the livestock systems and the rearing conditions in a generic manner. We could however extend that for in vitro and in vivo produced embryos to define the culture media, the origins of the gametes or the statuses of the dams that carry the embryos (breed, parity, energy balance, farming conditions). The combination of embryonic traits (in ATOL) and rearing conditions (in EOL) would enable a standardized and precise annotation of phenotypic databases linked to explicit metadata. Such ontologies also appear critical resources for automated semantic analyses that retrieve accurate information from relevant scientific or technical documents. Moreover, integrative and systemic approaches based on modelling would gain from a formal representation of knowledge that could then be automatically processed. The context and the content of both ontologies, as well as their putative application to embryo production and development, will be presented.

Detection of Genes Associated with Developmental Competence of Bovine Oocytes, Quantification of Transcript Levels Before and After Oocyte Maturation

Pesanova D[1]; Nemcova L[1]; Vodickova-Kepkova K[1]; Vodicka P[1,3]; Jeseta M[2]; Machatkova M[2]; Kanka J[1]*

[1]Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Czech Republic
[2]Veterinary Research Institute, Brno, Czech Republic
[3]Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, USA
*Presenting author: Tel.: +420315639551; fax: +420315639510. E-mail: kanka@iapg.cas.cz

Developmental competence of oocytes is acquired progressively during folliculogenesis and is linked to follicular size. It has been documented that oocytes originated from larger follicles show greater ability to develop to the blastocyst stage. The differences in cytoplasmic factors like mRNA transcripts could explain differences in oocyte developmental potential. In order to characterize differences in gene expression profiles of oocytes either from medium (MF, 6–10mm) or small (SF, 2–5mm) follicles at germinal vesicle (GV) and MII stage, we employed Bovine oligonucleotide microarrays (Missouri Consortium, http://www.ag.arizona.edu/microarray/BOM.html). Following normalization of the microarray data, analysis revealed changes in the level of 61 transcripts (≥ 1.4 fold) between the two groups, corresponding to 50 upregulated and 11 downregulated transcripts in MF oocytes compared to SF oocytes. The gene expression data were classified according to gene ontology, the majority of these genes was associated with regulation of transcription, translation, cell cycle, and mitochondrial activity. Subsets of 15 differentially expressed genes were validated by quantitative real-time RT-PCR. Before maturation, significant differences ($p < 0.01$) were revealed at the level of TAF1A, MTRF1L and ATP5C1 between MF and SF oocytes, the differences in other genes (NGDN, PRPF18 and ATP5F1) were on borderline of statistical difference. After maturation, real time RT-PCR quantification revealed different regulation of individual transcripts. The level of transcripts remained stable for ATP5F1 and BRD7 in MF and SF oocytes; in the case of TAF1A, the level of transcript remained stable only in SF oocytes. In all other investigated genes the quantification revealed a substantial drop in the level of individual transcripts in MF and SF oocytes after maturation. These results suggest that developmental competency may be quantitative trait, which is dependent on small changes in the RNA transcription profiles of many genes. Supported by grant NAZV QI91A018 and RVO: 67985904

Theme: Oocyte competence

FSH Signalling: Transactivation of Epidermal Growth Factor (EGF) Pathway Mediates Acquisition of Oocyte Competence in Bovine Cumulus Cells

Khan DR[1], Bilodeau-Goeseels S[2], Sirard MA[1] and Richard FJ[1]

[1]Centre de Recherche en Biologie de la Reproduction, Département des Sciences Animales, Université Laval, Sainte-Foy, Québec, Canada
[2]Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, Alberta, Canada

Folliculogenesis is governed by a complex crosstalk between endocrine and paracrine hormones. These factors regulate functional properties of follicular somatic cells (cumulus and granulosa) and herald better oocyte competence through the requisite cumulus-oocyte communication. In mammals, follicle stimulating hormone (FSH) induced, in vivo and in vitro, follicular cell proliferation, metabolism, steroidogenesis and better embryonic development—the processes widely investigated yet poorly understood. In current study we investigated the cell signaling pathways prompted by FSH in bovine cumulus cells through global transcriptome analysis and charted out possible network involved in acquisition of oocyte competence. In the first instance, functional genomic analysis of 677 upregulated and 792 downregulated transcripts indicated possible implication of PKA, PKC and EGF pathways in cumulus cells derived from cultured cumulus-oocyte complexes (COC). The genes involved in cumulus expansion, ECM stability, metabolism and oocyte competence markers were upregulated. Interestingly, EGF receptor (EGFR) tyrosine kinase inhibitor (AG) prevented these processes but not PKC inhibitor (GF) which denotes significant role of EGF pathway. In parallel, FSH elicited ERK1/2 phosphorylation which was abrogated by AG and remained unaffected by GF. Finally, FSH stimulation of EGFR was mediated by SRC as evidenced by SRC inhibitor (PP2). Collectively, our findings affirm that FSH transactivates EGF pathway which is interceded by SRC that steers ERK1/2 phosphorylation and modulates cumulus functions. This ERK1/2 activity ameliorates oocyte competence through optimisation of endocrine-paracrine interaction at the interface of cumulus cells which presages improved receptivity to oocyte secreted signals and provide better microenvironment.

Theme:

- Oocyte competence
- Embryo development
- Culture conditions / Asssisted reproductive technologies

Expression of Key Genes in a Trophoblastic Cell Line (Jag-1) Isolated From Porcine Day 14 Embryos

Kana Komatsu[1, 2], Stephen Tsoi[1], Walter Dixon[1] and Michael Dyck[1]

1 University of Alberta, Dept of Agricultural, Food and Nutritional Science, Edmonton, AB T6G 2P5 2 Laboratory of Reproductive Biology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan.

After hatching of blastocyst stage embryos, the trophectoderm (TE) starts an elongation process in parallel with the development of the inner cell mass into the embryonic disk. Different trophoblastic cell lineages continue to be established along with rapid phenotypic and functional changes to the conceptus. Successful implantation depends on proper development of the trophoblast within the uterine lumen which later develops into a simple epitheliochorial placenta in the pig. The molecular mechanisms underpinning this process in utero have yet to be fully elucidated in isolated trophoblastic cells from a specific lineage. We have use a porcine trophoblastic cell line derived from the bulbous tips of filamentous embryos at Day 14 to identify the molecular characteristics of Jag-1 cells (Ramsoondar et al., 1993). Both end point PCR and qPCR methods were used to characterize the RNA expression levels of two mammalian TE gene markers (CDX2 and KRT18) between Jag-1 and Day 30 embryos, along with two housekeeping genes (GAPDH and GUBS) and other embryonic stem cell markers (GATA4, BMP4, CD9 and VIM). A Low RNA expression level of two TE markers was found in Jag-1 cells when compared to Day 30 embryos. On the other hand, VIM, GATA4, BMP4 and CD9 were expressed at a similar level between Jag-1 and Day 30 embryos. Based on these results we conclude that high levels of expression of trophoblastic-determining genes found in human and mouse are not detected in pig trophoblastic cells isolated from TE of Day 14 embryos.

Thème - Embryo development

Bovine Oocyte Transcriptome in Relation to Chromatin Configuration of the Germinal Vesicle

Labrecque R[1] Lodde V[2] Luciano AM[2] Sirard MA[1]

[1]Centre de recherche en biologie de la reproduction (CRBR), Département des Sciences Animales, Université Laval, Québec, QC, Canada.
[2]Reproductive and Developmental Biology Laboratory, Department of Health, Animal sciences and Food Safety, University of Milan, Italy.

