Epigenetics and Periconception Environment

Proceedings of the EPICONCEPT Conference 2016

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Editors
Tiziana Brevini, Alireza Fazeli, 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:

- bottom up approach (the initiative of launching a COST Action comes from the European scientists themselves),
- 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 Epiconcept Members

This will be the final meeting of the FA1201 COST Action *'Epigenetics and Periconception Environment'*. We have had our conferences in Turkey, Portugal and Crete, and our workshops in Spain, Canary Islands, Croatia and Bulgaria. This last meeting will be held in Italy on the gorgeous island of Sicily which is described as the '*eternal crossroads of the Mediterranean'*, and *'an island with a dazzling diversity of landscapes'*. Both expressions illustrate what Epiconcept meant to us during the past four years.

Epiconcept was a crossroad where we met as scientists, but also as friends. The project was a reincarnation of epigenetics, the word which was first mentioned by Waddington the famous scientist. The secrets of the landscape of epigenetics have only partially been unveiled at our meetings but it was a good start! Finally, Epiconcept provided us with dazzling opportunities to continue and reshape our research. Now we are ready to continue this exploration to *'Where No (Wo)Man Has Gone Before'* (just like in the science fiction television series Star Trek in 1965).

For me it has been an honor to act as your chair in the past four years. I would not have been able to do it without the invaluable help from Alireza Fazeli who took over when needed, and from Laszlo Tecsi who looked after all administrative details. This time special words of thanks to Tiziana Brevini who volunteered to be the local organizer of this last meeting, and was able to surprise us with many hidden gems. I would also like to thank all other members of the executive committee, the working group leaders Trudee Fair, Alfonso Gutierrez-Adan, Kevin Sinclair, Anne Navarette-Santos, Pascale Chavatte-Palmer, Amos Tandler, Anita Franczak and Tiziana Brevini. Last but not least we are very grateful to Christine Aurich and Elin Kjorsvik who were managing the short term scientific missions.

I hope you will enjoy this meeting as much as I will do. And I am sure *'we'll meet again, don't know where, don't know when, but I know we'll meet again, some sunny day'*.

All the best

Ann Van Soom
Chair of Epiconcept

Organisers

Alireza Fazeli, United Kingdom
Ann Van Soom, Belgium
Laszlo Tecsi, United Kingdom
Tiziana Brevini, Italy

Programme

Day 1
Monday 26 September 2016

17:00 – 19:30 **Meeting Registration**

19:30 – 20:30 **Welcome Reception**

20:30 – 21:45 **Dinner**

Day 2
Tuesday 27 September 2016

07:45 – 09:00 **Breakfast**

09:00 – 09:15 (15:00)
Tiziana Brevini, University of Milano, Italy
Ann Van Soom, University of Gent, Belgium
Welcome Address

Working Group 1: Epigenomic Tools

09:15 – 09:45 (30:00)
Jerome Jullien, Gurdon Institute, United Kingdom
Sperm is epigenetically programmed to regulate gene transcription in embryos

09:45– 10:15 (30:00)
Pilar Coy, University of Murcia, Spain
Unvealing and palliating the epigenetic cost of assisted reproductive technologies

10:15– 10:30 (15:00)
Noelia Diaz, Max Planck Institute for Molecular Biomedicine, Germany
Characterization of the zebrafish 3D genome

10:30 – 11:30 **Coffee and Poster Presentation**

11:30– 11:45 (15:00)
Alfonso Gutierrez-Adan, Spanish National Institute for Agricultural and Food Research and Technology (INIA), Spain
Impaired folliculogenesis function in defective Zrsr2 mutant mice

11:45– 12:00 (15:00)
Jennifer Schoen, Leibniz Institute for Farm Animal Biology, Germany
Modelling the embryo-maternal contact zone in vitro: an air-liquid interphase approach to mimic the oviduct milieu

12:00 – 12:15 (15:00)
Mareike Pendzialek, Martin Luther University Faculty of Medicine, Germany
Maternal diabetes mellitus type 1 downregulates the embryonic microRNA biogenesis in trophoblast cells

