Epigenetics and Periconception Environment

Proceedings of the EPICONCEPT Workshop 2014
Epigenomic Toolbox: from Methods to Models

Las Palmas, Spain
07 - 09 May 2014

Editors
Marisol Izquierdo, Francisco Otero-Ferrer, Trudee Fair, Alfonso Gutiérrez-Adán, Alireza Fazeli and Ann Van Soom

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About the European Co-operation in Science and Technology

The European Cooperation in Science and Technology (COST) is the oldest and widest European intergovernmental network for cooperation in research. Established by the Ministerial Conference in November 1971, COST is presently used by more than 30 000 scientists of 35 European countries to cooperate in common research projects supported by national funds. The financial support for cooperation networks (COST Actions) provided by COST is about 1.5% (30 million EUR per year) of the total value of the projects (2000 million EUR per year). The main characteristics of COST are:

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- à la carte participation (only countries interested in the Action participate),
- equality of access (participation is also open to the scientific communities of countries which do not belong to the European Union) and
- flexible structure (easy implementation and management of the research initiatives).

As precursor of advanced multidisciplinary research COST has a very important role in shaping the European Research Area (ERA). It anticipates and complements the activities of the current Framework Programme for Research and Innovation (Horizon 2020). COST activities create a bridge between the scientific communities of countries and increases the mobility of researchers across Europe in many key scientific domains.
Parental stress before, during and after conception induces epigenetic changes in gametes and embryos. Such epigenetic changes may adversely affect the future health, development, productivity and fertility of the offsprings. Our cooperation in this COST Action focuses on the timeframes and mechanisms of these epigenetic modifications. We plan public engagement activities to inform the general public on the importance of the epigenome and the periconception environment in future food production, health and welfare. We aim to coordinate various European research activities on epigenetic control of development in order to avoid duplication, set targets and guidance for future research in this field through a large collaborative network.

www.cost.esf.org
www.cost-epiconcept-eu

Welcome from the Chairman

Dear Members of Epiconcept

I have been informed that the location for the workshop 2014 of WG1: 'Epigenomic toolbox: from Methods to Models' is amazing, and by the time you read this you will be able to see so for yourself. The only facts I knew about the Canary Islands before I arrived here is that they are volcanic, they have these little yellow birds (I presume) and they are well-loved by surfers. If you are staying a bit longer you will have plenty of time to find out if all of this is true. We are very thankful to Marisol Izquierdo and Fran Otero because they were able to find this astounding location for us, and they did all the work concerning logistics. We also do appreciate their contribution to the scientific committee including printing the conference book, organizing all these tasty meals and drinks, and organizing the presence of EL SOL!

But of course we are here for the science, and not for tourism. I am sure that the programme must be very appealing too, since we increased the number of registered attendants from less than forty a few days before the deadline, to almost one hundred. This is really impressive for such a specialized workshop.

With a glance at the programme I can imagine why this workshop is so popular. On the first day we will be informed on the latest advancements in the epigenomic toolbox, and we will learn how to analyze our data, which can indeed be challenging when you get thousands of genes being up- or downregulated. On the second day we will have an update on which methods can be used to analyse epigenetic changes, and we will also learn about the epigenetic regulation of development in oocytes and embryos. Eight specialised speakers from Canada, USA, Austria, Spain, Italy and Norway will enlighten us about their most recent research. Thank you Trudee and Alfonso for composing such an interesting programme with plenty of room for Early Stage Researchers to present their data, and to interact with the more senior scientists at this workshop.

Many thanks also to the Executive Committee, which was always stand-by during the preparation of this workshop and a special thanks goes to Laszlo Tecsi, our Epiconcept Secretary who is taking care of all administrative issues.

Bienvenido a Gran Canaria!

Ann and Alireza

Chairman of Epiconcept

Welcome from the Epigenomic Tools Working Group and the Organising Committee

As the study of epigenetics gains popularity, and the methodologies for whole epigenome investigations develop further, many newcomers to the field are faced with the challenge of working with rare and valuable gamete and embryo samples while at the same time maintaining best practice in the data analysis protocols that are used. It is from this perspective that we have designed the programme for our workshop. During the two days of our workshop we are delighted to welcome scientists with unmatched expertise in the areas of whole epigenome profiling and analysis and particularly the application of these technologies to oocyte and embryo samples, to share their knowledge and expertise with us. Please take every opportunity to benefit from their generous contribution to our meeting.

We wish to extend our gratitude to the speakers and also to Marisol and Fran who have worked so hard to arrange such a beautiful venue in a wonderful location for our meeting (encouraged most enthusiastically by Alireza).

We wish you an interesting and enjoyable meeting.

Members of the Scientific Committee

Trudee Fair, Ireland
Alireza Fazeli, United Kingdom
Alfonso Gutierrez-Adan, Spain
Patrice Humblot, Sweden
Marisol Izquierdo, Spain (Chairman)
Francisco Otero-Ferrer, Spain
Kevin Sinclair, United Kingdom
Ann Van Soom, Belgium

Programme

Day 1
Wednesday 07 May 2014

16:00 – 19:00 **Meeting Registration**

19:00 – 20:30 **Welcome Reception**

20:30 – 23:00 **Dinner**

Day 2
Thursday 08 May 2014

08:00 – 08:45 **Breakfast**

Opening of Meeting

08:45 – 09:00 **Welcome Address**

What's in the Epigenomic Toolbox?

09:00 – 09:40 **Claude Robert, Laval University, Canada**
Integrating the DNA methylome and transcriptome to better define the long term impacts of assisted reproductive technologies.

09:40 – 10:20 **Andre Eggen, Illumina, United States**
Last generation tools for the study of the genome and the epigenome.

10:20 – 10:35 **Gavin Kelsey, Babraham Institute, United Kingdom**
Profiling DNA methylation genome-wide at base-pair resolution in single oocytes.

10:35 – 10:50 **Dafni Anastasiadi, Institute of Marine Sciences, Spain**
Genome-wide alterations of DNA methylation in European sea bass (*Dicentrarchus labrax*) subjected to different environments.

10:50 – 11:20 **Coffee**

Analyse your Data!

11:20 – 11:35 **Laszlo Tecsi, University of Sheffield, United Kingdom**
Rules of Travel Reimbursements

11:35 – 12:15 **Christoph Bock, Research Centre for Molecular Medicine, Austria**
Methods for sequencing-based mapping and bioinformatics analysis of DNA methylation.

12:15 – 12:55 **Ana Conesa, Principe Felipe Research Centre, Spain**
The STATegra project: new statistical tools for analysis and integration of diverse omics data.

12:55 – 14:15 **Lunch**

Epigenetic Regulation of Development

14:15 – 14:55 **Valentina Lodde, University of Milan, Italy**
Oocyte large-scale chromatin configuration re-modelling: state of the art and perspectives.

14:55 – 15:35 **Philippe Collas, Oslo University Hospital, Norway**
Microchip assay and epigenetics of embryonic genome activation and early development.

15:35 – 15:50 **Ramiro Alberio, University of Nottingham, United Kingdom**
Paracrine effects of embryo-derived FGF4 and BMP4 during pig trophoblast elongation.

15:50 – 16:05 **Karolien Desmet, University of Antwerp, Belgium**
Elevated non-esterified fatty acid concentrations during bovine embryo culture influence DNA methylation patterns in blastocysts.

16:05 – 16:35 **Tea**

Epigenomic Toolbox: from Methods to Models

16:35 – 19:00 **Poster Presentation**

20:30 – 23:00 **Gala Dinner**

Day 3
Friday 09 May 2014

08:00 – 09:00 **Breakfast**

Epigenetic Reprogramming

09:00 – 09:40 **Manel Esteller, Bellvitge Biomedical Research Institute, Spain**
Cancer Epigenetics: from knowledge to applications.

09:40 – 09:55 **Marisol Izquierdo, University of Las Palmas, Spain**
Sparus aurata as a model for nutritional reprogramming of marine fish: Effectiveness of different developmental windows.

09:55 – 10:10 **Aneta Andronowska, Institute of Animal Reproduction and Food Research, Poland**
Does the presence of an embryo in the uterus prevent induction of luteolytic chemokines and chemokine receptors mRNA in the porcine CL?

10:10 – 10:25 **Antonio Gonzalez-Bulnes, Spanish National Institute for Agricultural and Food Research and Technology, Spain**
Modification of adiposity and metabolic features by trans-generational developmental programming in swine with thrifty genotype.

10:25 – 10:40 **Catherine Labbe, French National Institute for Agricultural Research, France**
DNA methylation pattern in fish spermatozoa after cryopreservation: a species specific alteration in cyprinids.

10:40 – 11:10 **Coffee**

11:10 – 12:00 **Roundtable**
12:00 – 13:00 **Application to Horizon 2020**

Closing of Meeting
13:00 – 13:15 **Farewell Address**

13:15 – 14:30 **Lunch**

15:00 – 18:00 **Sightseeing Tour**

Day 4
Saturday 10 May 2014

08:00 – 09:00 **Breakfast**

10:00 – 14:00 **Excursion**

Abstracts of Presentations

O-1

Oral Presentation

Alberio, Ramiro

School of Biosciences, University of Nottingham, United Kingdom

Valdez-Magana G, Rodriguez A, Zhang H, Webb R and Alberio R

Paracrine effects of embryo-derived FGF4 and BMP4 during pig trophoblast elongation

The crosstalk between the epiblast and the trophoblast is critical in supporting the early stages of conceptus development. FGF4 and BMP4 are inductive signals that participate in the communication between the epiblast and the extraembryonic ectoderm (ExE) of the developing mouse embryo. It is unknown whether a similar crosstalk operates in species that lack a discernible ExE and develop a mammotypical embryonic disc (ED). We investigated the crosstalk between the epiblast and the trophectoderm (TE) during pig conceptus elongation. FGF4 and FGFR2 were detected primarily on the plasma membrane of TE cells of peri-elongation embryos. FGF4 bound to its receptor triggered a signal transduction response that was evidenced by an increase in pMAPK in TE nuclei. Particular enrichment was detected in the periphery of the ED in early ovoid embryos. Gene expression analysis shows that CDX2 and ELF5, two genes expressed in the mouse ExE, are only co-expressed in the Rauber's layer, but not in the pig mural TE. Interestingly, these genes were detected in the nascent mesoderm of early gastrulating embryos. Analysis of BMP4 expression by in situ hybridization shows that this growth factor is produced by nascent mesoderm cells. A functional test in differentiating epiblast shows that CDX2 and ELF5 are activated in response to BMP4. The effects of BMP4 were also demonstrated in the TE cells, as shown by increased in pSMAD1/5/8. The results show that BMP4 produced in the extraembryonic mesoderm induces a SMAD response in the TE of elongating embryos. These results demonstrate that paracrine signals from the embryo induce a response in the TE prior to the extensive elongation. The study also confirms that expression of CDX2 and ELF5 is not conserved in the mural TE, indicating that although the signals that coordinate conceptus growth are similar between rodents and pigs, the GRN of the trophoblast lineage is not conserved in these species.

O-2

Oral Presentation

Anastasiadi, Dafni

Institute of Marine Sciences, Spanish National Research Council (CSIC), Spain

Anastasiadi D, Diaz N, Piferrer F

Genome-wide alterations of DNA methylation in European sea bass (Dicentrarchus labrax) subjected to different environments

An organism integrates the environmental information received during early development through epigenetic mechanisms, such as DNA methylation. This process may alter the gene expression or the ability to respond to environmental factors when experienced later in life. We have focused our studies on two types of environmental factors that confer changes in DNA methylation: temperature and farming conditions. First, we studied temperature effects on sea bass larvae using the Methylation Sensitive Amplified Polymorphism (MSAP) technique to detect global changes in DNA methylation. This method employs two isoschizomers, HpaII and MspI, the restrictive functions of which differ in their sensitivity to methylation. After two rounds of PCRs, the samples are run through capillary electrophoresis and the fragments detected are used for statistical analysis in order to compare the DNA methylation status of different groups of treatments. We applied eight temperature treatments and found statistically significant differences in global DNA methylation between all groups of fish. Next, the effect of farming conditions was examined in adult fish using next generation sequencing methodologies. Specifically, we used the Reduced Representation Bisulfite Sequencing (RRBS) technique which makes use of the restriction enzyme MspI in order to reduce the genome fraction analyzed to the 1-10% of the genome, focusing on CpG-rich regions. Samples from wild and farmed fish were run through RRBS and then sequenced by Illumina technology. The results allowed us to compare fish from different origins in terms of DNA methylation and to focus on gene promoters and gene bodies related to key biological pathways at a single nucleotide resolution. During this experimental process, we have investigated genome-wide DNA methylation alterations influenced by the environmental conditions, moving from a low resolution level informative for global differences to the single nucleotide resolution level for important genes.

O-3

Oral Presentation

Andronowska, Aneta

Department of Animal Reproduction and Food Research, Institute of Animal Reproduction and Food Research, Poland

Andronowska A, Witek KJ, Malysz-Cymborska I, Blitek A, Ziecik AJ

Does the presence of an embryo in the uterus prevent induction of luteolytic chemokines and chemokine receptors mRNA in the porcine CL?

There is a growing evidence that chemokines, produced by immune components such as leukocytes, may be directly or indirectly involved in corpus luteum (CL) formation, function and regression. The aim of the study was to verify the hypothesis that embryo signals derived from the uterus may protect CL against luteolysis. CLs were collected from mature crossbred gilts on days 8, 10, 12 and 14 of the estrous cycle and early pregnancy. Real Time PCR was used to determine chemokines (CCL2, CCL4, CCL8) and chemokine receptors (CCR1, CCR2, CCR5) mRNA expression. Data were analyzed using two-way ANOVA followed by Fisher's LSD post hoc test. During the estrous cycle the significant increase in CCL2 mRNA expression was found on day 14 ($P<0.05$), while during pregnancy CCL2 mRNA level declined on day 12 vs day 8 ($P<0.001$). Similarly to CCL2, CCL4 mRNA increased on day 14 of the estrous cycle ($P<0.0001$). Moreover, embryo presence on day 14 inhibited CCL2 and CCL4 mRNA expression ($P<0.001$). Expression of CCL8 mRNA raised on day 12 of the estrous cycle ($P<0.05$). During early pregnancy no significant differences in CCL4 and CCL8 mRNA expression were found. The significant decrease in expression of CCR1 mRNA was observed on day 12 both in the estrous cycle ($P<0,05$) and pregnancy ($P<0,05$) whereas CCR5 and CCR2 mRNA increase on day 12 and 14 of the estrous cycle ($P<0.05$), respectively. Moreover, expression of CCR1 mRNA significantly dropped on day 10 of pregnancy when compared with corresponding day of the estrous cycle. Similar profiles of CCR2 and CCR5 expression with visible increase on days 12 and 14 of the estrous cycle and pregnancy were observed. Summarizing, the obtained data demonstrate that embryonic signals lead to inhibition of CCL2, CCL4 and CCL8 mRNA expression which may protect CL against luteolysis.

Supported by NSC grant 2012/05/B/NZ9/03330

P-4

Poster Presentation

Ashwell, Christopher

Prestage Department of Poultry Science, North Carolina State University, United States

Ashwell CM, Lowman ZS

The effects of short-term exposure to low oxygen during incubation on long-term performance in broiler chickens.