Various physiological contexts have been used to study the mRNA composition of bovine oocyte in relation with the potential to reach the blastocyst stage. The chromatin configuration of the oocyte at the germinal vesicle (GV) stage has been positively associated with developmental competence, where a more compacted chromatin is correlated with a better chance to get an embryo. Four distinct and progressive states of chromatin condensation can be observed and are linked with the sequential acquisition of the developmental potential in bovine species. Therefore, GV stage oocytes were collected and separated in four groups according to their degree of chromatin condensation. Transcriptomic analyses were performed with the EmbryoGENE microarray platform in order to identify transcripts modulations during the gradual chromatin remodeling. A reference design was used where GV0 stage (diffused chromatin state) was compared with the other three groups (GV1; GV2 and GV3, the latter refers to the condensed chromatin state). The number of transcripts significantly different was relatively similar between the three contrasts (an average of 300 genes, fold change > 2; p-value < 0.01) with the majority of them were presenting a reduced level in oocyte with more compacted chromatin. However, among the transcripts presenting an increased mRNA level in these contrasts, many of them are associated with histone genes, chromatin organization and transcriptional regulation. These results suggest that even in a context of transcriptional repression, there is still an accumulation of specific transcripts. Adequate storage of specific histone mRNAs could provide an optimal maternal accumulation in order to fulfill the requirement for the subsequent cell divisions until embryonic genome activation. This dataset will provide a more complete characterization of the oocyte transcriptome modulations and will help us to better understand the competence acquisition in the oocyte at the end of the follicular growth.

Theme:

- Oocyte competence

Reference Gene Selection for Normalization of qPCR Data in Sexed Bovine Implantation Embryos Under Low and High Oxygen Tension

Li WW[1]; Goossens K[1]; Van Poucke M[1]; Van Soom A[2]; Peelman L[1]

[1]Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium
[2]Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, B-9820 Merelbeke, Belgium

Real-time quantitative polymerase chain reaction (qPCR) is an efficient and accurate tool for RNA quantification in preimplantation embryos. To normalize gene expression data, stable reference genes are needed. The stability of reference genes can vary under different developing stages or culture conditions, hence it is essential to determine reference gene stability before initiating a gene expression analysis. We evaluateded the stability of the 7 most commonly used bovine embryo reference genes in bovine preimplantation stages, considering oxygen tension and embryo gender. In brief, IVF bovine embryos were produced according to our standard laboratory procedures (Wydooghe et al, 2013). Cumulus cells were removed 21 hrs post-insemination (hpi), and presumed zygotes were cultured in serum-free media under 5% O2 or 20% O2. Embryos were collected at specific time points (2-cell at 36 hpi, 4-cell at 40 hpi, 8-cell at 64hpi, 16-cell at d5, morula at d6 and blastocyst at d8), and were sexed by a novel RNA-based method (Hamilton et al, 2011)from 8-cell onwards. Reference gene primers (GAPDH, ACTB, 18s RNA, SDHA, H2A, HPRT1, and YWHAZ) were taken from Goossens et al (2005). After single embryo RNA extraction and cDNA synthesis, qPCR data were analyzed using the GeNorm program (Vandesompele et al, 2002) to determine the most stable reference genes. The results show the candidate genes ranking varies among developmental stages. However, high oxygen tension and embryo gender do not change the ranking significantly. GAPDH and WYHAZ are stable reference genes with low expression variation values ($M<1.5$) in all stages regardless of sex and oxygen tension.

Theme:

- New technologies

Cumulus Cells Actively Transfer RNA to the Oocyte During Maturation

Macaulay AD[1], Gilbert I[1], Caballero J[1], Fournier E[1], Tossou P[1], Sirard MA[1], Clarke HJ[2], Khandjian EW[4], Richard FJ[1], Hyttel P[4], Robert C[1]

[1]Département de Sciences Animales, Centre de recherche en biologie de la reproduction, Institut sur la nutrition et les aliments fonctionnels, Université Laval, Ville de Québec, QC, Canada.
[2]Department of Obstetrics and Gynecology, McGill University Health Centre, Montréal, QC, Canada.
[3]Université Laval Département de Biologie médicale, Institute universitaire en santé mentale de Québec, Université Laval, Ville De Québec, QC, Canada.
[4]Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen Denmark.

The cumulus cells are directly connected to the oocyte by transzonal projections (TZPs) that are anchored to the oolemma by adherens-like junctions and gap junctions. During oocyte maturation, most noticeably after GVBD, these projections progressively lose all adhesion to the oolemma. Unlike many non-mammalian species that share large molecules through structures like ring canals, it is currently accepted that the communication between the oocyte and the cumulus cells is limited to small molecules like cyclic nucleotides that pass through gap junctions and regulate the oocyte's maturation. We hypothesize the contrary that large molecules can pass between the cumulus cells and the oocyte. We have shown that vesicles exist at the membrane interaction of the cumulus cell and the oocyte. We also demonstrated that de novo RNA foci are produced during maturation and localize to the projections. The accumulation of RNA in the projections coincides with the increasing levels of total RNA in the oocytes over the course of maturation, while the poly (A) RNA content remains constant. Using RNAseq, a subset of de novo RNA specifically enriched in the TZPs was identified and that RNA includes coding, long non-noncoding RNA, and rRNA. The RNA that is found in the projections codes for processes related to drive post-transcriptional modification, molecule trafficking, and RNA binding. Finally, the inhibition of vesicular function and also of RNA synthesis prevents oocyte maturation indicating their need for these processes. We demonstrate a novel form of intercellular communication for this cell type that is important to oocyte maturation and emphasizes the relationship of the oocyte and the supporting cumulus cells. The FQRNT and REDIH programs support this project.

Theme:

- Oocyte competence

Gene Expression Profiles of Pig Cumulus-Oocyte Complexes Cultured In Vitro with Follicle Stimulating Hormone or Epidermal Growth Factor-Like Peptides

Nemcova L[1]*; Blaha M[1]; Vodickova Kepkova K[1]; Vodicka P[1,2] and Prochazka R[1].

[1]Department of Developmental Biology, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Czech Republic
[2]Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, USA *Presenting author: Tel.: +420315639534; fax: +420315639510.

E-mail address: nemcova@iapg.cas.cz

Gonadotropin-induced maturation of mammalian oocytes in preovulatory follicles is mediated by epidermal growth factor-like peptides synthesized in mural granulosa cells and consequently also in cumulus cells. The aim of our work was to identify changes in gene expression profile of cumulus cells stimulated in vitro with FSH or amphiregulin (AREG) and epiregulin (EREG). For this purpose the cumulus-oocyte complexes were cultured in M-199 medium supplemented with recombinant human FSH or AREG and EREG for 3 h. At the end of the culture period, the cumulus cells were removed from oocytes by pipetting, lysed and stored at -80 °C. Three different samples (FSH treated, AREG/EREG treated and control untreated cumulus cells), each represented by 3 independently prepared biological replicates were hybridized to 9 microarrays. Microarray data background correction, normalization and statistical inference of changes in gene expression were performed using Bioconductor package Limma in R statistical environment. We have identified 2981 transcripts as over expressed and 3581 as under expressed in FSH stimulated cells compared to control cells. Comparison of AREG/EREG stimulated cells with the control cells revealed 1492 transcripts as over-expressed and 1783 transcripts as under-expressed. Altogether, 2899 transcripts were over-expressed and 2581 transcripts under-expressed in AREG/EREG group compared to FSH group. Only the transcripts with log2 fold-change above 1.5 were considered as being significantly changed in further analysis. Genes over-expressed in FSH-treated cells were involved in blood coagulation, response to oxidative stress, regulation of cell migration, gonad development and angiogenesis. Genes over-expressed in AREG/EREG stimulated cells were involved in blood coagulation, regulation of cell migration and angiogenesis, but also in apoptosis and regulation of neuron differentiation. The microarray results were confirmed with real-time RT-PCR. A panel of 16 genes were chosen. The differential expression of 13 transcripts was validated.