12:30 – 14:00 **Lunch**

Working Group 2: Periconception Environment

16:00 – 16:30 (30:00)	**Daniel Brison, University of Manchester, United Kingdom** The impact of ART environment on embryo and child growth and health
16:30 – 17:00 (30:00)	**Michael Skinner, Washington State University, United States** Environmentally induced epigenetic transgenerational inheritance of disease: ancestral ghosts in your genome
17:00 – 17:15 (15:00)	**Naveed Jhamat, Swedish University of Agricultural Sciences, Sweden** Identification of differentially methylated regions in the genome of bovine endometrial epithelial cells (bEEC) challenged by E. coli LPS and its effect on transcription
17:15 – 17:30 (15:00)	**Shaghayegh Basatvat, University of Sheffield, United Kingdom** Trophoblast outgrowth rate is dependent on the co-culture epithelial cell types and mode of the culture
17:30 – 17:40 (10:00)	**Alireza Fazeli, University of Sheffield, United Kingdom** Dedication Address
17:40 – 18:10 (30:00)	**Tom Fleming, University of Southampton, United Kingdom** Embryos: how they are made and what influences their future
18:10 – 18:40 (30:00)	**Discussion**
18:40 – 19:40 (60:00)	**Management Committee Meeting**

19:45 – 21:45	**Dinner**

Day 3
Wednesday 28 September 2016

07:45 – 09:00	**Breakfast**

09:00 – 09:15 (15:00)	**Laszlo Tecsi, University of Sheffield, United Kingdom** Rules of travel reimbursements

Working Group 3: Cross-species Epigenetics, Gametogenesis and Embryogenesis

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09:45 – 10:15 (30:00)	**Carlos Guerrero-Bosagna, Linkoping University, Sweden** Transgenerational epigenetic inheritance: implications from humans to farm animals
10:15 – 10:30 (15:00)	**Francesc Piferrer, Spanish Council for Scientific Research, Spain** Genetic, epigenetic and transcriptomic studies aimed at improving the breeding and control of reproduction in the european sea bass and the turbot

10:30 – 11:30	**Coffee and Poster Presentation**
11:30 – 11:45 (15:00)	**Wim Vanden-Berghe, University of Antwerp, Belgium** Does epigenetic control of zebrafinch birdsong reveals secrets of human speech and language development?
11:45 – 12:00 (15:00)	**Yoav Soen, Weizmann Institute of Science, Israel** Adaptation by natural improvisation
12:30 – 14:00	**Lunch**
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16:00 – 16:30 (40:00)	**Geert Opsomer, University of Gent, Belgium** Evidence for developmental programming in dairy cattle
16:30 – 17:00 (40:00)	**Bruce Whitelaw, Roslin Institute, United Kingdom** Genome editing technology
17:00 – 17:15 (15:00)	**Asim Qayyum Akbani, Swiss Federal Institute of Technology (ETH), Switzerland** Low-dose estrogen exposure modifies the environment for embryo development by epigenetic reprogramming of the female reproductive tract
17:15 – 17:30 (15:00)	**Ana Katusic-Bojanac, University of Zagreb, Croatia** Negative effect of valproate on embryonic growth in vitro can be compensated by subsequent transplantation in vivo
17:30 – 17:45 (15:00)	**Luca Palazzese, Universtiy of Teramo, Italy** Starvation improves sheep fibroblast chromatin remodeling in spermatide-like structure
17:45 – 18:00 (15:00)	**Jorge Lopez-Tello, University of Cambridge, United Kingdom** Determining the role of placental endocrine zone Igf2 in the 'tug of war' over resources between mother and fetus in mice
18:00 – 18:30 (30:00)	**Discussion**
18:30 – 19:00 (30:00)	**Alireza Fazeli, University of Sheffield, United Kingdom** Eight Years of COST; from a dream to the reality
19:00 – 19:15 (15:00)	**Tiziana Brevini, University of Milano, Italy** **Ann Van Soom, University of Gent, Belgium** Farewell Address
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Day 4
Thursday 29 September 2016

07:45 – 09:00	**Breakfast**
09:00 – 16:00	**Excursion**

Abstracts of Presentations

O-1

Oral Presentation

Akbani, Asim Qayyum

Institute of Agricultural Sciences, Swiss Federal Institute of Technology (ETH), Switzerland

Akbani AQ, Floter VL, Ulbrich SE

Low-dose estrogen exposure modifies the environment for embryo development by epigenetic reprogramming of the female reproductive tract