There have been tremendous advances in genetic selection of broilers resulting in the fast-growing, efficient modern-day bird. As a consequence of this selection, broilers have difficulty obtaining the oxygen supply needed to meet the demands higher body weights (BW) require. Birds can adapt to this challenge over time by increasing oxygen carrying capacities through angiogenesis, polycythemia, modification of hemoglobin composition, or by increasing hematocrit levels. In this study, we investigated the effects of short-term hypoxic conditions on the embryo and long-term body composition. Broiler embryos were exposed to a 48 h period of low oxygen (LoOx =16%) from day 16 to 18 of incubation. Birds were hatched and grown under standard brooding conditions to 14 d of age, sampling at internal pip, external pip, day 7, and day 14 (n=12). Acute effects of LoOx were observed at the end of the hypoxic treatment including an impact on heart rate, yolk utilization, and blood gas parameters. LoOx treatment had a long-term effect on body composition and growth rate. At day 7 control birds had a significantly higher breast % and body weight, but by day 14 these differences were lost and LoOx significantly increased heart % and liver %, determined by ANOVA ($P<0.05$). The observations made in this study may be useful to the broiler industry where birds are grown in varying environments. By manipulating incubation conditions including brief periods of hypoxia, the resulting birds may be better suited for specific environmental conditions or when specific modifications to body composition are desired

P-5

Poster Presentation

Astiz, Susana

Animal Reproduction, Spanish National Institute for Agricultural and Food Research and Technology (INIA), Spain

Astiz S, Gonzalez-Bulnes A, Sebastian F, Fargas O, Cano I, Cuesta P

Maternal age affects metabolic efficiency and life performance of progeny in a Holstein dairy cow model

The aim of the study was to determine the effects of maternal age at conception on the metabolic efficiency and performance of the offspring. The development and performance of 404 high-producing dairy cows was studied from birth onwards and during two lactations. Cluster analysis identified four performance 'types': high-yielding (HY) cows and persistently-high-yielding cows (PHY), which accounted for 33% of the animals; medium-yielding (MY) cows, 41%; and low-yielding (LY) cows, 26%. Prenatal determinants of performance were analyzed. Developmental and environmental factors were excluded as determinants (including birth weight, passive immunity transfer, growth rate, age at first parturition, reproductive efficiency). Life performance did show minor seasonal effects. Instead, the single most important factor influencing the life performance of daughters was maternal age. HY cows (12899±1635 and 15177±1929L/lactation for 1st and 2nd lactation) were born from the youngest mothers (1.89±1.14 parturitions), while LY cows (10082±1914 and 10438±2346 L/lactation for 1st and 2nd lactation) were born from the oldest (2.72±1.80 parturitions; $P<0.001$). Yield parameters correlated significantly between the first and second lactations, suggesting the influence of the individual. Mother's productivity was not associated with the productivity of the daughters. Although the sample of 404 animals is too small to discard genetic hereditability of the genetic merit, the difference of more than 20% of increase in milk yield between maternal and offspring productivity (average yield was 10137±2330 and 9515±3010 L/lactation for HY and LY-mothers, respectively) suggests that the genetic merit is not the main factor that affects production of the progeny. These results suggest that under optimal conditions maternal aging is a determinant of the life performance of progeny. These results argue for the need to identify conditions that contribute to health and disease in progeny according to the DOHaD-concept. Our findings may help the development of novel management guidelines for dairy farms.

P-6

Poster Presentation

Barandalla-Sobrados, Maria

Laboratory of Reproductive Technologies, Avantea, Italy

Barandalla M, Colleoni S, Lazzari G

Study on the viability of embryonic stem and somatic cells after exposure to hydrogen peroxide

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell. Hydrogen peroxide is generated in numerous biological processes, it is associated with the initiation and progression of oxidative stress-linked diseases and it is also implicated as the main transmitter of redox signals. For these reasons it has been commonly used as inducer of oxidative stress in vitro and it is known that its cytotoxic effect on cultured cells is largely influenced not only by its concentration, but also by the incubation time and the cell concentration. Given that, in this study the cytotoxic effect of hydrogen peroxide was evaluated on two different cell lines: HUES3 (Human Embryonic Stem Cells) and Hs27 (Human fibroblast). The cells were exposed for 72h at concentrations varying from 2 to 128uM. In both lines it was also determined the optimal cell concentration to induce and detect a dose-response effect. HUES3 cells were cultured in feeder-free maintenance medium mTeSR1TM, while Hs27 cells were cultured in DMEM:TCM199 Medium (1:1) supplemented with 10% fetal bovine serum. The cells were plated at different concentrations: 40000 cell/ml, 80000 cell/ml and, only for HUES3, 160000 cell/ml and 320000 cell/ ml. Exposure to H2O2 started 24h post plating and continued for 72h with daily change of medium. At the end of the exposure the cell viability was evaluated with the Alamar blue assay. The optimal plating density was estimated at 80000 cell/ml for Hs27 cells and 160000 cell/ml for HUES3 cells. In both lines cell death was gradually observed at concentrations ranging from 32uM to 64uM. At 16 uM and lower concentrations no cytotoxicity was detected. This preliminary study allowed to establish the conditions for an in vitro model of oxidative stress induction both in an embryonic and in a somatic cell line.

Poster Presentation

Beaujean, Nathalie
Developmental and Reproduction Biology, French National Institute for Agricultural Research (INRA), France

Chebrout M, Boulesteix C, Beaujean N

Identification of histone H3 post-translational modifications involved in embryonic chromosome condensation

Epigenetic modifications and nuclear architecture are globally rearranged after fertilization. Unlike somatic cells, mammalian embryos for example present a unique organization of pericentromeric heterochromatin. In mouse embryos, this part of the heterochromatin is not organized in clusters but in spherical structures around nucleolar precursor bodies forming a ‘cartwheel’. This pericentric heterochromatin is characterized by specific epigenetic marks, in particular trimethylation of histone H3 at Lysine 9 (H3K9me3), together with the heterochromatin protein 1 (HP1b). Surprisingly, we recently found out that this heterochromatin also contains histone H3 phosphorylated at serine 10 (H3S10P) from early interphase through mitosis over several embryonic cycles (Mason et al., 2012). In somatic cells, it is known that when histone H3 is phosphorylated at S10 at the end of the cell cycle, HP1b is ejected from the chromatin upon the entry in mitosis. We therefore questioned the ‘colocalization’ of H3S10P and HP1b we observed in early stage embryos, even in G1/S phases. To better understand the mechanisms behind HP1b association/ejection in early embryos, we performed immunostaining with several antibodies directed against HP1b, H3K9me3, H3S10P as well as with an antibody specific to the double modification H3K9me3S10P. In order to get a deeper insight into the co-localization between these epigenetic marks/proteins we also developed a new approach based on the in situ PLA technology (Proximity Ligation Assay). This technique allows the fluorescent detection of two targets when they are in close proximity (<40 nm) without any genetic manipulation of the cells in contrast to other techniques such as BRET or FRET. Altogether our results demonstrate that H3S10P and H3K9me3 can both co-localize with HP1b but that only the double modification H3K9me3S10P is responsible of HP1b ejection from chromatin only upon the first mitosis in mouse embryos.

Poster Presentation

Bermejo-Alvarez, Pablo

Department of Animal Reproduction, National Institute for Agricultural Research and Technology and Food (INIA), Spain

Bermejo-Alvarez P, Ramos-Ibeas P, Park K, Powell AP, Vansant L, Ramirez MA, Gutierrez-Adan A, Telugu BP

H19 imprinting is erased following conversion of mouse SSC into SSCiPSC in a Tet dependent manner

CpG methylation at imprinting control regions (ICR) determines the monoparental expression of a subset of genes. Imprinting marks are protected from the global demethylation taking place during preimplantation development, but it is unclear whether artificial reprogramming, induced by the expression of Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc), disrupts these marks. Our objective was to determine the methylation status of the paternally methylated ICR of H19 cluster in three different lines of mouse iPSC derived from fibroblasts (fiPSC) or spermatogonial stem cells (SSCiPSC). The iPSC were obtained from a mouse model expressing Yamanaka factors under a doxycycline-inducible promoter. SSC were obtained through Gfrα1 sorting of neonatal (6 days old) testis lysate. Methylation levels were analyzed by sequencing clones of PCR amplified bisulfite treated DNA. The region analyzed contained 15 CpG and 10 clones were sequenced per sample. The methylation level was slightly diminished in fiPSC (Fibroblasts 48 % vs fiPSC 20, 39.3 and 34 %), but was drastically reduced in SSCiPSC (SSC 68 % vs SSCiPSC 0, 0.6 and 2 %). SSCiPSC were able to generate chimeras and form teratomas with derivatives from the three germ layers. Interestingly, the teratomas maintained the reduced methylation levels at H19 ICR (subcutaneous 10.6 %, intramuscular 2 %). In order to determine the mechanism behind the imprinting erasure, we generated a double knock-out for the 5-methylcytosine oxidases Tet1 and Tet2 by CRISPR-mediated site-specific genome editing. We analyzed H19 ICR methylation in 3 clonal SSCiPSC lines harboring frame-disrupting mutations in both alleles and observed that demethylation did not occur following reprogramming (43.3, 48 and 70 %).
In conclusion, H19 imprinting is erased during reprogramming of SSC to SSCiPSC by a Tet-mediated mechanism.

Poster Presentation

Blitek, Agnieszka

Department of Hormonal Action Mechanisms, Institute of Animal Reproduction and Food Research, Poland

Blitek A, Morawska-Pucinska E, Szymanska M

Effect of PGI2 on proliferation and apoptosis in the conceptus trophectoderm and endometrial epithelial cells of the pig

Activation of prostacyclin (PGI2) signaling pathway plays an important role during implantation in rodents and ruminants. The present study aimed to examine the effect of PGI2 on proliferation and apoptosis in cultured conceptus trophectoderm (Tr) and endometrial epithelial (EE) cells of the pig. Tr cells from day 14 conceptuses (n=8) and EE cells from uteri of day 12 mature gilts (n=5) were used. The presence of PTGIR protein was confirmed with Western blot and immunofluorescency. For proliferation assay, Tr and EE cells were seeded in 96-well plates. After serum starvation, cells were pre-treated with CAY10441 to block PGI2 receptors, and then exposed to medium only (control), or iloprost (PGI2 analogue; 0.1 or 1 μM) in the presence or absence of CAY. Viable cells were stained with 0.2% crystal violet. To study apoptosis, Tr and EE cells were cultured in 6-well plates, pre-incubated with CAY and exposed to iloprost (0.1 or 1 μM) in the presence or absence of CAY. Cellular expression of BAX and BCL-2 mRNA was studied with qPCR. To investigate whether iloprost may protect from staurosporine-induced apoptosis, Tr and EE cells were pre-incubated in medium only, 0.1 μM iloprost, or 20% NCS. Subsequently, staurosporine was added and active Caspase-3 concentration was measured in cell lysates. Cultured Tr and EE cells possess PTGIR protein. Iloprost stimulated proliferation of Tr and EE cells ($P<0.001$ and $P<0.01$; respectively), and this effect was abolished in the presence of CAY. Neither the treatment with iloprost, nor the presence of PTGIR antagonist affected the ratio of BAX to BCL-2 mRNA in Tr cells. Iloprost did not protect Tr and EE cells from staurosporine-induced apoptosis.
In summary, PGI2 receptors are important component of a proper conceptus and endometrium function, and PGI2 acting via PTGIR may contribute to successful conceptus implantation in the pig.

Supported by NSC grant 2011/01/B/NZ9/07069

O-10

Oral Presentation

Bock, Christoph

Christoph Bock Laboratory, Research Centre for Molecular Medicine, Austria

Bock C

Analyzing and interpreting genome-wide DNA methylation data

DNA methylation is the most widely studied epigenetic mark. It plays important biological roles in vertebrates and invertebrates, in fungi, plants and in bacteria. Recent methodological advances have led to the development of many different protocols for large-scale DNA methylation analysis. I will review the underlying methodology and provide hands-on advice on how to select the most suitable protocols for DNA methylation analysis in the context of biological and biomedical research. Furthermore, I will highlight important practical considerations for analyzing and interpreting DNA methylation using genome-scale assays.

P-11

Poster Presentation

Canada, Paula

Abel Salazar Biomedical Sciences Institute, University of Porto, Portugal

Canada P, Engrola S, Conceicao L, Teodosio R, Mira S, Pinto W, Lopes AF, Barriga-Negra L, Sousa V, Fernandes J, Valente L

Using muscle cellularity and the expression of DNA methyltransferases to understand the effect of dietary protein composition on myogenesis regulation

There is evidence that prenatal nutritional supply affects skeletal muscle development in a variety of vertebrates, with possible long-term consequences on growth potential and flesh quality. The maternal dietary protein has been recently reported to influence muscle development in mammals, concomitantly with altered gene expression and the occurrence of epigenetic changes at transcriptional regulation level. These findings suggest that epigenetic events such as DNA methylation may underlie mechanisms of nutritional programming on muscle development and growth. For that reason we decided to investigate how could dietary protein composition influence skeletal muscle cellularity and growth in a fish species that undergoes a complex metamorphosis. In Senegalese sole larvae fed inert diets from mouth opening, increased dietary protein content showed a positive effect on larval growth from 2 to 51 DAH overcoming the effect of an imbalanced AA profile. Although the proliferative capacity of myogenic cells and the growth potential of Senegalese sole larvae remained unaffected at the metamorphosis climax (19 DAH), at juvenile stage (51DAH), the dietary protein composition did affect the muscle cross-sectional area, the total number of white muscle fibres, the fibre density and mean fibre diameter. We have also evaluated the effect of dietary protein content during Senegalese sole early development in the skeletal muscle expression patterns of key genes regulating myogenesis (Myf5, MRF4, MyoG, MyoD2, MyHC and Mstn1) and methionine metabolism (DNMT1, DNMT3a, and DNMT3b) in an attempt to highlight a possible influence of DNA methylation as regulator of gene expression affecting muscle growth during the metamorphosis climax and at the juvenile stage.

This work was supported by FEDER thought COMPETE – Programa Operacional Factores de Competitividade (POFC) and by FCT/MCTES (PIDDAC) under the project EPISOLE (PTDC/MAR/110547/2009). P Canada and S. Engrola are supported by grants from FCT (Portugal) SFRH/BD/82149/2011 and SFRH/BPD/49051/2008.

P-12

Poster Presentation

Chavatte-Palmer, Pascale

Developmental and Reproduction Biology, French National Institute for Agricultural Research (INRA), France

Rousseau-Ralliard D, Derisoud E, Tarrade A, Brat R, Rolland A, Thieme R, Navarrete-Santos A, Fischer B, Chavatte-Palmer P

A periconceptional maternal hyperglycemia disrupts the feto-placental membrane fatty acid profiles in a rabbit model.