Supported by GACR No. P502/11/0593 and RVO: 67985904.

The Use of Granulosa Cells in Culture to Investigate Gene Pathways Discovered by Transcriptomic Analysis in Bovine

Nivet AL, Gilbert I, Dufort I, Sirard MA

Centre de recherche en biologie de la reproduction, Université Laval, Québec City, QC, Canada

The development global transcriptomes capacity has now generated large amount of physiological data, ut, as far as we know, there has been no experiments studying exhaustively the direct effect of FSH on granulosa cell's gene expression in bovine. In order to answer this basic ovarian physiological question, we performed primary culture of bovine granulosa cells (n=4 replicates, 3 ovaries per replicates) from dissected follicles (2 to 6 mm diameter). The cells were cultured in vitro for 2 days and the last 4 hours the cells were treated or not with a biologically relevant dose of FSH (1ng/mL). Cells were collected, their RNA extracted, analyzed, amplified and hybridized on Embryogene complete bovine genome microarray slides. Data were analysed quantitatively then qualitatively versus previous knowledge on FSH signaling. Then, based on this validated model, we identified new major upstream regulators positively influenced by FSH in granulosa cells and their downstream targets. Results showed 1189 genes differentially modulated by FSH treatment in vitro (fold change >1.5, p-val 0.05), among them 678 genes were positively influenced by FSH and 511 negatively influenced by FSH. Interestingly, it is more than twice the previously known amount of genes influenced by FSH (based on Ingenuity Pathway Analysis database).
This study confirmed the positive role of FSH on PKA signaling (cAMP responsive element binding protein (CREB), Protein Tyrosine Phosphatase (PTP)) and Phosphatidylinositol-3 kinase (PI3K) signaling. In term of signalling pathway, interestingly, FSH has a positive influence on IL6 and IGF1 signaling. Upstream regulators were identified for the first time: Pancreatic and duodenal homeobox 1 (PDX1) which is upstream of early growth response 1 (EGR1), fibroblast growth factor 2 (FGF2), insulin-like growth factor 1 receptor (IGF1R), insulin-like growth factor binding protein 3 (IGFBP3). PDX1 is usually found in pancreatic cells but is over-expressed in our experiment. Also v-rel reticuloendotheliosis viral oncogene homolog (avian) (REL) is upstream cAMP responsive element modulator (CREM) and nuclear receptor subfamily 4, group A, member 1 (NR4A1) and upregulated in FSH treated granulosa cells. This study allow, for the first time, the characterisation of the short term effect of FSH on bovine granulosa cell transcriptome, as well as the discovery of new unknown influence of FSH on granulosa cell pathways which will permit to distinguish the specific effect of FSH on granulosa cells from other ovarian regulators. Finally FSH seems to inhibit the endothelial to mesenchymal transition.

Methyl-Seq Analysis of d30 Pig Placentae Derived from Sows Feed Restricted During Lactation

Oliver G [1, 3], Grant J [1], Wilkinson J[1], Plastow G[2], Dyck MK [1], Dixon WT[1] and Foxcroft GR[1]

[1]Swine Reproduction-Development Program, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, CANADA
[2]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, CANADA
[3] Presenting Author

Maternal nutrition during peri-conception period can have short-term effects on fetal development and long-term effects on adult health. Sows that are feed restricted during the last week of lactation produce smaller embryos and show altered muscle development, which persists beyond birth. Sows that utilise more body tissue to maintain high average daily gains in the suckling litter had significantly smaller average embryo weights than sows that utilised less (Patterson et al., 2011) , thus it could be considered that these sows were at ‘risk\' of compromising embryo development in the subsequent litter. This work aims to identify how the methylome influences nutritional programming during the peri-conception period, and in particular, how sow energy partitioning during lactation may risk impairing future embryo development. In the present study, sows were nutritionally restricted between d14 and d21 of lactation. At the first oestrus after lactation sows were rebred and at d30 of gestation placentae were collected. Sows were categorized as Risk (n=3) or Non-risk (n=3) based on the amount of energy mobilized from body tissue during this period (Risk>40 MJ ME/d; Non-risk<25 MJ ME/d), and gDNA from all viable placentae was pooled by sow. A MethylMiner kit was used with a 2 step elution process (600mM NaCl and 2000mM NaCl) to enrich for methylated sequences in each sow pool using 20mg of sheared gDNA. The libraries were then barcoded and prepared for SOLiD 5500 sequencing. MEDIPS software was used to identify DMRs that are associated with Risk. The present study identified DMR\'s that were associated with ‘Risk\', thus demonstrating that the manner in which the sow responds to lactational feed restriction influences the methylome of her next offspring. Risk was associated with hypomethylation compared to Non-risk, and Ingenuity pathway analysis of DMR\'s that were associated with genes identified genes associated with Metabolic Disease as being overrepresented.

Theme:

- Epigenomics

Rapidly Cleaving Bovine Two-Cell Embryos Have Better Developmental Potential and a Distinctive mRNA Pattern

Orozco-Lucero E,[1] Dufort I,[1] Robert C[1] and Sirard M-A[1,*]

[1]Centre de Recherche en Biologie de la Reproduction (CRBR), Faculté des Sciences de L'agriculture et de L'alimentation, Département des Sciences Animales, Pavillon INAF, Université Laval, Québec, Qc, Canada

*Corresponding author: Marc-André Sirard. Centre de Recherche en Biologie de la Reproduction, Faculté des Sciences de L'agriculture et de L'alimentation, Département des sciences animales, Pavillon INAF, Université Laval, Quebec city, Canada. G1V 0A6. Fax: 418-656-5877. E-mail: Marc-Andre.Sirard@fsaa.ulaval.ca

It is known that mammalian embryos that rapidly reach the 2-cell stage in culture have a better probability of becoming viable blastocysts. Our goals were to separate cattle 2c-embryos according to their zygotic cleavage speed and to acquire information of their global mRNA levels. Upon IVF, all embryos found cleaved by 29.5 hours post-insemination (hpi, early) were separately cultured from those that divided at 46 hpi (late-cleavers). The blastocyst rates were 46.1 ± 3.7% and 6.1 ± 3.4% for 29.5 and 46 hpi, respectively ($P < 0.01$). Seven replicates of selected 2c-embryos were collected at each time point for microarray characterization (n = 4), RT-qPCR (n = 3), and the rest left in culture for blastocyst evaluation. A total of 774 probes were preferentially present in early, and 594 in late-embryos (fold change ± 1.5, $P < 0.05$) with outstanding contrasts related to cell cycle, gene expression, RNA processing, and protein degradation. A total of 12 transcripts were assessed by RT-qPCR, from which ATM, ATR, CTNNB1, MSH6, MRE11A, PCNA, APC, CENPE, and GRB2 were in agreement with the hybridization results. Since most of these molecules are directly or indirectly associated not only with cell cycle regulation and DNA damage response, but also with transcription control, results here strongly suggest key roles of such biological functions in the mammalian preimplantation development. Project funded by the NSERC EmbryoGENE network. EOL is supported by a FRQNT studenship from the Government of Quebec and the EmbryoGENE network.