Early life exposure to endocrine disrupting chemicals (EDC) critically influences the endocrine-dependent ontogeny, leading to adverse consequences at later stages of life. However, the mechanisms of action, specifically of low-dose exposure, are still poorly understood. Recent findings have revealed perturbed DNA methylation patterns in offspring of animals after prenatal exposure to EDC, highlighting epigenetics as a possible underlying cause. In this study, we analysed the direct effect of EDC on the female reproductive tract; the environment for embryo development. Two different low doses and one high dose (0.05, 10 and 1000 µg/kg body weight/day) of estradiol-17β (E2) were orally applied in a pig model during the first 10 days of pregnancy. Potential effects on different tissues including liver, spleen, heart, skeletal muscle, endometrium and corpus luteum were analysed. Global methylation was determined by Luminometric Methylation Assay (LUMA), showing an overall trend of hypo methylation of up to 2% between control and other treatment groups. Interestingly, pronounced differential gene expression (Fluidigm, Biomark) was revealed of genes with functions related to cell cycle regulation and tumour suppression including BTG2, RERG, CDKN1A, CDKN2D, DAPK1, BUB1, GAS1, and SFRP1; showing a tissue and treatment specific lower expressions of up to 10-fold. The downregulation of such genes is often correlated with promoter hyper methylation, including CGI shores, that is considered a hallmark of carcinogenesis. Local methylation analyses using the PyroMark Q48 Autoprep, Qiagen for pyrosequencing are currently being performed to observe the methylation patterns possibly underlying this differential expression. Thus, by applying global and gene-specific DNA methylation we focus on E2-induced epigenetic reprogramming of the environment in which the developing embryo may experience impacts leading to transgenerational epigenetic effects.

P-2

Poster Presentation

Anastasiadi, Dafni

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

Anastasiadi D, Esteve-Codina A, Piferrer F

DNA methylation and relationships with gene expression in wild fish

In recent years, the regulation of gene expression by DNA methylation has been a central focus in research related to biomedicine. Only lately DNA methylation patterns are studied in non-model species and under an ecological context. Here, we used a wild marine fish, the European sea bass (Dicentrarchus labrax), to study natural patterns of DNA methylation and their relationship with gene expression in tissues of different cellular heterogeneity. Tissue samples were obtained from testis and muscle of wild sea bass caught off the Montgrí, Medes Islands and Baix Ter Natural Park, and were processed by Reduced Representation Bisulfite Sequencing (RRBS) to measure DNA methylation and RNA-seq to measure gene expression. The majority of CpG sites were either methylated or unmethylated in both tissues, although there were differentially methylated sites between tissues, while in specific genomic features, such as promoters and first exons, the majority of CpG sites were unmethylated. In testis there were more genes expressed than in muscle, while testis showed a more complex transcriptome including testis-specific genes showing low expression. In addition, while in both tissues there was in general low methylation of promoters and first exons independently of gene expression, in testis there was high variation in DNA methylation levels of promoters and first exons in the 20% of the least expressed genes. Tissue-specific genes containing differentially methylated regions (DMRs) showed both positive and negative correlation with gene expression, while these DMRs contained unique TF-binding sites depending on the direction of the correlation. Taken together, these results provide insights to the tissue-specific regulation of gene expression by DNA methylation in vertebrates in general and in marine fish in particular.

Supported by MINECO grant AGL2013-41047-R “Epifarm” to FP.

P-3

Poster Presentation

Andronowska, Aneta

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

Andronowska A, Malysz-Cymborska I

Changes in VEGF system in the porcine oviductal epithelial cells after exogenous gonadotropins treatment

The oviduct plays a crucial role in female reproduction by regulating gamete transport, providing a specific microenvironment for fertilization and early embryonic development. Beside its basic function, the oviduct secretes fluid, which is a complex mixture of plasma-derived products and proteins actively synthesized by oviductal tissue. Vascular endothelial growth factor (VEGF) is a well-known, potent angiogenic, and permeability-inducing factor. Our previous study revealed that induction of ovulation and superovulation affected the VEGF system in the porcine oviduct. However the mechanisms responsible for its regulation expression remain unclear. The aim of this study was to investigate the influence of hCG and/or FSH on expression of VEGF system in POEC. POEC, obtained in around ovulation period, were incubated with hCG (1ng/ml or 50 ng/ml), FSH (10 ng/ml) or hCG/FSH (1ng/ml or 50ng/ml) for 24 or 48 hours. Gene expression for VEGF, Flt-1 and KDR was done by qPCR. After 24 hours of stimulation VEGF mRNA was increased only after 1ng/ml of hCG($p<0,05$), transcripts for Flt-1 decreased under 50 ng/ml of hCG($p<0,05$) or FSH ($p<0,05$) alone, KDR mRNA expression dropped after FSH and hCG/FSH low dose treatment. Similarly, 48 hours incubation revealed the increase of VEGF transcripts after hCG (50ng/ml) FSH and hCG/FSH (1ng/ml) stimulation ($p<0,05$), decrease of Flt-1 mRNA expression after FSH ($p<0,05$) and hCG/FSH (50 ng/ml, $p<0,001$). After 48 hours both hCG and FSH alone or in combination demonstrated inhibitory effect on KDR mRNA expression ($p<0,0001$). Our study showed that stimulation with hCG and FSH affected the mRNA profiles of the VEGF system in POEC. Disrupted VEGF system expression may be crucial to many events occurring during the periovulatory period and consequently could lead to deprivation of VEGF-dependent factors that are necessary for proper fertilization, gamete transport and embryo development.