Type-1 diabetes (T1D) is caused by the reduction in pancreatic insulin secretion, inducing chronic hyperglycemia. Pre-gestational T1D increases the risk of miscarriage and congenital malformations and programs the offspring to develop metabolic syndrome at adulthood. Management of maternal diabetes is essential during the gestation but could be highly important around the conception. The aim of this study was to explore the effects of maternal TD1 during the periconceptional period on feto-placental phenotype at 28dpc (term=31days), according to the sex of the conceptus. Diabetes was induced by Alloxan in dams 7 days before mating. Glycemia was maintained at 15-20mmol/L with exogenous insulin injections. At 4dpc, embryos were collected and transferred into non-diabetic recipients. At 28 dpc, control (C) and diabetic (D) fetuses were collected for biometric records and lipid analysis of feto-placental tissues by gas chromatography. Data were analyzed by principal component analyses. D-fetuses were growth retarded, hyperglycemic and dyslipidemic compared to C. A specific fatty acid signature was observed in fetal plasma. The composition of placental and fetal liver membranes differed according to maternal status and fetal sex. Tissues from Dfetuses contained significantly more omega-6 polyunsaturated fatty acids compared to C. No biochemical signature was observed in the immature fetal heart, but docosahexaenoic acid was decreased and linoleic acid increased in the cardiac membranes of D-fetuses, indicating a higher risk of ischemia. This study demonstrates that an exposure to high plasma glucose during the short periconceptional period reduces fetal growth and alters the lipid profiles in all fetal tissues.

O-13

Oral Presentation

Collas, Philippe

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Collas P

Microchip assay and epigenetics of embryonic genome activation and early development

An important asset to understand the regulation of gene expression during embryo development is chromatin immunoprecipitation (ChIP). ChIP has traditionally required large cell numbers, limiting applications to early (mammalian) embryos. We have established a microChIP assay for histone and transcription factor ChIP from hundreds of cells. We have used microChIP to show differential marking of promoters by H3K4me3 and H3K27me3 genome-wide in the mouse inner cell mass and trophectoderm. We have adapted microChIP to identify chromatin determinants of zygotic genome activation (ZGA) in zebrafish embryos. Profiling of histone H3 on zebrafish promoters before, during and after ZGA provides evidence for epigenetic patterning of developmental gene expression. This involves pre-ZGA marking of inactive developmentally regulated genes by H3K4me3, with or without repressive histone modifications. We show a relationship between pre-ZGA H3K4me3 marking and transcription output after ZGA. We also show DNA methylation of thousands of promoters before ZGA and additional DNA methylation during ZGA. Developmentally-controlled hypomethylated promoters constitute a platform for H3K4 trimethylation. Histone and DNA methylation patterns on homeostatic and developmentally-regulated promoters are strikingly similar to those of sperm. Our results are consistent with a model of inheritance of epigenetic modifications though fertilization.
We propose from our findings that histone modifications play an instructive role for the developmental gene expression program.

O-14

Oral Presentation

Conesa, Ana

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Conesa A

The STATegra project: new statistical tools for analysis and integration of diverse omics data

Next-Generation sequencing has speed up genome analysis and brought omics research closer to many organisms and biological scenarios. Today an increasing number of research projects propose the combined use of different omics platforms to investigate diverse aspects of genome functioning. These proposals ideally seek to provide complementary sources of molecular information that eventually can be put together to obtain systems biology models of biological processes. Hence, it is not rare anymore to find experimental designs involving the collection of genome, transcriptome, epigenome and even metabolome data on a particular system. However, standard methodologies for the integration of diverse omics data types are not yet ready and researchers frequently face post-experiment question on how to combine data of different nature, variability, and significance into an analysis routine that sheds more light than the analysis of individual datasets separately. The STATegra project has been conceived to address these problems and provide the genomics community with user-friendly tools for the integration of different omics data types. STATegra targets several sequencing based functional genomics methods, proteomics and metabolomics. In this presentation I will present current results of the project that include the STATegraEMS, an experiment management system for storage and annotation of complex omics experiments, novel data integration visualization tools, statistical approaches to integrate RNA-seq data with different regulators of gene expression, transcriptomics measurements combined with downstream features such as proteomics and metabolomics, and data mining strategies to leverage public domain datasets in the integrative effort. I will also present the STATegRa, a new Bioconductor R package for integrative omics data analysis.

P-15

Poster Presentation

Correia-Alvarez, Eva

Genetic and Animal Reproduction, Regional Service for Agriculture and Food Research (SERIDA), Spain

Correia-Alvarez E, Gomez E, Martin D, Perez S, Peynot N, Giraud-Delville C, Caamano JN, Diez C, Otero J, Sandra O, Duranthon V, Munoz M

Early endometrial and embryonic transcription of Tumour Necrosis Factor α (TNF) and its receptor TNFR2 in cattle

Tumour necrosis factor α (TNF) is expressed in reproductive tissues of several mammals. In cattle, we identified TNF protein present in uterine fluid and expressed in endometrium and blastocysts. Therefore, TNF may be involved in embryo-maternal communication. In this work we have investigated the RNA expression of TNF and its receptor TNFR2 in bovine endometrium and blastocysts. On day 5 after estrus, cows were sham transferred (N=6) or transferred (N=5) with multiple in vitro produced (IVP) embryos (N=50). All cows were slaughtered on day 8. Embryos were flushed and endometrial samples were collected from caruncular and intercaruncular areas in the middle and cranial horn regions. Blastocysts that developed entirely in vitro were also collected. Endometrial samples and blastocysts were subjected to RT-qPCR and values were normalized by GeNorm using three reference genes (SLC30A6, C20RF29, and RPL19 for endometrium, and SDHA, GAPD, YWHAZ for embryos). Data were analyzed using the GLM procedure of SAS Version 9.2 and REGWQ test. Endometrial TNF and TNFR2 mRNA levels were not significantly affected by the presence of embryos. However, expression of TNF and TNFR2 transcripts was higher in IVP blastocysts than in blastocysts developed in the uterine tract ($p<0.05$). TNF transcript level increased in middle vs. the cranial uterine region ($p=0.03$). Our results suggest that TNF and TNFR2 mediate early embryo-maternal interactions in cattle.

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P-16

Poster Presentation

Depince, Alexandra

Fish Physiology and Genomics, French National Institute for Agricultural Research (INRA), France

Depince A, Jammes H, Gabory A, Labbe C

DNA methylation pattern after cryopreservation of goldfish cells: influence of the type of cryoprotectant.

Cryopreservation of fish cells is relying on the use of methylated cryoprotectant such as dimethyl sulfoxide, methanol, or dimethyl acetamide. It has been shown that some of these cryoprotectants behave as very strong DNA methylating agents provided that reactive oxygen species (ROS) are present during the chemical reaction. After cryopreservation, it is known that damaged cells and mitochondria release ROS, and it can be hypothesized that these ROS chemically interfere with the cryoprotectants to alter the DNA methylation pattern of the cryopreserved cells. It is not known however how the chromatin organization of the cells will prevent this damage, or whether some cryoprotectants are more active than others. The aim of the present study was to explore to which extent the cryopreservation of two cell types bearing different chromatin organization, spermatozoa and cultured fin cells, will alter their global DNA methylation pattern. Goldfish fin cells and spermatozoa were cryopreserved according to the protocol the most suitable for each cell type. Three different cryoprotectant bearing methyl groups or not - methanol, dimethylsulfoxyde, and propanediol - were tested. Changes in DNA methylation level when compared to the fresh control was assessed by the restriction enzyme assay (using MspI and HpaII), followed by LUMA (luminescence methylation assay). Snap freezing of the samples without any cryoprotectant was assessed as well. We observed that the global methylation level of the cryopreserved somatic cells was much more stable than that of the spermatozoa, and that the choice of the cryoprotectant influenced the effect of cryopreservation on the global DNA methylation level. However, this effect was independent of the methyl groups in the cryoprotectant. Consequences for the cell quality and properties will be discussed.

This work was partly funded by the program "Investissements d'Avenir" ANR-11-INBS-0003.

P-17

Poster Presentation

Depreester, Elke

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Depreester E, De Koster J, Dini P, Bogado O, Opsomer G

Periparturient obesity is associated with a higher inflammatory status of the uterus in high yielding dairy cows,some preliminary results

In human medicine, it has been shown that obesity is associated with a higher number of immune cells in the uterus of pregnant women. The present study was set up to examine the effect of overconditioning on the cellular immunity in the uterus of periparturient high yielding dairy cows, which is stated to be of decisive importance to determine the susceptibility to postpartum uterine diseases. Ten dairy cows across a range of body condition score (2,5 to 5) were euthanized approximately 12 days before parturition. During the dry period the body condition score and back fat thickness were evaluated every week. Quantitative Insulin Sensitivity Check Index (QUICKI) and Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) were determined in serum samples taken around 20 days before euthanasia. After euthanasia, tissue samples of the different fat depots were collected and the total weight of adipose tissue, in the different depots, was determined. The number of macrophages and lymphocytes in intercotyledonary endometrium and placentomes was evaluated immunohistochemically (resp. MAC387+ and CD3+). The total number of white blood cells in endometrium was positively correlated with the mean BCS during the dry period ($r=0.66$, $P<0.05$) and with the total weight of adipose tissue in the different depots ($r=0.66$, $P<0.05$).The highest correlations were noticed with the amount of adipose tissue in the intrathoracal ($r=0.768$), mediastinal ($r=0.747$), mesenteric ($r=0.725$) and perirenal ($r=0.701$) fat depots ($P<0.05$). Moreover, the number of macrophages (MAC387) in the endometrium was negatively correlated with RQUICKI ($r=-0.65$, $P<0.05$). Based on these preliminary results, we suggest that, like in women, obesity around parturition may bring the cow's uterus to a pro- inflammatory status, which may make them more susceptible to serious inflammatory uterine diseases postpartum. Further transcriptomic analyses on the uterine tissues will be done to confirm the upregulation of genes of pro-inflammatory cytokines.

O-18

Oral Presentation

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Desmet K, Van Hoeck V, Merckx E, Sirard MA, Bols PEJ, Leroy JLMR

Elevated non-esterified fatty acid concentrations during bovine embryo culture influence DNA methylation patterns in blastocysts

Elevated non-esterified fatty acid (NEFA) concentrations are a common feature during negative energy balance (NEB) in high producing dairy cattle, while metabolic stress is associated with subfertility. Previous research showed that such elevated NEFA concentrations during embryo culture hamper embryo development and alter gene transcription profiles in resultant blastocysts. For example, genes related to epigenetic markers (HIST1H1C, HIST1H2BN) displayed a different expression pattern in response to embryonic NEFA exposure. We therefore hypothesized that high NEFA concentrations during embryo culture affect epigenetic mechanisms, such as DNA methylation, in embryos. Bovine oocytes (n=1412; 4 replicates) were matured and fertilized following standard procedures. Zygotes were cultivated for 6.5 days under two different conditions: i) physiological NEFA conditions (mixture of 23 µM palmitic acid (PA), 28 µM stearic acid (SA) and 21 µM oleic acid (OA)) (BASAL) or ii) elevated NEFA concentrations as under lipolytic conditions (mixture of 230 µM PA, 280 µM SA and 210 µM OA) (HIGH). A selection of 10 blastocysts per treatment per replicate was analysed for DNA methylation patterns using the EmbryoGENE Bovine microarray platform and subsequent Ingenuity Pathway Analysis. The significance threshold was set to a fold-change > 1.5 and a P-value ≤ 0.05. The microarray data reveal significant differences in DNA methylation of 4671 genes of which 1912 genes were hyper- and 2759 genes were hypomethylated in blastocysts under BASAL conditions compared to HIGH NEFA conditions. Main pathways significantly affected are related to cell death and survival, lipid metabolism, carbohydrate metabolism, molecular transport and embryo development. Previous research revealed that maturing oocytes under high NEFA conditions resulted in a twenty times lower number of genes with an altered DNA methylation pattern.
We conclude that embryonic exposure to elevated NEFA concentrations changes the DNA methylation profile of the resultant blastocysts which may lead to an altered embryonic development.

P-19

Poster Presentation

Duranthon, Veronique

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DNA methylation of the POU5F1 regulatory region in rabbit first embryonic lineages.

The expression of POU5F1 is controlled by cis-regulatory elements located 5' upstream from the initiation start site. This regulatory region is highly conserved among species, and especially four conserved regions (CR1 to 4) have been identified. The minimal promoter is located in the CR1 region, while the proximal and distal enhancers involved in cell specific regulation of expression are located in CR2-3 and CR4 regions respectively. In the mouse the two enhancer regions contribute differently to gene expression depending on the developmental stage of the embryo. POU5F1 repression of expression is induced by regulatory factors binding to the 5' upstream region but also by DNA and histone methylation. DNA methylation of POU5F1 has been mostly analyzed in in vitro derived stem cells. However, promotor DNA methylation levels have been shown to differ between embryonic stem cells and the "in embryo" counterparts they are derived from. We thus decided to analyze DNA methylation of the four conserved regions of the POU5F1 upstream region in the first embryonic lineages. Therefore we took benefit from the rabbit embryo whose epiblast, hypoblast and trophoblast can be easily isolated. At that stage, POU5F1 expression is restricted to the epiblast.
We evidenced an hypomethylation of the four conserved regions of POU5F1 5' upstream region in the epiblast. Interestingly even the CR4 region which is supposed to be functional in the ICM but not in the epiblast conserved a very low methylation level in this tissue. CR1 methylation was lower in day 6 embryos than in fibroblasts.

O-20

Oral Presentation

Eggen, Andre
AgriGenomics, Illumina, United States

Eggen A

Last generation tools for the study of the genome and the epigenome

Identifying and characterizing heritable and reversible modifications of DNA and chromatin that do not change the primary nucleotide sequence is becoming an important field of research and is now critical for numerous fields of study, including disease biology and gene expression. It includes the ability to detect and quantify DNA methylation accurately and the identification of histone modifications. Recently, other modifications altering the ability to express genes such as microRNA have also gained increased interest. Many different methods have been optimized over the past years and with the tremendous reduction in sequencing costs, a lot of them have been recently adapted to genome scale approaches by combining it with high throughput sequencing (Next-Generation Sequencing). Latest developments associated with epigenetics research like DNA sequencing, DNA methylation analysis and the identification of microRNA will be presented and discussed as well as how these technologies are helping deciphering the molecular basis of traits of interest and advancing the development of new diagnostics and biomarkers.

O-21

Oral Presentation

Esteller, Manel

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Esteller M

Cancer Epigenetics: from knowledge to applications

For the last twenty-five years an increasing amount of evidence has shown the relevance of epigenetics in cell biology and tissue physiology, being DNA methylation aberrations in cancer the flag-ship for the recognition of its disturbance in human diseases. From the candidate gene approaches, new powerful technologies such as comprehensive DNA methylation microarrays and whole genome bisulfite sequencing has recently emerged that have reinforced the notion of epigenetic disruption in the crossroad of many sickness. From the poster-boy cases of MGMT and GSTP1 hypermethylation in the prediction of alkylating drug response and prostate cancer detection, respectively, to the personalized treatment of leukemia with small molecules targeted to fusion proteins involving histone modifiers such as DOT1L and MLL, the field has walked a long path. The current talk will focus in the epigenetic profiling, basically at the level of DNA methylation and histone modifications, that is starting to provide clinical value in the diagnosis, prognosis and prediction of response to drug therapies, with an emphasys in neoplasia, but without forgetting the novel advances in other human disorders. For cancer, we have already a wide view of the undergoing DNA methylation events that expand beyond classical promoter CpG islands of tumor suppressor genes and we have a growing list of mutated chromatin remodeler genes that contributes to the tumorigenesis process. It is time to apply this knowledge in practical clinical situations like the diagnosis of cancers of unknown primary, the screening of malignancies in high-risk populations or a biomarker selection of the patients that should receive treatment with epigenetic drugs.