Spatiotemporal Expression of DNA Demethylation Enzymes and Histone Demethylases in Bovine Embryo

Pagé-Larivière F; Sirard M-A

Département de sciences animales, Université Laval, Québec, Canada

Context: Fertilization in bovine causes profound changes in the epigenetic profile that affect both DNA methylation patterns and post-translational histone modifications. These dynamic changes have a great potential for activating pluripotency genes and unfolding certain chromatin regions to recruit different transcription factors. Surprisingly, while the fundamental function of epigenetic remodeling is well understood, the bases of the process are still unknown. Recent developments in epigenetics suggest a multi-step demethylation process that would imply the prior modification of the methylated cytosine or methyl group followed by a DNA repair mechanism implicating enzymes as Aicda and Tet dioxygenase. From one specie to the other, their functions seem to differ and are not yet well characterized in mammals. Histones have, for their part, many associated and specific lysine demethylases (KDM). Their expression profile in large mammals is not well characterized. Objective: Drawing a spatiotemporal expression profile for each of the genes studied to increase our understanding of the molecular interactions following fertilization in early bovine embryo stages. Method: Bovine oocytes and embryos at various preimplantation stages were collected following in vitro fertilization protocol. Total RNA for Aicda, Tet1, Tet2, Tet3, KDM3A, KDM4A, KDM4C and KDM5B was amplified by real-time PCR. Other embryo pools were collected and protein localization of the genes studied was characterized. Results: Tet3 dioxygenase was present in the very first embryo stages, in contrast to Tet1 and Aicda. Histone demethylases KDM3A, KDM4A and KDM4C were expressed before and after embryonic genome activation while KDM5B was mainly expressed during the blastocyst period. Conclusion: DNA demethylation following fertilization in bovine is not accomplished by Aicda but most probably by TET3. Histone demethylation is carried out by, among others, KDM3A, KDM4A and KDM4C, which could act in sequence to demethylate histones prior to DNA demethylation of the female chromosomes

Theme:

- Embryo development
- Epigenomics

Cumulus Cell Transfer, Poly(A) mRNA Degradation and Phosphocreatine as Drivers of ATP Synthesis During Oocyte Maturation

Scantland S, Cagnone G, Fournier E, Macabelli C.H, Gagne D, Robert C

Laboratoire de génomique fonctionnelle du développement embryonnaire, Centre de recherche en biologie de la reproduction, Pavillon des services, Faculté des sciences de l'agriculture et de l'alimentation, Université Laval, Québec, Canada

Background: Oocytes need large amounts of ATP to allow maturation, fertilization and, later on, early embryonic development. However, energy production within the oocyte faces many obstacles: mitochondria take on an immature form with limited oxidative phosphorylation potential, and glycolysis is almost absent, with oocytes having to obtain its sub-products from the cumulus. To figure out how the oocyte paliates this energetic deficit during this crucial stage of its development, we turned ourselves toward the adenosine salvage pathway to determine if it might represent an alternative mechanism for energy production. Results: Many enzymes from the adenosine salvage pathway (adenylate kinases and creatine kinases) are present and active during oocyte maturation. When they are inhibited, oocytes show an energetic phenotype of apoptosis. This supports the hypothesis that this pathway plays an important role in energy production. To stimulate the adenosine salvage pathway, considerable sources of AMP must be available. When oocytes are cultured under denuded form or when the enzyme responsible for degradation of the poly(A) tail is inhibited, available quantities of ATP fall drastically. These results support our hypothesis that cumulus cells can transfer molecules which can lead to the accumulation of AMP and ATP within the oocyte and that degradation of the poly(A) tail represents an important source of AMP. When an energetic deficit is induced within the cell, addition of AMP to the culture media allows the restoration of ATP levels above the normal and creates a rescue phenotype. Conclusion: Adenylate kinases and creatine kinases are both enzymes which lead to the de novo formation of ATP within the oocyte. To achieve this, cumulus cells must transfer molecules leading to the accumulation of AMP within the oocyte. AMP molecules released by the degradation of the poly(A) tail of mRNAs during maturation also play an important role in this process.

Theme:

- Oocyte competence

Activity of NRF2 Mediated Oxidative Stress Response and Embryo Survival Under Oxidative Stress Condition

Amin A[1,2]; Held E[1]; Gad A[2]; Salilew-Wondim D[1]; Prastowo S[1]; Hoelker M[1]; Rings F[1]; Tholen E[1]; Udin J[1]; Looft C[1]; Schellander K[1]; Tesfaye D[1].

[1]Animal Breeding and Husbandry Group, Institute of Animal Science, University of Bonn, Bonn, Germany
[2]Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt

In the current study, we aimed to examine whether the preimplantation bovine embryos are able to activate Nrf2-mediated oxidative stress response and trigger their competence under oxidative stress conditions. For this, zygotes, 2-, 4-, 8-cell and blastocysts stage embryos were generated under low (5%) or high (20%) oxygen in vitro culture conditions. The Nrf2 and its downstream genes were analyzed from each stage and embryo groups and the level of reactive oxygen species (ROS) was evaluated in the blastocysts using H2DCFDA fluorescent probe. The expression level of Nrf2, Nrf2 inhibitor (Keap1) and selected Nrf2 downstream antioxidant genes were measured using qPCR. High ROS level was observed in the blastocysts in 20% compared to the 5% oxygen culture groups. The mRNA transcript level of Nrf2 and its target antioxidant genes (CAT1, HMOX1, NQO1, PRDX1, SOD1 and TXN1) were found elevated in 8- and 16-cell and blastocyst stage embryos under high oxygen compared to low oxygen group, but Keap1 showed opposite expression pattern. In order to investigate the association of NRF2 activity with embryo competence we have compared the expression of Nrf2 and Keap1 mRNA and protein in early versus late cleaving 2-cell embryos and early versus late blastocyst stage embryos. Subsequently, Nrf2 and its antioxidant target genes expression was increased in early compared to late blastocyst stage embryos. Similarly, a more active nuclear Nrf2 protein has been observed in early or competent embryos compared to the late ones. This was accompanied by reduced level of ROS in the early developing embryo compared to the late ones. In conclusion, this study demonstrated that under oxidative stress conditions, pre-implantation bovine embryos are able to activate the NRF2-mediated oxidative stress response pathway, which is found to be correlated with their survival under in vitro condition.