O-4

Oral Presentation

Basatvat, Shaghayegh

Department of Human Metabolism, University of Sheffield, United Kingdom

Basatvat S, Maslehat Lay N, Elliott S, Fazeli A

Trophoblast outgrowth rate is dependent on the co-culture epithelial cell types and mode of the culture

Studying the implantation in human is a great challenge as using in vivo models is not ethical and the animal models are not fully representing the events taking place in the human. Trophoblast outgrowth is a critical event in the establishment of a close relationship with the mother for a successful pregnancy. Using human 2- and 3-Dimentional cell culture models, we studied the outgrowth of trophoblast spheroids on endometrial (RL95-2 and Ishikawa) and non-endometrial epithelial (HEK293T) cells over the course of 24, 48 and 72 hours. The 3D model consisted of human stromal endometrial cells (HESC) seeded in a gel with either RL95-2 or Ishikawa or HEK293T overlaying the gel as a monolayer coincubated with the Trophoblast (JAr) spheroids to mimic human embryos. The in vitro 2D model consisted of JAr spheres cocultured with either of the epithelial cell lines. The JAr outgrowth was analysed by the image J software. All the epithelial cells tested, supported the outgrowth of JAr spheroids. But the rate of spheroids outgrowth on RL95-2 cells was significantly higher compared to Ishikawa and HEK293T cells in the 2D and the 3D models. The outgrowth rate of JAr spheroids on RL95-2 cells and HEK293T cells in 2D model was higher than their outgrowth rate in the 3D model. Although, all the epithelial cells tested supported JAr spheroids outgrowth, the RL95-2 cells which represented receptive endometrium induced the highest rate of trophoblast outgrowth, indicating that trophoblast proliferation to form connection with the endometrial cells is better supported by a receptive endometrial epithelial cells. Further investigations are needed to understand if a higher rate of trophoblast growth is necessarily synonymous with better quality of interaction between trophoblast and epithelial cells.

P-5

Poster Presentation

Blitek, Agnieszka

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

Blitek A, Szymanska M

Peroxisome proliferator-activated receptors (PPARs) affect porcine trophoblast cells in a ligand-specific manner - in vitro study

Peroxisome proliferator-activated receptors (PPARs) belong to the nuclear receptor super-family and function as transcription regulators. PPARs are involved in glucose homeostasis, lipid metabolism, and affect cellular growth and differentiation. There are increasing evidence showing its important role for conceptus development. In the present study, cultured porcine trophoblast (Tr) cells were used to examine the effect of PPARα, β/δ and γ activation on proliferation as well as estradiol and prostaglandin (PG) E2 synthesis. Eight gilts at early implantation period (days 14-16) were used as a source of Tr cells. For proliferation assay, Tr cells were seeded in 96-well plates and exposed to GW9578 (1-100 nM; PPARα agonist), L-165,041 (0.1-10 μM; PPARβ/δ agonist), or rosiglitazone (0.1-10 μM; PPARγ agonist). Viable cells were stained with 0.2% crystal violet. To study the effect of PPARs activation on estradiol and PGE2 synthesis, Tr cells were cultured in 48-well plates and treated with PPARs agonists. Medium was collected to analyze concentrations of estradiol (RIA) and PGE2 (ELISA), while cells were used for mRNA analysis of PTGS2, mPGES1 and P450arom (qPCR). One-way ANOVA was performed for data analysis. GW9578 and rosiglitazone, but not L165,041, stimulated proliferation of Tr cells ($P<0.05$). Rosiglitazone increased P450arom mRNA expression ($P<0.05$), however did not affect estradiol secretion. Moreover, activation of PPARγ by rosiglitazone stimulated mPGES1 transcript abundance in cells and PGE2 accumulation in medium ($P<0.05$). In summary, PPARs are important components of porcine conceptus function. Activation of PPARα and PPARγ may contribute to successful implantation by increasing cell proliferation and PGE2 synthesis; respectively.