P-22

Poster Presentation

Gomez, Enrique

Animal Biotechnology Centre, Regional Service for Agriculture and Food Research (SERIDA), Spain

Diez C, Trigal B, Caamano JN, Munoz M, Correia E, Carrocera S, Martin D, Moreno JF, Gomez E

Normal morphometry at birth after transfer of bovine IVP blastocyst cultured in synthetic oviduct fluid and vitrified in ethylene-glycol, dymethyl-sulfoxyde and sucrose

Under specific conditions, in vitro embryo culture and cryopreservation may lead to developmental abnormalities. In this work we analyzed the effects of vitrification and warming on Day-60 pregnancy onwards and birth rates and morphometry of born calves. Oocytes matured and fertilized in vitro with Holstein sex-sorted sperm (75% oocytes with female sperm) were cultured in SOFaaci + 6 g/L BSA. Day-7 blastocysts were loaded in 7.5% ethylene-glycol (EG) + 7.5% DMSO, and subsequently in 16.5% EG + 16.5% DMSO + 0.5M sucrose prior to vitrification in fibreplugs. After warming in 0.25 and 0.15 M sucrose, vitrified embryos and fresh controls were transferred to estrus-synchronized crossbred recipients in experimental herd. Pregnancy and birth rates were previously reported (Trigal et al, Theriogenology, 2012, 78:1465-75). Calving ease (CE; 1-5 scale), body weight (BW), height at withers (HW), thorax circumference (TC), and gestation length (GL) were recorded at birth. No significant differences (ANOVA; $p>10$) were shown between parameters of calves born from fresh and vitrified embryos. Pregnancy losses (PL) were numerically higher in vitrified embryos. One calf showed spastic paresia and another calf immaturity with death in 15 days (from fresh and vitrified embryos, respectively). Due to our limited sample size, it is yet unknown whether this vitrification procedure may result in increased PL. However, our records indicate that birth of phenotypically normal calves can be expected.

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O-23

Oral Presentation

Gonzalez-Bulnes, Antonio

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Gonzalez-Bulnes A, Astiz S, Ovilo C, Lopez-Bote CJ Perez-Solana M, Ayuso M, Torres-Rovira L, Barbero A

Modification of adiposity and metabolic features by transgenerational developmental programming in swine with thrifty genotype

The swine industry has currently two main trends; intensive breeding of highly-selected breeds for value-for-money pork production and a minority extensive breeding of traditional breeds, like Mangalica and Iberian, for distinctive high-quality dry-cured products. These products, mainly the ham, are worldwide famous by their unique taste, which it is due to the abundance of the intramuscular fat. This pattern of fat storage is the result of a thrifty genotype developed for alleviating seasonal undernutrition in extensive free-range breeding. However, in case of food excess and highly-caloric diets, the ability to store fat causes obesity and metabolic disorders, as found in the Iberian pig. At juvenile periods, induces the prodrome of metabolic syndrome (obesity with impairments of glucose regulation) and an earlier puberty; however, young animals are still able to develop adaptive responses for regulating homeostasis. On the contrary, at adulthood, the animals display the five components of the metabolic syndrome (central obesity, dyslipidaemia, insulin resistance and impaired glucose tolerance, and elevated blood pressure) and even the prodrome of type-2 diabetes; there is no other animal model developing more than three components at the same time. In case of pregnancy, the maternal dyslipidaemic state induces excess of triglycerides and cholesterol at the fetoplacental unit, affecting the developmental trajectory of the foetuses. The offspring exposed to maternal overnutrition during pregnancy are prone to obesity at so early postnatal stages as during lactation; in case of obesogenic diets at juvenile stages, metabolic changes are even more severe than in non-challenged piglets, with increased adiposity, evidences of metabolic syndrome and even prodrome of type-2 diabetes. The second generation have also early-postnatal increases in adiposity and disturbances in lipids profiles compatible with the early prodrome of metabolic syndrome and paediatric liver disease, evidencing transgenerational programming.

P-24

Poster Presentation

Gonzalez-Serrano, Andras Felipe

Biotechnology, Institute of Farm Animal Genetics, Germany

Gonzalez-Serrano AF, Ferreira CR, Pirro V, Lucas-Hahn A, Hadeler KG, Aldag P, Baulain U, Heinzmann J, Piechotta M, Danicke S, Cooks RG, Niemann H

May lipid content of oocytes and embryos determine success of preimplantation embryo development?

Assisted reproductive techniques (ARTs) such as cryopreservation and in vitro embryo production are strong influenced by the lipid content of oocytes and embryos. Therefore a sensitive and accurate lipid characterization of female gametes and preimplantation embryos seems to be of great importance. As a first step of this study, lipid profiling of single in vitro (IVTB) and in vivo (IVVB) preimplantation bovine embryos was performed by desorption electrospray ionization mass spectrometry (DESI-MS). Relative abundance of transcripts related to the oocyte development capacity (IGF1R and GJA1) and lipid metabolism (FASN, SCAP, SREBP1 and CPT1b) was assessed by RT-qPCR. Additionally, similar analyses were performed from oocyte collected by OPU from Holstein-Friesian heifers after long-term supplementation of the daily diet with either rumen-protected conjugated linoleic acid (CLA) or stearic acid (SA). Follicular fluid and blood samples were collected from the supplemented heifers for monitoring lipid profiles by gas chromatography (GC). Results showed clear effects of the in vitro culture system on lipid profiles and the gene expression pathern of preimplantation embryos when compared to their in vivo counterpart. In more details, the saturated fatty acids palmitic (C16:0) and stearic (C18:0) were more abundant in IVTB in comparison to IVVB. The fatty acid supplementation experiments showed modification of the lipid profile of oocytes collected from supplemented donors. Specifically, oocytes collected from the CLA-supplemented heifers showed higher accumulation of triacylglycerols of unsaturated fatty acids while oocytes from the SA-supplemented group acumulated higher amounts of palmitic acid and plasmalogen species. Changes in lipid profiles after in vitro embryo production and fatty acid diet supplementation of female donors were observed in the present study. These results pave the way for improving in vitro embryo production systems and support the role of the bovine experimental model for identifying fertility impairments associated to nutritional performance in humans and other mammals.

P-25

Poster Presentation

Grybel, Katarzyna

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Grybel K, Knelangen J, Schindler M, Gurke J, Haucke E, Pendzialek M, Baisch H, Fischer B, Navarrete-Santos A

The histone-lysine N-methyltransferase SETDB1 is upregulated in rabbit blastocysts during a diabetic pregnancy

The embryo and embryonic cells are sensitive to nutritional and hormonal changes. They have an astonishing capacity to adapt within a short time to metabolic changes to secure survival and differentiation. DNA methylation and histone modifications are two epigenetic mechanisms, which are likely candidates for metabolic programming effects. Diabetic pregnancies are associated with higher embryo loss and more newborns with metabolic disorders. These features might be the result of metabolically programmed epigenetic changes in early embryonic development. We used the rabbit model to investigate the influence of a maternal insulin-deficient diabetes mellitus on embryonic histone modifications and the expression of methyltransferases SETDB1, SUV39H1 and the histone deacetylase HDAC1. SETDB1 and SUV39H1 are members of the histone-lysine N-methyltransferase Suvar3-9 subfamily. Whereas SETDB1 specifically trimethylates lysine 9 of the histone H3 (H3K9), SUV39H1 dimethylates H3K9. The acetylation of H3K9 is removed by HDAC1. All three enzymes have key functions in heterochromatic gene silencing. In blastocystsfrom diabetic rabbits the lysine 9 methylation of H3 (H3K9me2) was increased and the lysine acetylation (H3K9ac) decreased as measured by specific antibodies. SETDB1, SUV39H1 and HDAC1 were expressed from the morula until the blastocyst stage at day 6. In 6 day old blastocysts from diabetic rabbits the transcript numbers of SETDB1 was increased, whereas SUV39H1 and HDAC1 were decreased in their expression. The upregulation of SETDB1 in embryos form diabetic rabbits may contribute to the higher methylation of histone H3K9, indicating that maternal diabetes influences histone modifications and the expression of related enzymes in the preimplantation embryo, providing a potential mechanism for early metabolic programming.

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P-26

Poster Presentation

Hayrabedyan, Soren

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DNA methylation landscape in Sertoli cells play role in its specific inflammasome regulation with implications to male fertility

DNA methylation is a crucial epigenetic modification of the human genome beyond the DNA sequence level that is involved in the regulation of many cellular processes. DNA methylation also occurs beyond promoter regions at non-CpG islands within the heterochromatin. We have recently found that Sertoli cells have an active NALP3 inflammasome signaling, responsible for inflammatory IL-1beta maturation and secretion. NALP3 is HSP90 dependent, the latter under HDAC6 control. In this study we questioned wether the inflammasome signaling is DNA methylation dependent. Using 3D DNA methylation image based analysis we assessed the ie-DAP and ATP induced global methylation status at cellular level resolution. We further tested if this changes were NALP3 dependent by NLRP3 siRNA mediated knockdown. The expression of NOD1, NOD2 and NALP3 inflammasome pathway members was followed by RT-qPCR and flow cytometry in native and 5-AzaCytozine total de-methylated context. We further investigated which pathways related to epigenetic regulation are enriched after NOD1 induced NALP3 signaling using nanoproteomics approach. Then we compared the abundance of NALP3 in macrophages and Sertoli cells analyzing genome wide transcriptome and methylome (RNASeq, 5hmC) public datasets. We conclude that DNA methylation is one of the key mechanisms to dynamically regulate NALP3 signaling and its upstream activation affects the global 3D DNA methylation phenotype. Thus epigenetic modulators would affect Sertoli biology under stress impairing fertility.

P-27

Poster Presentation

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miR-13b influences female reproductive success in Drosophila

The miR-2 microRNA family, which is widespread among invertebrates, is the largest family of microRNAs in Drosophila melanogaster. It includes the microRNA genes of miR-2 and miR-13, clustered over 8 loci in the Drosophila melanogaster genome. We discovered in a preliminary microarray analysis that miR-13b is expressed in the reproductive system of unmated Drosophila females and that its level is down-regulated at 6 hrs after the start of mating. To examine the role miR-13b plays in female reproduction, we mis-expressed miR-13b in the spermatheca secretory cells (SSC) pre- and post-mating by silencing or overexpressing miR-13b using Send-1-GAL4 and Send-2-GAL4, respectively. We show that mis-expression of miR-13b pre-and-post-mating significantly reduced female reproductive success. To ascertain the function of miR-13b, we used TargetScan to identify predicted targets from our microarray dataset of mating-responsive transcripts that are expressed in the lower reproductive tract of unmated and mated females. miR-13b predicted targets are enriched in genes that are related to developmental processes. Interestingly, neural development related GO term represented a significant number of up-regulated predicted targets. Taken together, our results suggest that miR-13b regulates processes that are essential for female reproductive success, possibly through mediating targets in the central nervous system. We are currently experimentally verifying several predicted targets of miR-13b to determine its plausible function and involvement in setting up the periconceptional environment.

P-28

Poster Presentation

Heras-Garcia, Sonia

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Heras S, Smits K, Van Soom A

Optimization of DNA methylation immunostaining in equine zygotes produced after ICSI

In the past two decades, the dynamics of the DNA methylation of many mammalian species have been revealed. To the best of our knowledge, no studies have been made on the DNA methylation pattern of equine embryos, probably due to the difficulties in obtaining equine zygotes. Proper unmasking of epitopes is crucial for revealing the dynamics of the methylation pattern of zygotes. In this study three different epitope retrieval conditions were tested to optimize the DNA methylation immunostaining for equine zygotes.Equine oocytes were matured for 28h in a DMEM/F12 - based medium. Only oocytes with a visible polar body were fertilized by means of piezo drill-assisted ICSI, cultured in vitro in DMEM/F12 with 10% FCS for 22-24h, fixed with 4% paraformaldehyde and permeabilized. For 5-mC epitope retrieval three different conditions were used: denaturation with 4N HCl for 30 min (A), denaturation with 4N HCl for 1h (B) and digestion with 0.25% (w/v) trypsin for 20s after 30 min denaturation with 4N HCl (C). Zygotes were subsequently blocked and incubated with the primary antibody (mouse anti-5-mC) and the secondary antibody (goat anti-mouse FITC). DNA was counterstained with EthD-2. In the three conditions tested, DNA methylation could be observed in both pronuclei. After 30 min of denaturation (A), although DNA methylation was observed, epitope retrieval was not complete and the visualization of both pronuclei was unclear. After application of conditions B or C, the DNA methylation pattern of both pronuclei could be correctly visualized. The results obtained with condition 3, which included tryptic digestion, were more stable between all the zygotes. In conclusion the optimal immunostaining conditions for 5-mC epitope retrieval in equine zygotes are denaturation with 4N HCl for 30 min followed by tryptic digestion for 20s.

This work was funded by IWT grant 111438.

P-29

Poster Presentation

Ivask, Marilin

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RNA-seq analysis uncovers transcriptomic variations between SCNT and IVF bovine blastocysts

Transgenic animals provide useful tools for the pharmaceutical industry and agricultural applications. Several methods are used to generate transgenic production animals, including nuclear transfer using genetically modified donor cells. While somatic cell nuclear transfer (SCNT) has been successfully implemented in several species, its efficiency is extremely low, possibly due to insufficient reprogramming of somatic nuclei. To investigate potential effects of SCNT on embryonic development, this study compared bovine blastocysts derived from in vitro fertilization (IVF) and SCNT to determine transcriptomic variations using RNA sequencing (RNA-seq) with 5500 SOLiD System. Two groups of SCNT blastocysts were used, one created using a transgenic fetal fibroblast cell line expressing human insulin (SCNTtg), and the other one with non-transgenic fetal fibroblasts (SCNTwt). Both groups comprised 5 female blastocysts. The two groups of SCNT blastocysts displayed similar gene expression profiles, having differences in 15 genes with false discovery rate (FDR) < 0.05. Biological pathways associated with cellular signaling and molecular transport were found to be different. Comparison of SCNTwt with IVF embryos revealed differences in expression in 512 genes (FDR < 0.05). Functional annotation indicated variation in “Cell-to-cell signaling and interaction, cell signaling, nucleic acid metabolism” and “Developmental disorder, hereditary disorder, skeletal and muscular disorders” pathways. There were 394 differentially expressed genes (FDR < 0.05) identified among SCNTtg and IVF embryos. Top associated networks were “Cell-to-cell signaling and interaction, cell signaling, nucleic acid metabolism” and “Auditory and vestibular system development and function, cellular development, embryonic development”. In conclusion, SCNTwt and SCNTtg embryos have quite similar gene expression profiles which substantially differ from IVF embryos. These findings provide valuable knowledge of the developmental changes in SCNT embryos and a possible explanation why the majority of SCNT embryos fail to develop into live offspring.