Theme:

- Embryo development

EDMA: A Platform for Parallel Analysis of Bovine Embryo Genome-wide Methylome and Transcriptome

Shojaei Saadi H.A , Gagné D, Fournier E, Laflamme I, Sirard MA, Robert C

Centre de Recherche en Biologie de la Reproduction (CRBR), Département des Sciences Animales, INAF, Université Laval, Québec, Canada

Recently, we developed EmbryoGENE DNA Methylation Analysis (EDMA) platform to survey mainly embryonic microenvironment by contrasting the methylome and transcriptome of bovine blastocysts using dedicated microarray based platform. The EDMA allows robust mapping of genome-wide bovine DNA methylation using a minimum requirement of input gDNA (7-8 ng corresponding to gDNA obtained from 10 expanded blastocysts). Furthermore, EDMA is working for any bovine tissues, including sperm. Here, using EDMA we examined the effects of S-adenosyl methionin (SAM) as the global methtyl donor on in vitro produced bovine embryos. Furthermore, in a separate study using frozen-semen we investigated the genome-wide DNA methylation profile of twin bulls (n=4) produced by embryo splitting as one of the current ART method applied on bovine embryos for duplication of high genetic merit bulls. For the first study, the results showed global DNA methylation was increased in SAM-treated vs. control embryos. Several genes as well as noncoding RNAs were affected by SAM treatment. The global parallel analysis of both methylome and transcriptome highlighted several commonly affected functions and molecular networks. By overlaying transcritomic and methylomic data limiting the loci identification to the ones fitting the general dogma of DNA methylation control over gene expression (more methylation = les expression or vice-versa), a subset of 13 highly relevant genes/loci were identified amongst which the Beckwith-Wiedemann locus. In conclusion, this study showed that SAM effects both the embryonic DNA methylation and transcriptome profiles. Overlaying both types of information provides a powerful tool reducing background noises to highlight highly relevant targets. For the second study, we observed genome-wide differences in DNA methylation between twin bulls and these differences were in loci where QTLs were found. At this stage we do not know the extent of the involvement of the bull sperm DNA methylation on the performance of the offspring.

Theme:

- Embryo development
- Culture conditions / Asssisted reproductive technologies
- Epigenomics
- New technologies

EmbryoGENE: A Transcriptomic Approach to Understanding Embryonic Stress During In Vitro Culture

Cagnone G[1,2,], Sirard MA[1]

[1]Centre de Recherche en Biologie de la Reproduction (CRBR), Département des Sciences Animales, INAF, Université Laval, Québec, Canada
[2]Monash University, Australia

The culture of early embryo is well recognized to impact the development and quality of resulting blastocysts; however the signaling pathways that are involved in the embryo’s stress response are poorly understood. Here, we present the data from molecular analysis of embryonic transcriptome in regard to the stress induced by the culture environment. Transcriptome analysis allows to determinate the expression profile of numerous genes and to uncover putative pathways that are significantly affected in stressed embryos. From these pathway analyses, it appears that mitochondrial signaling is central in embryonic stress response and would represent a key factor of embryonic plasticity under energetic compromise. Moreover, inflammatory signaling and modifications of the extracellular matrix are also observed in the transcriptomic response to external stress, and these responses could interfere with proper embryonic recognition of the maternal immune system. All together, these results allow a better understanding of the culture impact on early development as well as defining potential embryonic markers of developmental compromise.

Relative Abundance of Extra-Cellular miRNAs in Bovine Follicular Fluid: Implication for Cell-Cell Communication During Oocyte Growth

Sohel MMH, Seifi Noferesti S, Salilew-Wondim D, Hoelker M, Rings F, Tholen E, Udin J, Looft C, Schellander K, Tesfaye D

Animal Breeding and Husbandry Group, Institute of Animal Science, University of Bonn, Germany

Here we aimed to investigate the expression pattern of the circulating extra-cellular miRNAs in exosome and non-exosomal fraction of follicular fluid consisted of fully grown or growing oocytes and to validate exosome mediated cell-cell communication between follicular cells. For this, follicles of 5-8 mm diameter (n=120) were isolated and individual COCs were subjected to brilliant cresyl blue (BCB) staining and classified as BCB+ (fully grown, n=60) and BCB- (growing, n=60) groups. The corresponding follicular fluid, granulosa cells and theca cells were used for further molecular analysis. MiRNAs isolated from exosomal and nonexosomal portion of follicular fluid from the two categories was used for cDNA synthesis and subsequent analysis using a human miRNA PCR array (with 745 miRNA). Results revealed that 25 miRNAs (16 up and 9 down regulated) in exosomes and 30 miRNAs (21 up and 9 down regulated) in non-exosomal portion of follicular fluid were differentially expressed (fold change ≥ 2 and $p < 0.05$) between growing vs. fully grown oocyte group. Among these miRNAs, miR-654-5p and miR-640 were found to be enriched in exosomal portion of follicular fluid containing growing oocytes, while miR-526b* and miR-373 were highly abundant in exosomal portion of follicular fluid containing fully grown oocytes. In-silico analysis of miRNAs enriched in follicular fluid containing growing oocytes revealed that genes are involved in different signaling pathways like ubiquitin mediated proteolysis, focal adhesion, oocyte meiosis, MAPK signaling pathways to be potential targets, which are crucial for follicular development. Co-culture of granulosa cells with PKH67 dye labeled exosomes showed successful uptake of exosomes by those cells and subsequently elevated the endogenous miRNAs level in 2.5-5.5 fold. In conclusion, the present study highlighted the oocyte growth status dependent differences of circulating miRNA profiles in follicular fluid and exosome mediated uptake of those molecules by surrounding cells in follicular microenvironment.

Bovine Ovarian Hyperstimulation Induced Changes of Circulating Extra-Cellular miRNAs in Follicular Fluid and Blood Plasma

Seifi Noferesti S, Hölker M, Sohel MMH, Salilew-Wondim D, Rings F, Schellander K, Tesfaye D

Institute of Animal Science, Animal Breeding and Husbandry Group, University of Bonn, Germany

Circulatory noncoding small RNAs (miRNAs), which are present in various body fluids, are reported to be potentially used as biomarkers for disease and pregnancy. The present study was conducted to investigate the effect of ovarian hyperstimulation on the expression pattern of circulatory miRNA in follicular fluid and blood plasma. For this, Simmental heifers (n=12) were synchronized using a standard synchronization protocol and six of them were hyperstimulated using a standard superovulation protocol. Following this, while blood samples were collected at 0 (onset of oestrous), 3rd and 7th day, follicular fluid samples were aspirated from dominant follicles at the day 0 from all animals. Total RNA including miRNA was isolated from plasma samples of both groups at day 7 and follicular fluid at day 0 and expression profiling of miRNA was performed using the human miRCURY LNA™ Universal RT miRNA PCR array system with 745 miRNA primer assays. Of the 24 miRNAs, which were differentially expressed in blood plasma between hyperstimulated and unstimulated animals, 9 miRNAs including miR-127-3p, miR-494, miR-147, miR-134 and miR-153 were down regulated and 15 miRNAs including miR-34a, miR-103, let-7g, miR-221 were found to be up regulated in the hyperstimulated animals. Similarly, 66 miRNAs were found to be differentially expressed in follicular fluid derived from hyperstimulated and unstimulated groups. Out of these, while 32 miRNAs, were down regulated, while 34 were up regulated in follicular fluid aspirated from hyperstimulated animals. Ingenuity pathway analysis (IPA) of potential target genes of candidate miRNAs, which are dysregulated due to ovarian hyperstimulation, revealed axonal guidance signaling and Wnt ß-catenin signaling pathways to be the dominant ones. In conclusion, this study revealed ovarian hyperstimulated resulted in changes in expression profile of circulatory miRNA in blood and follicular fluid.