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O-30

Oral Presentation

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Sparus aurata as a model for nutritional reprogramming of marine fish: Effectiveness of different developmental windows

Studies in mammals and humans show that dietary influences exerted at critical developmental stages early in life (neonatal nutrition, post-natal nutrition) may have long-term consequences on physiological functions in later life. Nutritional programming phenomenon is largely studied in mammalian models for the understanding of diseases such as the metabolic syndrome or diabetes. The functioning of certain metabolic pathways such as fatty acid metabolism in juvenile fish also depends on specific nutritional signals during the critical larval periods, demonstrating that the concept of metabolic programming also exists in fish. Modulation of key enzymes for fatty acid synthesis is possible by feed given to juveniles. The present study will examine the influences early nutritional programming either during embryonic phase or during metamorphosis. To affect nutrient intake during these two periods either broodstock or early weaning diets were modified and their effects studied on sea bream performance, lipid metabolism, gene expression and response after a feeding challenge. Specific genes such as those involved in essential fatty acids metabolism, were markedly affected by the nutritional programing at both developmental windows. For instance an up regulation of delta-6 desaturase gene expression was obtained when fish was conditioned with moderate levels of vegetable oils, whereas extreme conditioning conditions inhibited the expression. Despite it was potentially possible to condition fish during metamorphosis, the larvae were very sensitive during this period and high mortalities occurred during the treatment. When fishes were programmed through broodstock nutrition, a very high survival rate was obtained. Even extreme conditioning produced reliabe survival rates. After treatment with different conditioning during the embryonic period, all fish were fed standard fishmeal diet for three months and, afterwards, challenged with vegetable oils. A significant positive effect of the nutritional programming was observed in lipid metabolism response as well as a better utilization of the feeds.

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P-31

Poster Presentation

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The effect of L-carnitine on in vitro fertilization and subsequent development of bovine embryos

It was documented that energy status is associated with the developmental competence in bovine oocytes. The objective of this study was to characterize the effects of mitochondrial stimulator L-carnitine on oocyte maturation, fertilization and early development of bovine embryos. Specifically, the effects of L-carnitine treatment on oocyte nuclear maturation, efficiency of in vitro fertilization after 16 hours and embryonic development 8 days were examined. In IVM oocytes, mitochondrial activity levels (Mito Tracker Orange) and lipid droplet contents (Nile Red) were also measured. During IVM culture, immature bovine oocytes were either treated or not treated with 2.5 mM L-carnitine. L-carnitine treatment did not improve the nuclear maturation of oocytes but significantly increased mitochondrial activity levels and decreased lipid droplet contents. Oocytes treated with L-carnitine showed higher ($P < 0.01$) mitochondrial activity after maturation (64.6 vs. 49.1; average MiTo Tracker Orange intensity/oocyte equatorial section) and lower ($P < 0.01$) contents of lipid droplets in the cytoplasm (36.7 vs. 43.9; average Nile Red intensity/oocyte equatorial section). Indeed, oocytes treated with L-carnitine showed a higher ($P < 0.05$) penetration rate (91.5 % vs. 79.1 %) and mainly higher ($P < 0.05$) total efficiency of fertilization (84.3% vs. 74.5%). Eight days after IVF, the proportion of blastocysts from inseminated oocytes was significantly higher ($P < 0.05$) in the L-carnitine treated group than in control group of oocytes (33.1 % vs. 25.7 %). Indeed, blastocysts from both groups were very similar in the total cell number (112.4 cells/blastocyst vs. 115.7 cell/blastocyst) and also in the proportion of inner cell mass cells (31.6 % ICM/blastocyst in control and 30.5 % ICM/blastocyst in the L-carnitine treated group). Our results demonstrate that L-carnitine treatment during IVM has a positive effect on in vitro fertilization and early development of bovine embryos without affecting blastocyst differentiation.

Supported by grant LD14104.

P-32

Poster Presentation

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Effect of Prostaglandin F2α on global gene expression profile in the porcine conceptus

Proper interactions between uterus and conceptuses are necessary for establishment of pregnancy and the development of the placenta. Key factors in these regulations are prostaglandins (PGs). During the peri-implantation period, the uterus and conceptuses synthesize elevated amounts of PGF2α. We showed elevated expression of PGF2α receptor (PTGFR) protein in the porcine endometrium on days 15 and 18 of pregnancy. We also demonstrated elevated expression of PTGFR gene in porcine conceptuses on days 14-15 when compared to days 10-12 of pregnancy, with a highest level of expression on days 16-19 of pregnancy. Thus, the aim of this study was to determine the effect of PGF2α on global gene expression profile in conceptus cells during the implantation period. Cells of conceptuses collected from gilts (n=5) on day 14 of pregnancy were flushed from uterine horns with Medium 199 (M199) supplemented with 1% Bovine Serum Albumine (BSA) and dissolved by pipetting. Cells were washed with M199, then plated on 6-well plates coated with collagen I. Plated cells were cultured in medium M199 containing 5% Newborn Calf Serum and 0.1 % BSA and antibiotics. After reaching 80-90% confluence, cells were treated with 100 nM PGF2α or vehicle for 24 h at 37°C in a humidified atmosphere (95% air and 5% CO2). After incubation cells were lysed with Fenozol and total RNA was isolated for microarray analysis. Agilent Sus scrofa 4x44k microarrays were used to determine the effect of PGF2α on global gene expression profiles. Statistical analyses were conducted using LIMMA package in BioConductor software. We identified 37 differentially expressed genes (26 upregulated and 19 downregulated; lfc > 0.585; FDR = 0.1) that were involved in different processes such as TGF-beta, WNT, MAPK, VEGF, GnRH signaling pathways and others. Concluding, our results suggest an important role of PGF2α during the embryo implantation process in pigs.

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P-33

Poster Presentation

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SOMICS - Genetics, genomics and evolution of prolific breeds of domestic sheep (Ovis aries)

Ovulation rate and litter size in sheep (Ovis aries) are complex traits affected by endocrinological, genetic and environmental conditions. We analyzed factors affecting fecundity of the prolific Finnsheep breed and searched for related structural (SNP genotyping) and functional (mRNAs and miRNAs) variations in its genome. A total of 31 ewes representing three breed groups (Finnsheep, Texel and their F1-crossbreds) and two diets (control and flushing) were included. Experiments were focused on two different time points during establishment of pregnancy: follicular growth phase (the first phase) and early pregnancy prior to implantation (the second phase). Blood parameters were used to monitor ovary function and energy status of the ewes. In the first phase, one ovary of each ewe was surgically removed determined by individual progesterone profiles. After estimation of follicular counts, a sample of each ovary was used for RNA extraction followed by mRNA and miRNA sequencing using HiSeq200 Illumina technology. This revealed a total of 13 537 ovine genes expressed in all 31 samples. The gene expression profiles were then compared within and between breed-diet pairs. In the second phase, the sheep were inseminated and slaughtered followed by collecting another set of tissue samples (pituitary gland, CL, oviductal and uterine epithelial cells, preimplantation embryos) for RNA extractions and sequencing. In the final phase, SNP genotyping data will be correlated to transcriptome and phenotypic data. Preliminary results from the first phase show no significant effects of the diet (control vs flushing) on follicular counts within pure breeds ($p>0.05$). However, 503 genes showed significantly different expression levels between Finnsheep and Texel having flushing diets compared to one gene significantly differentially expressed between Finnsheep and Texel having control diets. This study provides new information on effects of flushing diet on fertility in the high-prolific Finnsheep breed, a valuable genetic resource for global sheep farming.

O-34

Oral Presentation

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Profiling DNA methylation genome-wide at base-pair resolution in single oocytes

Epigenetic modifications in gametes, such as DNA methylation, can pass on information beyond DNA sequence from one generation to the next, as in genomic imprinting. By a combination of genetic and epigenomic studies, we have learnt a great deal about the landscape of and the mechanistic basis for DNA methylation in oocytes in the mouse as a model organism. Necessarily, much less is known about methylation in oocytes of other mammals, in particular human. The possible vulnerability of epigenetic marks in gametes and early embryos to procedures associated with assisted reproduction techniques underscores the importance of understanding methylation establishment and maintenance in humans. The general conservation of imprinting between mouse, livestock species and human suggests that the principles of methylation in oocytes should be similar amongst mammals; indeed, a few imprinted genes have been analysed in human oocytes, and their methylation patterns found to be similar to those in mouse. However, there are significant differences in the expression of some of the epigenetic modifiers involved in de novo methylation in human compared with mouse oocytes, suggesting the existence of species-specific mechanisms. We have developed a method to profile DNA methylation genome-wide in single cells by Illumina sequencing. Crucially, by merging the data from as few as a dozen single-cell libraries, we are able to fully reproduce the methylation landscape of mouse oocytes with base-pair resolution. This advance opens the way to map in detail and to understand the methylation landscape of human oocytes and pre-implantation embryos.

P-35

Poster Presentation

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Proteome Analysis Of Porcine Endometrium During Maternal Recognition Of Pregnancy - Preliminary Study

Progressive changes in the uterine microenvironment between days 10 and 16 of gestation determine embryonic survival following trophoblast elongation and embryo implantation. Pregnancy recognition in pigs occurs around days 12-13 when embryos undergo rapid elongation and produce estradiol 17β. The establishment and maintenance of pregnancy involves a number of integrated signals among the ovary, uterus and conceptus. Alterations in the synchronous development of the uterine environment with the growing conceptus during maternal recognition of pregnancy can result in a failure to establish pregnancy. Embryo-induced uterine changes can be manifested by the alteration in endometrial transcriptome and epigenome resulting in the modulated expression of various proteins. The aim of the current study was to compare the proteomic profiles of porcine endometrium harvested from the time of maternal recognition of pregnancy (days 12-13) with proteomic profiles of the endometrium from the same days of estrous cycle. Total proteins were extracted from pregnant (n = 4) and cyclic (n = 4) endometrial tissues directly into a rehydration buffer. Tissue extracts were used for two dimensional gel electrophoresis (2DE). Then, MALDI TOF mass spectrometry was performed. To identify the proteins ProFound search engine was used. We identified 3 unique - present only in pregnant pigs - endometrial proteins: DNA mismatch repair protein Msh3, breakpoint cluster region protein (partial) and adenylate cyclase type 1. The selected proteins are involved in DNA repair, GTPase activation and cAMP biosynthesis, respectively. We suppose these proteins might be important for successful endometrial development in pregnant pigs to create the proper intrauterine environment for developing embryos. However, further research is needed to confirm/establish the importance of identified proteins for the support of early pregnancy.

Supported by the National Science Centre, Poland, grant 2012/05/N/NZ4/02343.

P-36

Poster Presentation

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Epigenetic effects of prenatal estradiol-17β exposure on the prostate gland in pigs

In a variety of species, there is evidence that prenatal exposure to environmental estrogens can have adverse effects on health later in life. One possible target is the prostate gland, which is highly dependent on steroid hormones during embryonic development. It has been hypothesized that early life exposure to endocrine disrupting chemicals with estrogenic activity might increase the risk of abnormal growth and carcinogenesis of the prostate in adults. In rats it was shown that perinatal exposure to 17β-estradiol-3-benzoate (EB) or bisphenol A (BPA) increases the formation of precancerous prostate lesions. Furthermore, EB or BPA exposure resulted in increased expression of the putative oncogene high mobility group nucleosome binding domain 5 (Hmgn5) und reduced expression of the putative tumor suppressor hippocalcin-like 1 (Hpcal1) from postnatal day 10 on. We investigated the effects of prenatal exposure to the most potent natural estrogen, estradiol-17β (E2), in pigs. The pig was chosen as a model because its placental estrogen synthesis is comparable to that of humans. In our study we applied E2 orally to pregnant sows at three different concentrations (0.05, 10 and 1000 µg/kg body weight/day). In the offspring, we analyzed gene expression of Hmgn5 and Hpcal1 in the prostate gland of 8 week old male piglets by RT-PCR. Surprisingly, no profound changes in the expression of the two genes were observed in a preliminary approach. This could be due to several reasons. Differences between pigs and rodents in the uterine hormonal environment and prostate development might be of importance, as well as the substances used or the time point of analysis. To verify the primary results and to get a more global view on gene expression changes we will analyze further possible targets of endocrine disruption, including components of the DNA methylation machinery.

O-37

Oral Presentation

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DNA methylation pattern in fish spermatozoa after cryopreservation: a species specific alteration in cyprinids

Cryopreservation of fish sperm is relying on the use of methylated cryoprotectant such as dimethyl sulfoxide, methanol, or dimethyl acetamide. It has been shown at a chemistry level that some of these cryoprotectants behave as very strong DNA methylating agents provided that reactive oxygen species (ROS) are provided to the media and it can be hypothesized that this property is valid in cellular systems. After cryopreservation, damaged cells and mitochondria release ROS, and in some species (catfish, some cyprinids), fertilization with cryopreserved sperm was shown to induce some malformation of the produced fries. It cannot be excluded that fries defects are due to altered methylation patterns of the fertilizing spermatozoa. The aim of the present study was to explore to which extent cryopreservation of spermatozoa in two cyprinid species, the goldfish and the zebrafish, will alter the global DNA methylation pattern of these cells. Spermatozoa were cryopreserved according to the most suitable protocol for each species, with 8 % methanol as the best cryoprotectant. Changes in DNA methylation level when compared to the fresh controls were assessed by the restriction enzyme assay (using MspI and HpaII), followed by LUMA (luminescence methylation assay). We observed that the average methylation level was quite similar between zebrafish and goldfish in fresh samples, where 86 and 84 % of the sites were methylated respectively. After goldfish sperm cryopreservation, the average methylation pattern was not modified. However, at an individual level, some sperm showed an increase in their methylation level, some were unchanged, and some had a lower methylation level. In zebrafish on the contrary, the methylation pattern after cryopreservation was unaffected. These results raise the question of the chromatin stability in the two species, and of the consequences on the embryo development.

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P-38

Poster Presentation

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Insulin during oocyte maturation induces changes in the transcriptome and epigenome of day 8 bovine blastocysts

In dairy cows suffering either from negative energy balance or from obesity and excessive growth rate, deviation from physiological levels of insulin in blood and follicular fluid occurs, leading to impaired fertility. In this study the effect of elevated insulin levels during oocyte maturation on gene expression in Day 8 bovine blastocysts (BC8) was investigated. Abattoir-derived oocytes (n=882) were in vitro matured for 22 h with High (H: 10µg/ml); Low (L: 0.1 µg/ml) insulin concentrations or under control conditions (Z: Zero). Pools of 10 frozen (-80°c) BC8 were used for parallel gDNA and total RNA extraction (4 replicates per group). Transcriptome and epigenome data was obtained by microarray hybridization (EmbryoGENE platform / bovine transcriptome array (BESTv1) and EDMA oligo-array for study of the bovine epigenome). An empirical Bayes moderated t-test and the `limma' package in R were used to search for the differentially expressed genes between the control and the insulin treated groups (those with a 1.5 fold-change difference between treatment /control and a p-value < 0.05). The analysis of gene expression differences between insulin treated versus control groups revealed differences for 202 transcripts in H (8 down, 198 up regulated) and 142 transcripts in L (4 down, 138 up regulated) with 104 of the changed transcripts present in both groups. The analysis of the epigenome showed that 7632 and 3914 regions were hypo-methylated in the L and H groups vs Z whereas 6026 and 8504 regions were hyper-methylated in L and H groups vs Z. Further investigations will be performed to study the relationships between these numerous insulin related molecular changes and phenotypes for embryonic development.