Theme:

- Maternal environment

Global Transcriptome and Proteome Analysis of Bovine Cumulus Cells in Relation to Maturation and Developmental Competence of Enclosed Oocyte

Uzbekova S, Gargaros A, Auclair S, Harichaux G, Labas V, Dalbies-Tran R

Physiology of Reproduction and Behaviour research unit, INRA, Nouzilly, France

During maturation, mammalian oocyte is metabolically supported by surrounded cumulus cells (CC) through bilateral exchange of different nutritive and regulatory molecules. Age of animals and maturation conditions affect oocyte potential to accomplish meiosis and to develop in vitro. We compared transcriptomic and proteomic profiles of CC enclosing either immature oocytes from prepubertal calves (IMP) and adult cows (IMA), or in vivo mature oocytes (MAT) and those after in vitro maturation (IVM). Differential genes and proteins ($p<0.05$) were identified by quantification of 10106 genes detected using bovine microarrays (300ng total RNA, 6 replicates) and of 2378 proteins identified using nano-liquid chromatography coupled to high resolution mass spectrometry (10µg total proteins, 3 replicates). In CC, abundance of 30.3% of transcripts and 19.6% of proteins differed between IMA and MAT, whereas IVM changed 16.3% of transcriptome and 18.0% of proteome as compared to MAT. Oocyte transcriptome (25 oocytes, 4 replicates) displayed 20.1% of difference between IMA and MAT, and 1.8% of transcripts were affected by IVM as compared to in vivo ($p<0.05$). Different metabolic functions (fatty acid and pyruvate metabolism, insulin signaling, etc) and cell-to-cell interaction (extra-cellular matrix - ECM, focal adhesion) were enriched in differentially expressed genes in CC. Differential proteins were mostly involved in regulation of transcription (chromosome organisation, nuclear acids binding) and in cellular metabolism (protein and lipid binding, enzyme inhibitor and regulator activity, secretion and glycosylation). In the oocytes, list of differential genes was enriched in those encoded by mitochondrial genome and involved in nuclear acids binding and metabolic functions. All these data corroborate with functioning of CC as metabolic support for the oocyte during maturation and in production of ECM to prepare ovulation and fertilization; also a number of novel factors related to oocyte maturation and quality was revealed. Sponsored by OSCILE and Ovogenae-2 projects (ANR and Apis-gene).

Theme:

- Oocyte competence

FECUND EU-project: Optimisation of Early Reproductive Success in Dairy Cattle Through the Definition of New Trait and Improved Reproductive Biotechnology

Iamartino D[1], Lonergan P[2], Ramelli P[1], Forde N[2], Fair T[2], Besenfelder U[3], Blanco A[4] and Williams J[1]

[1] Fondazione Parco Tecnologico Padano, Via Einstein, 26900, Lodi, Italy;
[2] University College of Dublin, Belfield 4, Dublin, Ireland;
[3] University of Wien, Veterinaerplatz 1, 1210 Vienna, Austria;
[4] University College of Dublin, Conway Institute, Flow Cytometry Core, Belfield 4, Dublin, Ireland.

The FECUND project, funded by the European Union, is investigating the problems of reproductive success in cattle using interdisciplinary approaches of biology, physiology, -omics and bioinformatics to integrate in vivo and in vitro studies. The project will address factors affecting early reproductive success and use the results to facilitate genomic selection for improved fertility, and to improve artificial reproductive biotechnology. FECUND is studying the mechanisms underlying differences in reproductive success between heifers and cows, and will define the genetic vs management factors involved. The central theme is to discover the reasons for fertilization failure and events prior to implantation resulting in embryonic loss. Focussed “animal models” will address the factors impacting on the establishment of a pregnancy, in order to assess whether dairy cow infertility is primarily a genetic or metabolic problem. The FECUND Energy Balance model compares lactating Holstein cows vs non-lactating cows and heifers, all with similar production and fertility EBVs. Twenty five pregnant cows and 6 heifers were managed under strictly controlled and standardised conditions, and at calving 13 of the cows entered a normal lactation while 12 were dried off. Heifers and cows were synchronised, between 35-45 days post partum for cows, using a GnRH/CIDR/PG protocol with GnRH administered 36 h after CIDR removal and embryos were transferred 7 days later. Blood samples were collected at days 0, 3, 10, 13, 16,19 and analysed for immune parameters. All animals were slaughtered at day 19 when 5 (38%) of the lactating cows were found to be pregnant, 8 (66%) dry cows and 4 (66%) heifers. Reproductive organs, follicular and oviduct fluid, uterus and embryos were collected and stored. Samples are being analysed to reveal molecular and physiological variation among the three groups: lactating vs dry cows and heifers.

Gene Expression Profiling of In Vivo-Derived Female and Male Day 11 Porcine Embryos

Zhou C, Tsoi S, Dixon WT, Foxcroft GR, Dyck MK

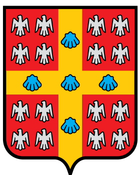
Swine Reproduction-Development Program, Swine Research & Technology Centre, University of Alberta, Edmonton, Alberta, CANADA T6G 2P5

Male and female mammalian embryos display differences in growth rates, metabolism, and gene expression level during the pre-implantation period of embryonic development. These differences may be associated with further development and survival of the embryos. To better understand the mechanisms of the sex-associated differences during early embryonic development, comparative transcriptomic analysis of in vivo-derived porcine day 11 (D11, before elongation) embryos was performed with a custom designed porcine embryo-specific microarray (EMPV1 array http://embryogene.ca/). Total RNA samples were extracted from pools of 5 identical embryos, and 3 biological replicates from female and male D11 embryos were analyzed following a reference design. Microarray data was analyzed using the FlexArray software, Ingenuity® Pathway Analysis (IPA) software and DAVID functional annotation tool. The microarray analysis positively detected 11419 genes from the D11 embryos, and 64 of these genes displayed significant (|fold change|≥2, p-value<0.05) differential expression between female and male embryos. Significant changes in genes associated with important biological functions including "Cellular Growth and Proliferation", "Embryonic Development", "Tissue Morphology", and "Nervous System Development" were observed between female and male embryos. In addition, "histone modification"-associated genes were significant enriched in the differentially expressed genes between female and male D11 embryos. More than a 2 fold up-regulation of three histone and chromatin modification-associated genes (BRCC3, KDM1A, and RNF20) were observed in female D11 embryos. A 2.3 fold down-regulation of HIST2H2BE, a member of the histone H2B family, was also observed in female D11 embryos. Results from the present study revealed several significantly altered gene networks and biological processes between female and male D11 embryos. In addition, our results indicating that the female and male embryos may have differences in the regulation of histone and chromatin modification during the early embryonic development. This research is supported by the EmbryoGENE Strategic Research Network (NSERC).