P-39

Poster Presentation

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Functional analysis of miR-145 and miR-218 in bovine blastocysts

MicroRNAs are small non coding RNAs that regulate gene expression on a posttranscriptional level. The 20 to 25 bp long RNAs are able to bind to certain messenger RNAs due to base pair complementarities in a 5 to 8 bp long region called the seed region. The binding of microRNAs to the messenger RNA results in inhibition of its translation. The microRNA regulation system plays therefore a crucial role for fine tuning spacio/temporal gene expression as is required in the rapidly developing early embryo. The similarities and contrasts of human and bovine microRNAs-145 and 218 in their binding capacity of the target genes OCT4, SOX2 and KLF4 respectively the target genes CDH2 and NANOG were explored. The binding capacities were tested by means of a luciferase assay. Human Embryonic Kidney cells were transfected with seed region containing psiCHECK2 vectors and synthetic microRNAs using a Dharmafect transfection system. For reading out the luminescence the Dual-Luciferase Reporter Assay System 1-pack was used. The results showed remarkable species specific regulation of these target genes. To evaluate the results obtained in a human context the possibility of re-testing the bovine constructs with a luciferase assay in bovine cells was investigated. Transfection of bovine cumulus cells and Madin Darby Bovine Kidney cells (MDBK) was performed with the Dharmafect transfection system and electroporation respectively. However, the standard deviation from the three replicates was in every case too vast to be useful. So it remains the question if these bovine cell types will be useful for luciferase assays with the microRNAs.

O-40

Oral Presentation

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Lodde V

Oocyte large-scale chromatin configuration re-modelling: state of the art and perspectives

During meiotic arrest, and particularly during the oocyte growth phase leading to the formation of the fully-grown and differentiated oocyte, the chromatin enclosed within the Germinal Vesicle (GV) is subjected to several levels of regulation controlling both its structure and function. These events include mechanisms acting both locally, on specific loci, and on a large scale to remodel wide portions of the oocyte genome. Morphologically, the chromosomes lose their individuality as well as their characteristic appearance and form a loose chromatin mass, which in turn undergoes profound and dynamic rearrangements within the GV before the meiotic resumption. These 'large-scale chromatin configuration changes' are temporally correlated with the process of transcriptional silencing in the oocyte nucleus as well as with epigenetic modifications such as histone tail modifications and changes in the global level of DNA methylation. Moreover, chromatin configuration rearrangements are tightly associated with the acquisition of meiotic and developmental competence. The molecular mechanisms governing changes in large-scale chromatin configuration still remain largely unknown. Most likely, strategies set in place for the control and coordination of these events are part of a complex physiological process that ultimately confers the oocyte with meiotic and developmental competence. Here, we summarize some studies intended to explain the mechanism(s) regulating this complex process, including recent findings indicating that ovarian granulosa cells and their coupling with the oocytes through gap junctions are implicated in such a process. During meiotic arrest, and particularly during the oocyte growth phase leading to the formation of the fully-grown and differentiated oocyte, the chromatin enclosed within the Germinal Vesicle (GV) is subjected to several levels of regulation controlling both its structure and function. These events include mechanisms acting both locally, on specific loci, and on a large scale to remodel wide portions of the oocyte genome. Morphologically, the chromosomes lose their individuality as well as their characteristic appearance and form a loose chromatin mass, which in turn undergoes profound and dynamic rearrangements within the GV before the meiotic resumption. These 'large-scale chromatin configuration changes' are temporally correlated with the process of transcriptional silencing in the oocyte nucleus as well as with epigenetic modifications such as histone tail modifications and changes in the global level of DNA methylation. Moreover, chromatin configuration rearrangements are tightly associated with the acquisition of meiotic and developmental competence. The molecular mechanisms governing changes in large-scale chromatin configuration still remain largely unknown. Most likely, strategies set in place for the control and coordination of these events are part of a complex physiological process that ultimately confers the oocyte with meiotic and developmental competence. Here, we summarize some studies intended to

explain the mechanism(s) regulating this complex process, including recent findings indicating that ovarian granulosa cells and their coupling with the oocytes through gap junctions are implicated in such a process.

P-41

Poster Presentation

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Effect of second cleavage speed of mice embryo on implantation potential

Selection of high quality embryos is of crucial importance in assisted reproduction techniques (ARTs). Our hypothesis is that first cleavage speed after embryo genome activation could be a good indicator of embryo development and implantation competence. To analyze this, in vivo 2-cell stage embryos were recovered at 1.5 dpc and were classified into three different groups according to their division speed to the 4-cell stage: early cleavage (the fastest, 33% of the total of embryos for each female), moderate cleavage speed (32% medium) and slowest cleavage (final 35%). Once the groups were classified, embryos were differentially transferred to 1.5 dpc pseudopregnant females (n=366). Implantation and resorption rates as well as fetal and placental weights were examined at 15 dpc. Implantation rates were significantly higher ($P<0.05$) in medium cleavage embryos (82.35%, n=102) compared to fastest cleavage embryos (67.26%, n=113), but not to slowest cleavage embryos (75.50%, n=151). Early cleavage embryos presented higher esorption rates (20%) compared to medium (15%) and late cleavage embryos (13%) although differences were not significant. No differences were found in fetal and placental weights among groups. In vitro embryo development up to blastocyst stage and hatching rates were also recorded. Medium cleavage speed embryos showed significantly higher ($P<0.05$) hatching rates (66%, n=164) compared to fastest (56%, n=168) and slowest cleavage embryos (52%, n=180).

In conclusion, first cleavage speed after embryo genome activation can be used to predict development to the blastocyst stage and implantation competence in mice embryos.

P-42

Poster Presentation

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Long-lasting changes of E2 and P4 in the blood serum and ESR1, ESR2, PGR, LHCGR and FSHR in the porcine oviduct after ovarian stimulation with hCG and eCG

The objective of this study was to evaluate whether synchronization of oestrus or superovulation using eCG and hCG influences E2 (oestradiol) and P4 (progesterone) levels in blood serum and their receptors (ESR1, ESR2, PGR) as well as LH/hCG and FSH receptors in the porcine oviducts on day 3 after insemination. Mature crossbred gilts were divided into groups: inseminated, synchronized/inseminated (750 IU eCG + 500 IU hCG) and superovulated/inseminated (1500 IU eCG + 1000 IU hCG) and slaughtered on day 3 after insemination. Blood samples were collected during slaughter for oestradiol (E2) and progesterone (P4) levels analysis (RIA). Oviducts (isthmus and ampulla) were collected for mRNA and protein analysis. Examination of mRNA expression of ESR1, ESR2, PGR, LHCGR and FSHR was done using Real-Time PCR. Protein levels of LHCGR and FSHR were performed by Western Blot. Data were analyzed by one-way and two-way ANOVA followed by the Bonferroni's post hoc test. The E2 and P4 levels significantly increased in blood serum of superovulated group of gilts ($P<0.01$, $P<0.05$ for E2 and P4 respectively). ESR1 but not ESR2 mRNA expression significantly dropped in oviductal isthmus of synchronized ($P<0.05$) and superovulated ($P<0.001$) gilts. Similarly, PGR mRNA levels were also decreased in isthmus of superovulated gilts ($P<0.01$). LHCGR mRNA analysis did not reveal any changes between groups. However, protein level of LHCGR was reduced in ampulla of superovulated gilts ($P<0.05$). The FSHR mRNA levels were not affected by eCG and hCG. Whereas, protein level of FSHR distinctly dropped in both hormonally stimulated group of gilts ($P<0.05$, $P<0.01$; for synchronized and superovulated respectively). Collectively, our results indicate that synchronization of oestrus and superovulation affect E2 and P4 serum levels and their receptors. Moreover, hCG/eCG treatment influence LHCGR and FSHR protein expression in the porcine oviduct. Altogether, these changes may alter proper functioning of oviduct and in turn fertilization and early embryo development.

Supported by NSC grant 2012/05/N/NZ9/02444.

P-43

Poster Presentation

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Developmental potential of rabbit SCNT embryos derived from adult fibroblasts or mesenchymal stem cells and treated with Scriptaid

The aims of the present study were: a) to assess the in vitro developmental ability of cloned embryos reconstructed with rabbit adult fibroblasts (RAF), adult or fetal bone marrow mesenchymal stem cells (ABM- and FBM-MSC) and b) to evaluate the effects of Scriptaid (SCR), a novel histone deacethylase inhibitor with low toxicity on the developmental competence of such embryos. Previously reported methods were used for RAF, ABM- and FBM-MSC isolation, in vitro culture, morphology evaluation, assessment of proliferation and differentiation potency and SCNT procedure. SCR was added at 500 nM for 14 -16 h after activation. The proportion of cleaved embryos was significantly higher in ABM-MSC-derived embryos compared with RAF- (96% vs 84.9%; $P<0.01$) and FBM-MSC ones (96% vs 84.7%; $P<0.01$). However, significantly more RAF-derived embryos developed to the blastocyst (Bl) stage than those derived from ABM-MSC (day 5-Bl 45% vs 27%; $P<0.001$; day 6-Bl 46% vs 32%; $P<0.01$). Blastocyst formation rate of FBM-MSC-derived embryos tended to be higher than that of ABM-MSC (38% day 5-Bl and 41% day 6-Bl), even with no statistical difference. The proportions of hatched blastocyst were not affected by the type of donor cells. Irrespective of the cell type, development to the blastocyst stage was significantly increased by treatment of cloned embryos with SCR. In RAF-derived embryos, SCR increased the rates of day 3 morulae ($P<0.01$), day 5 Bl ($P<0.001$), day 6-Bl ($P<0.001$) and hatched Bl ($P<0.01$). In ABM- and FBM-MSC-derived embryos, SCR increased the rates of day 5-Bl and day 6-Bl ($P<0.001$ in ABM-MSC- and $P<0.05$ in FBM-MSC-derived embryos).

In conclusion, this study demonstrates that treatment of rabbit cloned embryos with Scriptaid significantly improves their in vitro developmental potential irrespective of the type of donor cells.

P-44

Poster Presentation

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Expression level of DNMT, MBD and TET gene families in adult and prepubertal ovine oocytes

DNA methylation is an important epigenetic mark that plays a role in gene regulation. Despite being relatively stable in somatic cells, DNA methylation is subject to reprogramming during embryo development and gametogenesis. Enzymes and cofactors responsible for the regulation of DNA methylation can be divided in two principal groups on the basis of their function: enzymes that methylate the DNA by maintaining the methyl marks during DNA replication or by establishing de novo methylation and enzymes involved in the DNA demethylation. Aim of this work was to analyze the expression of a panel of genes involved in DNA methylation and demethylation [DNA methyltransferases (DNMTs), methyl-CpG-binding proteins (MBD) and ten-eleven translocation (TET) dioxygenase gene families] in a model of differential developmental competence consisting in ovine oocytes derived from adult and prepubertal donors, in order to better understand the wave of de- and re-methylation that occurs during gametogenesis after birth and early development. RNA was isolated from pools of 10 immature (GV) and in vitro matured (MII) oocytes from adult and prepubertal donors (4 replicates per group). Gene relative quantification was performed by Reverse Transcription followed by Real-Time PCR. Lower expression of TET1, TET2 and TET3 genes was observed in prepubertal GV oocytes (ANOVA; $P < 0.05$), while no differences were observed for the enzymes involved in methylation (DNMT1,DNMT3A, DNMT3B, MBD1, MBD3,MBD4). TET1, TET2, and TET3, whose expression has never been studied in ovine, generate 5-Hydroxymethlcytosine (5hmC) by oxidation of 5-methylcytosine (5mC), and are thought to be involved in active DNA demethylation, which occurs during the very early steps of mammalian development. Prepubertal GV oocytes show reduced developmental capacity and our observation of lower expression of the TET genes in lower competence oocytes suggest that epigenetic mechanisms may be involved herein and paves the way to better understand methylation dynamics during sheep pre-implantation development.

P-45

Poster Presentation

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Proteomic analysis of porcine endometrial tissue reveals altered protein expression during early pregnancy

Successful embryonic development is a time sensitive process and depends upon communication between endometrium and embryo. In pigs, this communication is believed to begin after the surge of estrogens released by conceptuses around days 11-12 of pregnancy and this triggers changes in the uterine environment inclusive of secretion of proteins by the endometrium. The objective of this study was to elucidate changes in expression of proteins induced by conceptus estrogens. We analysed endometrial tissue lysates from pigs on days 9 and 12 of cycle and on days 9, 12 and 15 of pregnancy by 2-D DIGE gel electrophoresis. Several proteins were found to be up- or down-regulated depending upon the pregnancy status of animals. A number of these proteins were identified using tandem mass spectrometry (MALLDI-TOF/TOF) analysis. We identified a unique protein profile for early pregnant endometrium. Whereas, 62 proteins were significantly altered in the 12 day of pregnant endometrium as compared to 12 day cycle, a total of 107 proteins significantly altered between day 9 and day 12 of pregnancy. Some of the identified down-regulated protein included annexin 4, Hsp 90, Hsp 60, keratin type II, cytosol amino peptidase like protein, prolyl 4-hydroxylase beta polypeptide and chaperonin containing TCP. The up-regulated proteins were related to specific aspects of embryonic development including 1) growth and remodeling: actin, alpha 1 glycoprotein, alpha 2 macroglobulin, vinculin, 2) immune system regulation: immunoglobins and complement factor B, 3) oxidative stress balance: thioredoxin peroxidase, hemopexin, 4) nutrition: apolipoprotein 1, ceruloplasmin and 5) protease inhibitors: different isoforms of serpins and trypsin inhibitor heavy chain. These data provide information on dynamic physiological processes associated with pregnant endometrium and may potentially demonstrate a protein profile associated with successful pregnancy.

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P-46

Poster Presentation

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Low-molecular-weight metabolites as developmental indicators in IVP culture media

Assisted reproduction has an important role in dairy and beef industries. The in vitro embryo production system contributes to the implementation of genetic selection strategies and improvement of pregnancy rates in herds with low fertility. The traditional criteria for embryo evaluation, like embryo morphology and speed of blastomere divisions, are often insufficient to predict bovine embryo viability. We tested the hypothesis that changes in low-molecular-weight metabolites of culture media are related to the developmental ability of embryos. Single bovine embryos were cultured in 60µl SOF+0.4%BSA droplets under mineral oil. Twenty µl of culture media was removed at day 2, 5 and 8 post-fertilization. A total of 70 samples were analyzed using liquid chromatography-mass spectrometry (Q-Trap 3200). Our preliminary results indicate that embryos that reached only the morula stage secreted a significantly broader range of low- molecular- weight metabolites into the culture media than embryos that advanced into the blastocyst stage. Principal component analysis showed that the metabolomic profiles of the culture media containing embryos with higher developmental ability were more similar to the control culture medium not containing embryos.

This study was supported by Project EU29023 of Enterprise Estonia, CCRMB, IUT 8-1 and Project 3.2.0701.12-0036 of Archimedes Foundation.

P-47

Poster Presentation

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Serum free maturation medium influences the vitrification of mature bovine oocytes

The presence of serum in oocyte maturation medium influences the vitrification results of sheep blastocyst in a negative way. It is also known that bovine embryos produced in serum-free media contain less lipids and are more tolerant to cryopreservation injury, data on oocytes however, are lacking. Moreover, serum can result in the improper maintenance of methylation and chromatin imprints. The aim of this study was to determine if oocyte maturation in serum-free medium can improve the cryotolerance of bovine oocytes during vitrification. Bovine cumulus oocyte complexes (COCs) (n= 795, 3 replicates) were matured in the presence of serum (TCM199+ 20% FBS) or in serum-free condition (TCM199 + 20 ng/ml EGF). COCs were vitrified in 15% EG+ 15% DMSO + 0.5M sucrose (Kuwayama, Vajta et al. 2005). After warming, the oocytes were subjected to IVF and IVC. To assess the fertilization rate, the oocytes were fixed and stained with Hoechst. Cleavage rate was recorded at 48 hours post insemination and blastocyst rate was assessed 8 day post insemination. Embryo development was significantly impaired after oocyte vitrification compared to control oocytes ($p<0.05$). However, serum-free oocyte maturation tended to increase embryo development rates after vitrification of mature oocytes. After vitrification, fertilization, cleavage and blastocyst rates for oocytes matured in serum-free or serum containing conditions were 52.9% vs. 40.7% ($p =0.11$), 61.5% vs. 29.2% ($p <0.05$) and 3.5% vs. 0.7% ($p=0.13$) respectively.

In conclusion, serum-free maturation seems to have a positive effect on vitrification outcome of mature oocytes. At present, we are investigating effects of the vitrification on methylation at the zygote stage to know if the different methylation pattern between vitrified and control oocytes can be responsible for the low development to the blastocyst stage.

P-48

Poster Presentation

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Oocytes from bitches with normal and impaired reproductive performance display comparable transcript level of maternal effect genes and mtDNA content

Developmental potential (quality) of mammalian oocytes is shaped by a wide range of genetic factors including maternal effect genes (Oct4, Gdf9) and mtDNA content. Oct4 and Gdf9 significantly influence oocyte quality and Oct4 is essential for development of the future embryo and maintenance of blastomere pluripotency. Developmental potential of oocytes increases with the number of mtDNA copies as shown in mouse, human and pig. Since reproductive disorders of a donor female impair oocyte and embryo quality it can be assumed that such conditions affect gene expression and mtDNA replication. This study aims to investigate whether reproductive performance of a donor bitch affect relative transcript abundance (RA) of Oct4 and Gdf9 genes and mtDNA copy number in oocytes. Cumulus oocyte complexes of a proper morphology were obtained from ovaries of 33 bitches (21 with normal reproductive performance NR - C, 12 with impaired reproduction - IR). Denuded oocytes were frozen individually (mtDNA) or in groups of 25 (mRNA). Procedures were performed by real-time PCR (absolute and relative quantification with standard curves). For mtDNA analysis a fragment of mitochondrial CYTB gene was amplified whereas RA investigation (Oct4, Gdf9) included Actb as reference gene. The present experiment included 813 oocytes (mtDNA 63 individual oocytes; RA 30 pools of 25 oocytes). The mean mtDNA copy number equaled 405 968 and it was significantly higher (471 696) in oocytes from IR bitches (NR females 368 175, $P<0.005$). With respect to transcript content reproductive status did not affect RA of analyzed genes (Gdf9 $P = 0.13$; Oct4 $P = 0.458$).
In summary, quality of oocytes from bitches with impaired reproduction was similar (RA) or even better (mtDNA) than that of oocytes from females of normal reproductive status. Although results of this study do not corroborate evidence published for oocytes of other mammals, in many reproductive aspects the dog is an unique species and as such needs a special attention.

P-49

Poster Presentation

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Piras C, Guo Y, Roncada P, Soggiu A, De Canio M, Urbani A, Bonizzi L, Humblot P

Proteomic responses associated to proliferation phenotypes of uterine epithelial cells after challenge with E coli LPS

E coli is the most common bacteria associated with cow post partum metritis and endometritis. Its pathogenic effects are induced by the outer-membrane component Lipopolysaccharide (LPS). Strong proliferative properties of LPS have been demonstrated in different cell types including recently bovine endometrial cells (Guo et al., 2014). The objective of this work was to characterize the proteomic responses associated with proliferative phenotypes in endometrial epithelial cells.
In vitro culture of bovine endometrial epithelial cells (EEC) and fibroblast were performed following collection of bovine endometrium at the slaughterhouse. EEC and fibroblast were separated before primary culture providing pure cell culture of epithelial cell (>95%). Bovine epithelial cell were challenged with 0, 8 and 16 µg/ ml E coli LPS. The variation in cell number (numbers of cells LPS treated - numbers of cells Controls)/numbers of cell Controls) was calculated 72 hours after challenge. Cells were collected and pellets deep frozen until use. The proteomic profiles was determined by 2D electrophoresis followed by MALDI TOF-MS and SHOTGUN-MS from controls and in pellets obtained following LPS treatment. As found previously, cell growth rate was steadily increased for cells exposed to 8 µg/ ml when compared to controls (range + 20% to +40%; $p<0.001$); The response for cells exposed to 16 µg/ ml was extremely variable. Stimulation of cell growth following exposure to 8 µg/ml LPS was associated to significant differential protein expression of Interferon-induced dynamin-like GTPase, Protein disulfide-isomerase A3, and of transketolase. A larger set of proteins are differentially expressed but significance of the results should be verified form a larger set of samples. These preliminary results indicates that LPS induced proliferation is associated with changes in protein profiles associated to resistance to pathogens, general metabolism, membrane activity and regulation of DNA metabolism.

P-50

Poster Presentation

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A system to establish biopsy-derived trophoblastic cell lines for bovine embryo epigenotyping

Preimplantation embryo development is a crucial period in which epigenetic marks are established and persist throughout posterior life. It has been described that epigenetic alterations occasionally occur in embryos produced by assisted reproductive technologies (ARTs). The identification of early markers of these alterations would avoid posterior epigenetic syndromes or diseases in the animals and would improve livestock efficiency. However, embryo epigenotyping has critical limitations as reduced sample amount and embryo destruction. To solve these limitations, we have developed a successful culture system of trophectoderm (TE) biopsies to screen for epigenetic deregulations before the embryo is transferred. Day 8-9 blastocysts were biopsied and they showed a survival rate of 82.2±2.6% (n=190). A limiting factor for trophoblastic cell lines establishment was the initial rapid adhesion of the biopsy to the culture plate, which was attained with a microdrop culture system on a gelatin-coated surface. Different culture media were examined, and conditioned medium from mouse embryonic fibroblasts showed significantly higher biopsy adherence and cell proliferation at day 4, when microdrop confluence was reached (73.9±1.6%; n=31/42), and at day 10 of culture in P96 (48.2 ±4.3%; n=15/31). Trophoblastic cell lines mimicked in vivo TE behavior, since mononucleate and binucleate cells were present and trophoblastic-specific genes were expressed. Cell lines were able to proliferate for more than two years, apoptotic cells rarely appeared and pluripotency-related genes expression was detected. This system will initially allow us to determine the diagnostic value of TE culture epigenetic basis and later to develop both, strategies to reduce the effects of ART in embryos, and screening epigenetic merit for animal selection.

P-51

Poster Presentation

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Effects of a sodium butyrate supplemented diet on the expression of genes related to epigenetic regulatory mechanisms and immune response in the European sea bass (Dicentrarchus labrax)

Butyrate is a short chain fatty acid extensively used in animal nutrition since it promotes increases in body weight and other multiple beneficial effects on the intestinal tract, including anti-inflammatory effects. Butyrate supplementation is then an option to consider when feeding carnivorous fish with diets where fish oils have been partially substituted by vegetable oils. However, butyrate effects in fish have been poorly studied and little is known about the metabolic processes underlying these effects. In the present study, we have evaluated the effects of a butyrate-supplemented diet through the expression of several inflammation-related genes in the intestine and liver of European sea bass juveniles. Moreover, since butyrate acts as a histone deacetylase inhibitor, some genes related to epigenetic regulatory mechanisms were also analyzed. As expected, sea bass fed with the butyrate supplemented diet showed a significant increase in weight. Furthermore, dicer1 high expression in the intestine was linked to a decrease in the irf1 levels, suggesting that dicer1 may be somehow inhibiting an interferon response against external influences, in this case dietary ones. In addition, high expression levels of the euchromatic histone-lysine-N-methyltransferase 2 (ehmt2) were related to il6 low levels in both examined tissues. Since ehmt2 is known to methylate histone residues, here it may be repressing il6 promoter and therefore reducing il6 expression. In addition, hdac11 levels (known to downregulate il10 expression in antigen-presenting cells) in the intestine were low, accompanied by a high expression of il10, suggesting the inactivation of the T-cell response. Together, these results are in agreement with the expected anti-inflammatory effects of butyrate and provide support for the expected persistent phenotypic effects of appropriate nutritional programming of carnivorous fish with the goal of contributing to a more sustainable fish farming.

O-52

Oral Presentation

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Robert C

Integrating the DNA methylome and transcriptome to better define the long term impacts of assisted reproductive technologies

Oocytes and early embryos contain only minute amounts of DNA, RNA and proteins, making the study of early mammalian development using common methods highly challenging. The study of the embryo epigenome, in particular the DNA methylome, has been made accessible thanks to the possibility of amplifying specific sequences. We describe a novel platform dedicated to the genome-wide study of bovine DNA methylation, including a complete pipeline for data analysis and visualization. The platform allows processing and integrating of DNA methylome and transcriptome data from the same sample. Procedures were optimized for genome-wide analysis of 10 ng of DNA (10 bovine blastocysts). This is the first developed early embryo compatible genome-wide epigenetics platform for bovine. Such platforms should improve the study of the potential epigenetic risks of assisted reproductive technologies (ART), the establishment sequence of embryonic cell lines and potential deviations in both gene expression and DNA methylation capable of having long-term impact.

P-53

Poster Presentation

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Epiproteomics in reproduction

Different cellular types of multicellular organisms present an identical genome and a different phenotype according to the tissue and to the function. Epigenetic pattern regulates differential gene expression and is strictly related to the structural organization of genome depending on chromatin and histone structure. Proteomics is a complementary technology that allows the global study of protein expression and regulation in a biological system including all the gene products regulated by epigenetics factors.

If epigenetic factors are able to control protein expression and synthesis, proteomics is able to evaluate the whole protein expression on the basis of different protein expression linked to different tissues or to a pathological status. Moreover, through a proteomic approach, it is possible to study histones present a high number of post-translational modifications that give their contribution to the formation of the epiproteome correlated with cellular identity (Dai and Rasmussen 2007). The pattern of the different histone post-translational modification pattern can be analyzed through a proteomic approach in order to study the “histone code” (Eberl, Mann et al. 2011). Epigenetics could also be linked to uterine health, and, more precisely, epigenetic abnormalities have been found to be linked with pregnancy failure (Brown, Bonney et al. 2013), in turn, LPS stimulation can modify epigenetic regulation (Doherty, O’Farrelly et al. 2013). Sperm cells as well contain specific histone variants in comparison to somatic cells (Schagdarsurengin, Paradowska et al. 2012). Proteomic expression of sexed sperm cells has already been studied highlighting the differential protein expression of several structural and metabolic proteins (Soggiu, Piras et al. 2013, De Canio, Soggiu et al. 2014). Epiproteomics differences of sexed bovine semen still needs to be evaluated in order to unravel the gender-related histone code transfer to the offspring and their putative application for sexed semen differentiation.

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P-54

Poster Presentation

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DNA methylation-mediated control of osteonectin gene expression in fish metamorphosis

Osteonectin is a multifunctional matricellular glycoprotein, which is expressed in embryonic and adult tissues that undergo active proliferation and dynamic morphogenesis. However, the precise function of osteonectin and the regulatory elements required for its temporally and spatially specific expression in particular during post-embryonic development are largely unknown. The present study was undertaken to explore the molecular mechanisms that regulate osteonectin gene expression by in-vitro and in-vivo functional characterization of the osteonectin promoter and identification of possible putative regulatory elements that govern basal promoter activity. We report here transient expression analyses of eGFP expression from transgenic zebrafish containing a osteonectin-iTol2-eGFP-BAC or a 7,25kb-osteonectin-Tol2-eGFP promoter gene constructs. We found that in-vitro methylation of the 5′-flanking region of the osteonectin gene caused suppression of reporter activity, implying that osteonectin gene expression could be regulated by DNA methylation-mediated gene silencing. In addition, in-vivo analysis of DNA methylation indicated that stage-specific CpG methylation of the osteonectin core promoter was associated with gene expression during embryo post-embryonic remodelling. Our results suggest that osteonectin might serve specific biological functions in regulating the post-embryonic development process of the zebrafish embryo, and that methylation of the 5′-flanking region of the osteonectin gene may play an important role in regulating osteonectin at a transcriptional level. Acknowledgements. We thank Prof. M. Suster and Prof. K. Kawakami for providing the iTol2 constructs.

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P-55

Poster Presentation

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Methyl supplements in salmon diets, project description and preliminary results

During the last decade there has been a gradual change from marine to plant based feed ingredients for fish in the aquaculture industry. This changes the ratios of essential nutrients in the feeds and influence the metabolism of several amino acids and B vitamins including folate and B12. These b vitamins are important for cellular methylation reactions. Among these methylation reactions is the methylation of DNA. DNA methylation is the most studied epigenetic mechanism and is a biochemical process whereby a methyl (1C) group is added to the cytosine nucleotide which might influence the potential for gene expression. The plant based feed alternatives have previously shown to influence both growth performance and flesh quality, but how the fish feed composition affect the epigenetic regulation of gene transcription during the different life stages and across generations, remains an important and unexplored field. In cooperation with a EU project ARRAINA we are currently running a feeding trial to study how plant based feed enriched with methyl donors regulates gene expression patterns by DNA methylation. A transgenerational (F0-F1) salmon feeding trial is currently ongoing, using plant based raw materials. Two diets has been designed, one that has high and one that has low levels of the nutrients important for methylation reactions. Dissected samples of mature salmon liver are currently being sampled. Livers from salmon (F0) will be analysed for global DNA methylation and preliminary results from the feeding trial will be presented.

P-56

Poster Presentation

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Co-culture with equine embryos affects gene expression in equine oviduct epithelial explants

The equine embryo spends an exceptionally long time in the oviduct. While studies in other species demonstrated an effect of the presence of embryos on oviductal gene expression and protein production, little is known about this interaction in the horse. The aim of this study was to determine the effect of co-culture of horse embryos and oviduct epithelial explants (OEE) on function and gene expression of OEE. Equine OEE and embryos were produced in vitro as described previously and cultured in 50µl-droplets of DMEM-F12 with 10% FCS at 38.5°C in 5% CO2, 5% O2 and 90% N2. Culture of 1µl OEE was conducted in the absence (control) or presence of 10-20 embryos (co-culture). On day 9, OEE were evaluated and frozen. Expression of 11 embryotrophic genes was determined by RT-qPCR. The ciliary beating of the OEE was not affected, but co-culture affected gene expression. The OEE cultured in the presence of embryos showed significant upregulation of MMP2, PGRE2-2, TIMP1 ($0.0005<p<0.005$) and TGFA ($0.01<p<0.05$). A tendency ($0.05<p<0.2$) of upregulation was observed for CSF1, GLUT1, PAI1, PGRE2-4 and VEGFA. Expression of HIF1A, TGFa and uPA was not changed significantly. Co-culture with equine embryos stimulated the expression of embryotrophic genes in OEE. While in vivo the equine embryo produces PGE2 to mediate its own transport to the uterus, in vitro a higher expression of the PGRE2 was observed. The other upregulated genes, TIMP1, TGFA and MMP2, as well as CSF1, GLUT1, PAI1, PGRE2-4 and VEGFA, have been described to stimulate embryonic development and quality, and they modulate oviductal matrix turnover. In conclusion, co-culture did not affect function of OEE, but expression of 4 embryotrophic genes was significantly upregulated.