Theme:

- Embryo development

List of Participants



List of Participants

4th Mammalian Embryo Genomics meeting

Name	Oganization	Email	Pages
Goran Andersson	Swedish University of Agricultural Sciences	Goran.Andersson@slu.se	
Fazl Ashkar	University of Guelph	fashkar@uoguelph.ca	36
Luis Manuel Baldoceda Baldeon	Université Laval	luis-manuel.baldoceda-baldeon.1@ulaval.ca	32, 37, 38
Patrick Blondin	L'Alliance Boviteq	blondinpa@boviteq.com	32, 37, 38
Rodrigo Bohrer	McGill University	rodrigocbohrer@hotmail.com	
Vilceu Bordignon	McGill University	vilceu.bordignon@mcgill.ca	
Hannah Brown	University of Adelaide	hannah.brown@adelaide.edu.au	33, 39
Julieta Caballero	Université Laval	julieta.caballero.1@ulaval.ca	40, 59
Carolina Cardenas	Université Laval	carocar2002@hotmail.com	21
Rozenn Dalbies-Tran	INRA Centre Val de Loire	Rozenn.Dalbies@tours.inra.fr	71
Isabelle Dufort	Université Laval	isabelle.dufort@fsaa.ulaval.ca	28, 36, 41, 43, 47, 49, 52, 61, 63
Véronique Duranthon	INRA - Jouy en Josas	veronique.duranthon@jouy.inra.fr	24
Michael Dyck	Univeristy of Alberta	michael.dyck@ualberta.ca	20, 48, 56, 62, 73
Trudee Fair	University College Dublin	trudee.fair@ucd.ie	25, 31, 72
Eric Fournier	Université Laval	eric.fournier@fsaa.ulaval.ca	40, 41, 48, 59, 65, 67
George Foxcroft	University of Alberta	george.foxcroft@ualberta.ca	20, 48, 62, 73
Ahmed Gad	Cairo University	ahmed.y.gad@gmail.com	28, 43, 44, 66
Dominic Gagné	Université Laval	dominic.gagné@fsaa.ulaval.ca	32, 43, 44, 67
Tracy Gartner	University of Alberta	tracy.gartner@ualoerta.ca	
Annie Girard	Université Laval	annie.girard.4@ulaval.ca	17, 46, 47
Karen Goossens	Ghent University	Karen.Goossens@ugent.be	45, 58
Jason Grant	University of Alberta	jgrantk@gmail.com	20, 48, 62
Nicolas Guillemin	Université Laval	nicolas-philippe-alexandr.guillemin.1@ulaval.ca	49
Sonia Heras	Ghent University	sonia.herasgarcia@ugent.be	34, 50
Morteza Hosseini	Université Laval	sayyed-morteza.hosseini.1@ulaval.ca	51
Isabelle Hue	INRA - Jouy en Josas	isabelle.hue@jouy.inra.fr	29, 53
Patrice Humblot	Swedish University of Agricultural Sciences	patrice.humblot@slu.se	
Poul Hyttel	University of Copenhagen	poh@sund.ku.dk	22, 59
Peter J. Hansen	University of Florida	pjhansen@ufl.edu	16

Embry

Genomics Epigenomics
Nutrition Environment

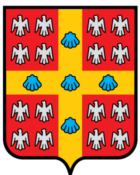
List of Participants

4th Mammalian Embryo Genomics meeting

Name	Oganization	Email	Pages
Jiri Kanka	Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic	kanka@iapg.cas.cz	54
Rémi Labrecque	Université Laval	remi.labrecque.2@ulaval.ca	57
Serge Lacaze	MIDATEST	serge.lacaze@midatest.fr	
Denise Laskowski	Swedish University of Agricultural Sciences	denise.laskowski@slu.se	
Jo Leroy	University of Antwerp	jo.leroy@ua.ac.be	18
Lyne Létourneau	Université Laval	lyne.letourneau@fsaa.ulaval.ca	21
Wenwen Li	Ghent University	wenwen.li@ugent.be	58
Alberto Luciano	University of Milan	alberto.luciano@unimi.it	12, 57
Angus Macaulay	Université Laval	angus.macaulay.1@ulaval.ca	59
Alan Mileham	Genus	amileham@pic.com	
Erik Mullaart	CRV BV	astrid.van.der.a@crv4all.com	
Lucie Nemcova	Institute of Animal Physiology and Genetics CAS, v.v.i.	nemcova@iapg.cas.cz	30, 54, 60
Julie Nieminen	Université Laval	julie.nieminen@fsaa.ulaval.ca	
Anne-Laure Nivet	Université Laval	anne-laure.nivet.1@ulaval.ca	17, 61
Gina Oliver	University of Alberta	goliver@ualberta.ca	62
Ernesto Orozco-Lucero	Université Laval	ernesto.orozco-lucero.1@ulaval.ca	63
Florence Pagé-Larivière	Université Laval	florence.page-lariviere.1@ulaval.ca	64
Graham Plastow	University of Alberta	plastow@ualberta.ca	62
Randall Prather	University of Missouri	pratherr@missouri.edu	15
Francois Richard	Université Laval	francois.richard@fsaa.ulaval.ca	17, 55, 59
Claude Robert	Université Laval	claude.robert@fsaa.ulaval.ca	23, 32, 37, 38, 48, 59, 63, 65, 67
Peter Sandøe	University of Copenhagen	pes@sund.ku.dk	22
Sara Scantland	Université Laval	sara.scantland.1@ulaval.ca	40, 65
Karl Schellander	University of Bonn	ksch@itw.uni-bonn.de	19, 27, 28, 43, 66, 69, 70
Habib Shojaei	Université Laval	habib-allah.shojaei-saadi.1@ulaval.ca	67
Kevin Sinclair	University of Nottingham	kevin.sinclair@nottingham.ac.uk	
Jaswant Singh	University of Saskatchewan	jaswant.singh@usask.ca	13, 17

Name	Oganization	Email	Pages
Marc-André Sirard	Université Laval	marc-andré.sirard@fsaa.ulaval.ca	13, 17, 28, 41, 43, 46, 47, 48, 49, 52, 55, 57, 59, 61, 63, 64, 67, 68
Dawit Tesfaye	University of Bonn	dtes@itz.uni-bonn.de	19, 27, 28, 43, 66, 69, 70
Stephen Tsoi	University of Alberta	stsoi1@ualberta.ca	56, 73
Christopher Tuggle	Iowa State University	cktuggle@iastate.edu	
Svetlana Uzbekova	INRA Centre Val de Loire	svetlana.uzbekova@tours.inra.fr	71
Brian Van Doormaal	Canadian Dairy Network	Brian@cdn.ca	
Adriana Verduzco	L'Alliance Boviteq	averduzco@boviteq.com	
Christian Vigneault	L'Alliance Boviteq	vigneaultc@boviteq.com	32, 37, 38
John Williams	Parco Tecnologico Padano	john.williams@tecnoparco.org	32, 72
Eckhard Wolf	LMU Munich	ewolf@lmb.uni-muenchen.de	14
Chi Zhou	University of Alberta	czhou4@ualberta.ca	20, 73

Invited Speakers Bios



Alberto Luciano

Dr Luciano received is PhD in Biotechnology applied to Veterinary Sciences from the University of Milan, Italy, in 1998. He then pursued his studies through 2 post-doctoral fellowships on Nephrology and Reproductive Physiology at S. Carol Borromeo Hospital, Milan and the University of Connecticut, respectively. He holds an associate professor position at the Faculty of Veterinary Medicine of the University of Milan since 2006. His research interests cover Reproductive and developmental biology as well as Biotechnology applied to assisted reproduction in domestic mammals and biomedicine.

Jaswant Singh

Dr Singh received his undergraduate and master degrees in Veterinary Science and Animal Husbandry from Ludhiana, India. After teaching applied anatomy and embryology to veterinary and graduate students at Punjab Agricultural University, he moved to Canada to complete his PhD studies at the University of Saskatchewan. Now professor of Veterinary Biomedical Sciences at Western College of Veterinary Medicine, University of Saskatchewan, his research interests are centered on the use and further development of the bovine model for the study of ovarian function in humans.

Eckhard Wolf

Dr Wolf studied Veterinary Medicine at the Ludwig-Maximilian University (LMU), Munich, Germany (1982-87) and received his Dr. med. vet. from the LMU Munich in 1990. After postdoctoral research in Munich and Vienna he finished his Habilitation for Animal Breeding and Genetics at the University of Veterinary Medicine, Vienna, Austria (1994). Since 1995 he is Head of the Institute for Molecular Animal Breeding and Biotechnology, Gene Center, LMU Munich, and since 2003 Director of the Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU Munich. His research interests are interactions of gametes and embryos with the maternal environment. In addition his lab is specialized in the generation and characterization of genetically engineered pigs for xenotransplantation, diabetes research and human monogenic diseases.

Randall S. Prather

Dr. Prather's earned his BS and MS from Kansas State University, and PhD and Postdoc from the University of Wisconsin-Madison. While at Wisconsin he cloned by nuclear transfer the first pigs, and some of the first cattle. His lab has since made over 800 cloned pigs at MU representing over 30 different genetic modifications for agriculture and medicine. He is the Director of the NIH-funded National Swine Resource and Research Center. He is currently a Curators' Professor with the title Distinguished Professor of Reproductive Biotechnology in the Division of Animal Science at the University of Missouri.

Peter J. Hansen

Peter J. Hansen is L.E. “Red” Larson Professor of Animal Sciences in the Dept. of Animal Sciences at the University of Florida. His research interests center around the basic mechanisms controlling the establishment and maintenance of pregnancy and development of methods to improve fertility. Particular emphasis is placed on elucidating effects of elevated temperature on early embryonic development, identifying genes controlling embryonic survival and characterizing interactions between the immune system, the reproductive tract, and the embryo. Another focus is on development of methods to increase profitable uses of embryo transfer. Dr. Hansen joined the faculty at Florida as an assistant professor in the College of Veterinary Medicine in 1984 and transferred to the Dairy Science Department (now Animal Sciences) in 1986. His formal education was at the University of Illinois and University of Wisconsin. He received postdoctoral training in the Department of Biochemistry and Molecular Biology at the University of Florida and spent a sabbatical leave at the University of Guelph. He currently serves as President of the International Embryo Transfer Society and International Congress of Animal Reproduction and is Past-President of the American Society for Reproductive Immunology.

Marc-André Sirard

Dr Sirard received his doctorate in Veterinary Medicine from University of Montreal in 1981 and went on to earn a PhD in Experimental Medicine at Laval University followed by postdoctoral studies at University of Wisconsin, Madison. The methods he developed to produce the first cows conceived in vitro are now used around the world. Since 2000, he holds a Canada Research Chair in Animal Genomics Applied to Reproduction. His laboratory now focuses mainly on oocyte competency in human and bovine. He is the principal investigator and co-director of the EmbryoGENE Network through which new transcriptomics and epigenetics tools were developed and which have led to countless collaborations throughout the world.

Karl Schellander

Dr Schellander studied Veterinary Medicine in Vienna where he got his PhD in 1981. He then went on to receive his habilitation qualification in 1991 in Vienna. He is a Professor at the Institute of Animal Science at the University of Bonn, Germany since 1996. His research focus is on embryogenomics in cattle, with special emphasis on embryo transcriptome related to developmental competence.

Michael Dyck

Michael Dyck obtained a B.Sc. (Ag.), with the honour of distinction, from the University of Manitoba and then pursued graduate studies at the University of Guelph where he obtained a M.Sc. with a specialization in reproductive physiology. Dr. Dyck then went on to work in the field of artificial insemination in swine at First Choice Genetics in Woodstock, Ontario and later joined ReproMed Ltd. in Toronto, Canada's largest human sperm banking facility. He then enrolled in Laval University's Ph.D. program where he was a FCAR and NSERC Scholar. During his Doctoral training, Dr. Dyck conducted research into the use of transgenesis to alter physiological traits in swine. Upon completing his PhD he joined TGN Biotech Inc., Quebec City as an NSERC Industrial Research Fellow and eventually assumed the position of Director of Transgenics and Cell Biology for this company. Dr. Dyck then joined the Faculty of Agricultural, Life and Environmental Sciences at the University of Alberta in 2004 and has been conducting research focused on the development and application of molecular techniques and reproductive technologies, in collaboration with the pork production industry, to improve reproductive efficiency in swine. He has been a lead researcher with the Pan-Canadian NSERC Strategic Research Network – EmbryoGENE since its inception and assumed the role of Co-Director of the Network in July 2012.

Lyne Létourneau

Lyne Létourneau is Full Professor in the Department of Animal Science at Laval University, where she teaches agriculture and food ethics. She holds a doctorate in law from the University of Aberdeen (2000). Combining her legal background with an expertise in ethics, her research interests focus on the interface between regulation and ethics in agricultural biotechnology and animal protection.

Peter Sandøe

Dr Sandoe studied Philosophy at the University of Copenhagen and the University of Oxford where he received his PhD in 1988. He then held Associate Professor and Research professor at the University of Copenhagen in the fields of philosophy and bioethics. He serves as Chairman of th eDanish Ethical Council for Animals and is an Honorary Professor of Animal Ehtics at the University of Nottingham.

Claude Robert

Dr Robert earned his undergraduate degree in the field of biochemistry from Sherbrooke University and went on to earn his MSc dregree at the facilities of the Federal Government within the Agri-Food Canada research station in Lennoxville. His research focused on finding biomarkers for backfat thickness in pigs. He continued his studies at the PhD level at Laval University looking for biomarkers of oocyte developmental competence. His Post-doctoral studies were conducted at Guelph University on the transcriptomic comparison of ART derived embryos. Dr Robert is running his independent research program since 2003 on the study of early embryonic development and the consequences of Assisted Reproductive Technologies. He led the team that developed EmbryoGENE's technological platforms.

Véronique Duranthon

After training as an agronomist engineer, Dr. Duranthon specialized in molecular developmental biology during her PhD studies at the Pasteur Institute. She started her research on the maternal-to-embryo transition and embryonic genome transcriptional activation in non-murine mammalian embryos, taking advantage of the rabbit and bovine as models given their delayed onset of embryonic transcription. More recently, she developed studies on epigenetic reprogramming and on the long-term effects of embryo microenvironment on fetal and post-natal phenotypes (embryo metabolic programming). She is currently a group leader (INRA Director of Research) and assistant director of the Laboratory of Developmental Biology and Reproduction _INRA Jouy en Josas. Her research group works on genome reprogramming and the control of pluripotency during early development, mainly in non-murine mammalian species.

Trudee Fair

Dr Fair obtained her undergraduate and master degrees from University College Dublin with a specilization in Animal production. She received her PhD from the Royal Veterinary and Agricultural University Denmark in 1996. She then went on to complete postdoctoral studies ath INAR, Nouzilly in France and at University College Dublin in Ireland where she now holds a permanent academic position at the School of Agriculture & Food Science. She is a principle Investigator for the SFI Funded Reproductive Biology Research Cluster: Investigating the Factors affecting dairy cow fertility. Her research interests focus on bovine oocyte growth and maturation, key checkpoints and pathways associated with acquisition of oocyte developmental competence, establishment and stabilization of maternal imprints during bovine oocyte growth, epigenetic effect of the maturation environment, progesterone regulated networks associated with oocyte competence and the role of the maternal immune system in cow fertility.

4th Mammalian Embryo Genomics meeting

October 9 to 11, 2013, Hilton Quebec City

Thank you to our sponsors!

Hosted by the EmbryoGENE Network / Université Laval