P-57

Poster Presentation

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Soen Y

The microbiome aspect of epigenetic regulation

The symbiotic microbiome is known to have a profound effect on the development, physiology, and health of its host. Yet, current studies of environmental epienetics often ignore the potential contribution of the microbiome to the induction (and potential inheritance) of host-intrinsic epigenetic changes. We hypothesize that disruption of the microbiome by environmental stress tends to increase the epigenetic response of the organism, thereby contributing to elevated epigenetic and phenotypic variability. Moreover, inheritance of microbial changes could further contribute to transgenerational inheritance of epigenetic changes. To address these issues we introduced a new experimental framework and used it to investigate the mechanisms and meaning of interactions between epigenetic regulation and the microbiome. Preliminary results in flies provide a dramatic example in which this interaction contributes to increased developmental variability in response to environmental stress. I will discuss the importance of the microbiome for the stability of the normal epigenetic patterns and the potential meaning of microbiome-epigenetic interactions for the establishment of new adaptive programs.

P-58

Poster Presentation

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Dietary methionine in salmonid fish feed alters the expression of genes involved in methionine metabolism and epigenetics

We have previously studied the influence of exposure of fish embryos to methionine on embryonic development and epigenetics. In order to deepen the knowledge to this argument, we investigated the effect of dietary methionine (Met) concentration and alternating feeding strategies in methionine delivery, on the mRNA transcript levels of genes involved in Met resynthesis (betaine-homocysteine methyltransferase, BHMT; S-adenosylhomocysteine hydrolase, SAHH) and net Met loss (taurine synthesis) (cystathionine beta-synthase, CBS) in Atlantic salmon (Salmo salar) liver. Salmon alevins (265 ± 3 mg) were distributed into 24 tanks (50 fish per tank; 3 replicates). The experimental diets were supplemented with L-methionine at 0, 1.9, 5.8, and 17.4 g/kg (M0, M1/3, M1, and M3, respectively). The M3 diet without glycine was prepared to examine Met toxicity (M3-G). These diets were provided via `mono-feeding strategy' meaning fish were fed a designated single diet. This experiment also included alternative feeding groups with 'duo-feeding' strategy: AF1 (fish fed M0 for 2 days followed by M1 for 1 day), AF2 (fish fed M0 for 2 days followed by M3 for 1 day), and AF3 (fish fed two meals of M0 followed by one meal of M3). Salmon fed M0 diet had smaller weight compared to all other groups. There was no effect of alternative feeding on the growth except with the M0 group. The highest expression of CBS gene was found in the M0, M1/3, and AF-1 groups compared to M3 and M3-G groups. The expression of CBS gene in the M1 group was lower compared to the AF-1 group. The expression of SAHH gene was the highest in the M3 group compared to M1, M1/3, and AF-3 groups. The highest expression of BHMT gene was found in the M0, M1/3, and AF-1 groups indicating enhanced re-methylation of homocysteine by betaine to Met. The lowest BHMT expression in M1, M3, M3-G, AF-2, and AF-3 groups compared to other treatments can be indicative of downregulation of remethylation in the liver.

P-59

Poster Presentation

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Genome disarrangement cell line models reveal an epigenetically driven miR-204 regulation of androgen receptor

Androgen receptor (AR) signaling has a central role in normal male reproduction and in pathologies such as prostate cancer (PCa) being implicated cell survival, proliferation arrest, apoptosis, PCa initiation and metastatic progression. Cancer progression global genome re-arrangement affects epigenetic and non-coding regulation producing controversial micro-RNA mediated AR signaling phenomena. In this study we tired to address the role of miR-204 in modulating functional AR signaling in the context of total de-methylation in cell line models of lymph node (LNCaP) and advanced vertebral metastasis (VCaP), using RT-qPCR and flow cytometry (FCS) followup of AR after exogenous introduction of miR-204 chemically synthesized mimics or inhibitors. We followed-up the pathways under epigenetic control affected by miR-204 using nano-proteomics and the AR promotor methylation status in both cell lines. We found that while miR-204 artificial overexpression stimulated AR (qPCR, FCS) in both lymph node and vertebral metastasis cell lines, in concert with expected miR-204 tumor suppressor modality, its inhibition differentially affected AR, upregulating it in VCaP cells. The total demethylation using 5-AzaCytidine (5-AzaC) revealed an epigenetic based miR-204 modality shift towards AR independence. While 5-AzaC alone resulted in AR upregulation in both cell lines, confriming AR trasncript level control trough its promoter methylation, miR-204 over-expression preserved its AR stimulatory effect in LNCaP but not in VCaP cells. In latter, miR-204 over-expression resulted in both AR transcript and protein level reduction. We further found that in VCaP cells AR promoter is hypomethylated and that miR-204 further reduced its methylation 10 times. Our data suggested that methylation status is one of the mechanisms implicated in miR dysregulation in PCa.

P-60

Poster Presentation

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Effects of preimplantational estradiol-17β exposure on the expression profile of miRNAs in the porcine endometrium

The exposure to estrogenic substances during the preimplantational phase has been shown to alter or even disrupt pregnancy establishment in the pig. Several studies demonstrated endometrial mRNA expression changes starting at day 10 post insemination and degeneration of the embryos. In the pig, the synthesis of estrogens, especially estradiol-17b (E2), by the conceptus between days 11 and 12 is the signal for maternal recognition of pregnancy. In the present study, we analyzed the effects of preimplantational oral estrogen exposure on endometrial expression of miRNAs, as key players in posttranscriptional regulation. In addition, potentially E2 regulated target miRNAs were analyzed in the endometirum during the porcine estrous cycle. From insemination on, sows were fed the model estrogen E2 until examination at day 10 of pregnancy. Low and high dose effects were analyzed as either 0.05, 10 or 1000 µg E2/kg body weight/day, or ethanol carrier only, was given. The endometrial concentration of E2, measured using ELISA, was significantly increased in the highest dose group compared to the control (111 ± 4 and 3065 ± 830 pg/g tissue, $p < 0.05$). After high throughput-sequencing of small ncRNA libraries, the statistical analysis of treatment groups versus the control indicated no significantly different expressed miRNAs. Similarly, 13 potentially E2 regulated miRNAs analyzed by RT-qPCR, were also not significantly altered. However, the abundance of endometrial miR-205 ($p = 0.002$) and miR-146b ($p= 0.005$) varied significantly between days 0, 3, 6, 12 and 18 of the estrous cycle. A target prediction scan for miR-205 resulted in 182 target mRNAs. Interestingly, some of the latter are known to be involved in processes such as angiogenesis, adhesion, cell differentiation, and cell cycle control. This study demonstrated that despite cyclic changes of miRNA occurred in gilts, an oral E2 administration during preimplantation did not disrupt the endometrial expression profile of miRNAs during early pregnancy in the pig.

P-61

Poster Presentation

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Differential proteomic analysis of bovine oocytes and cumulus cells: zoom on histones abundance and modifications during maturation

During oocyte meiotic maturation, prophase immature oocytes (IO) progress to metaphase-II and became mature oocytes (MO) and this process is accompanied by significant molecular changes in the oocyte and in surrounding cumulus cells (CC). In vitro maturation (IVM) significantly affected gene expression in both oocytes and CC and also oocyte ability to regulate the epigenetic reprogramming, critical for successful meiosis and subsequent embryonic development. Using quantitative nano-liquid chromatography coupled to high resolution mass spectrometry (nano-LC-MSMS), we compared the proteomes of immature and in vitro mature oocytes and the proteomes of CC surrounding immature, in vivo mature (preovulatory) and IVM oocytes. Proteins from 200 IO, 200 MO and 10 micrograms of CC total proteins per condition were resolved by SDS-PAGE, trypsin-digested and analyzed using LTQ Orbitrap MS and Scaffold software. 380 and 1687 proteins were identified and quantified in oocytes and in CC, respectively. Among them, different linker histones (H1), core histones (H2A, H2B, H3 and H4), histone acetyltransferase HAT1 and histone deacetylase HDAC2 were identified. N-terminal acetylation was detected on HIST1H1C in oocytes and on HIST1H1A, HIST1H1D, HIST1H1E, H1F0, H2AFY and H2B in CC. Among the proteins differently represented in oocytes before and after maturation, histones H2B1C and H4 were significantly more abundant in MO as compared to IO ($p<0.05$). In CC, significant decrease of HIST1H1A was observed in preovulatory follicles as compared to immature ones, and HIST1H1E and HAT1 were more abundant in CC after IVM than after in vivo maturation. By using Intact Cells MALDI-TOF MS (ICM-MS) of oocytes and CC we observed several m/z peaks which may correspond to histones according to experimental m/z values. Direct fragmentation of intact molecular species (Top down approach) might be carried out to obtain sequence and structural information of these peaks in order to detect eventual modification of histones (methylation, acetylation, etc) and compare them between different maturation conditions.

P-62

Poster Presentation

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Effect of conceptus on prostaglandin catabolizing enzyme, 15-hydroxyprostaglandin dehydrogenase in the porcine endometrium

Prostaglandin (PG) E2 and F2α, synthesized by porcine endometrium and conceptus are important regulators of reproductive functions. However, effect which prostaglandins exert depends not only on their synthesis but also on catabolism. The objectives of present study were: to determine the gene expression profile of the main enzyme of prostaglandin catabolism, 15-hydroxyprostaglandin dehydrogenase (HPGD) in the endometrium; to analyze the effect of conceptus on endometrial HPGD gene and protein expression in vivo; and to determine HPGD gene expression in conceptus/trophoblast during early pregnancy. Endometrial samples were collected from gilts on days 9, 11, 12, and 15 of the estrous cycle (n=33) and pregnancy (n=28). Conceptuses/trophoblasts were obtained on days 10-11, 12-13, 14-16, 18-20 and 22-25 of pregnancy (n=3-6 per group). Endometrial expression of HPGD gene was decreased on day 12 of pregnancy (vs. corresponding day of the estrous cycle; $p<0.05$). To study effect of embryo on HPGD expression, an unilateral model of pregnancy was used. Gene and protein expression of HPGD was reduced in the endometrium from intact uterine horn on days 11 and 14 of pregnancy when compared with surgically isolated horn at the corresponding stage of the estrous cycle ($p<0.05$). The local down-regulation of HPGD gene and protein expression by conceptus on day 11 of pregnancy was observed in the endometrium from the gravid horn (vs. the isolated horn; $p<0.05$). Gene expression of HPGD in conceptus/trophoblast was low on days 10-13, intermediate on days 14-16 and elevated on days 18-25 of pregnancy ($p<0.001$). Summarizing, our results indicate local inhibition of PGE2 degradation by porcine conceptus during maternal recognition of pregnancy. Elevated expression of HPGD gene in the conceptus/trophoblast after day 14 may be involved in creating a protective barrier for embryo against active prostaglandins. It can also limit the effect of prostaglandins on myometrium contractility during embryo implantation.

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Poster Presentation

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Maternal effect genes expression in porcine oocytes and embryos

Maternal effect genes play crucial role in events such as oocyte meiosis completion, successful processing of the sperm nucleus, organization of the two pronuclei, first embryonic divisions, and finally activation of the embryonic genome. The purpose of the present study was to: 1) determine the expression of selected maternal effect genes: maternal antigen that embryos requires (MATER), zygote arrest 1 (ZAR-1) and nucleoplasmin 2 (NPM2) in the in vitro cultured porcine oocytes and embryos at the consecutive stages of development (2-, 4-, 8-, morula and blastocyst); 2) examine the influence of hormonal estrus induction on the mentioned maternal effect genes expression in porcine embryos at 4-cell stage, when the embryonic genome is activated. For the first experiment, porcine oocytes and embryos obtained in vitro and in vivo were used. In the second experiment, gilts were subjected to hormonal estrus induction with two different protocols: (1) treated with PMSG/hCG and (2) with PMSG/hCG+PGF2α. Then animals were inseminated and embryos at the stage of 4-cell were flushed from the uterine horns. Transcripts levels of the investigated maternal effect genes were determined with the use of qPCR. We found all the examined transcripts both in porcine oocytes and embryos. However, their expression in in vitro cultured embryos decreased at the earlier stage of development (starting from 2-cell stage), compared to in vivo obtained porcine embryos (4-cell stage). The hormonal induction of estrus either with PMSG/hCG or with inclusion of PGF2α, did not affect significantly the transcripts levels of the investigated genes in 4-cell porcine embryos. Summarizing, the lower levels of maternal effect genes transcripts in embryos obtained in vitro (comparing to those observed in in vivo embryos) and their drop before embryonic genome activation, can be the reason for the inferior developmental competence and lower yields of in vitro produced porcine embryos.

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Poster Presentation

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Can porcine embryos induce the expression of estrogen receptor α (ESR1) simultaneously in the uterus and peripheral blood mononuclear cells?

Embryo-maternal cross-talk results in significant alterations in the regulation of genes expression in the endometrium and the myometrium. These alterations lead to structural and functional adaptation of uterine tissues for maintenance of pregnancy, e.g. modulation of both tissues remodeling, regulation of synthesis and secretion of steroids and prostaglandins or immune response in the uterus. Pregnancy, as a physiological state, manifests itself also systematically and some explicit changes can be visible in peripheral blood. Adaptations observed in peripheral blood include fluctuations of steroid hormones in blood plasma and changes in the expression of number of genes in peripheral blood mononuclear cells (PBMC). Estradiol-17β is one of most potent steroid hormone which acts multidirectional via specific receptors - ERα and ERβ. The current study aimed to determine: 1) whether the embryos induce changes in ERα gene (ESR1) expression in porcine endometrium and myometrium, and 2) if these putative changes in ESR1 expression in the uterus are reflected in PBMC. The adult crossbred gilts were used during maternal recognition of pregnancy (days 12 to 13; n = 5) and on days 12 to 13 of the estrous cycle (n = 5). The total RNA was isolated from the endometrium, the myometrium and PBMC and reverse-transcribed. The amplification of ESR1 was performed using real-time PCR. The expression of ESR1 was higher in pregnant vs. cyclic endometrium and did not differ in pregnant vs. cyclic myometrium. In PBMC from pregnant gilts the ESR1 expression was decreased ($P \leq 0.05$) in comparison with ESR1 expression in PBMC of cyclic gilts. The results clearly indicate on tissue-dependent regulation of ESR1 expression. The comparative transcriptomic profiling will be used to describe in details if porcine embryos can simultaneously induce the expression of other uterine and PBMC genes.

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