Supported by NSC grant 2013/11/B/NZ9/00806

O-6

Oral Presentation

Brison, Daniel

Department of Reproductive Medicine, Manchester Academic Health Sciences Centre, United Kingdom

Brison DR, Path FRC

The impact of ART environment on embryo and child growth and health

Clinical Assisted Reproduction Technology (ART) is now considered routine treatment with an estimated 5 million babies born globally since 1978. However, the pace of scientific and technological advances means that ART practitioners now have access to an increasing array of new and invasive technologies. In parallel with this, wider scientific and medical advances mean that we are becoming increasingly aware of the potential impact of ART on embryonic development, gene expression, epigenetics, and the long-term health of ART children according to the Barker hypothesis and the Developmental Origins of Health and Disease (DOHaD). I will describe our research on the impact of ART on the transcriptome of human preimplantation embryos and cells, and on the birthweight and early growth of children arising from ART treatment. This work is funded by the UK MRC and the EU FP7 Health programme as part of the EpiHealth consortium.

Poster Presentation

Calderari, Sophie

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

Calderari S, Archilla C, Jouneau L, Peynot N, Daniel N, Mourier E, Richard C, Tarrade A, Gatien J, Chavatte-Palmer P, Duranthon V

High fat maternal diet impacts the nutrient sensing signaling network in rabbit blastocyst

The periconceptional period is recognised as a particularly vulnerable period for environmental programming. Maternal nutrition during developmental stages may trigger effects on several physiological functions of the organism. We have previously shown that a maternal lipid and cholesterol-enriched diet (HH diet) administred to rabbit does from prepubertal period and throughout gestation induces fetal growth retardation and components of the metabolic syndrome in adult offspring. HH diet induces lipid droplet accumulation at the blastocyst stage. We aimed to identify the impact of maternal HH diet on rabbit blastocyst gene expression. We performed transcriptomic profiles of blastocysts obtained from rabbit does fed with either a HH diet (n=15) or a control diet (n=12). For this purpose, customized rabbit microarray (Agilent Technologies, GEO platform GPL16709) was used. Statistical analysis (Limma) revealed 49 probes differentially expressed in HH vs control blastocysts (padj<0.05). We were able to annotate 26 genes, 16 up and 10 down-regulated. Functional classification based on GO terms indicated that differentially expressed genes are mainly implicated in lipid transporter activity (RBP4, SCP2) and glucose metabolic process (RBP4, PGD, OMA1). In addition to this approach, we used Gene Set Enrichment Analysis (GSEA) to identify significant enrichment of gene sets (FDR<0.25, p<0.05). Beyond the confirmation of deregulation of glucose and lipid metabolisms, GSEA highlighted enrichment of mTORC1, a master growth regulator that senses growth factors, amino acids, energy status levels to regulate cell growth, proliferation and lipid metabolism. In conclusion, we demonstrated that maternal HH diet altered nutrient sensing and cellular metabolism in blastocyst. These transcriptomic datas will be soon integrated with metabolomic datas obtained on HH and control blastocoelic fluids using ultrasound biomicroscopy puncture by NMR.
Funded by INRA PHASE: ACI 2015 program.

Poster Presentation

Canon, Eugenie

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

Canon E, Blachere T, Jouneau L, Peynot N, Daniel N, Boulanger L, Godet M and Duranthon V

Progressive establishment of differential methylation on POU5F1 upstream sequence over blastocyst development

The POU5F1 gene encodes one of the "core" transcription factors necessary to maintain pluripotency but very few data are available concerning the precise epigenetic regulation of its expression in early embryo. We analysed the progressive modifications of DNA methylation of POU5F1 upstream region in the different compartments of the developing blastocyst. Therefore we used the rabbit embryo as a model for most mammalian blastocysts, where contrarily to the mouse, epiblast differentiates in a plane embryonic disk at the surface of the conceptus and in direct contact with maternal environment. Embryos were micro dissected at Day4,5, and6. POU5F1 expression was quantified by RTqPCR. Methylation profile of four regions encompassing conserved cis-elements (Kobolak et al. 2009 BMC Mol. Biol.10 88.) was determined by bisulfite treatment, cloning and sequencing. POU5F1 is highly expressed in Day4 trophectoderm but significantly enriched in the inner cell mass. At Day5, the POU5F1 relative expression decreased but the difference between embryonic and trophectoderm compartments tends to increase. At Day6, POU5F1 transcripts were restricted to the epiblast, with a very reduced expression in the hypoblast. Noticeably POU5F1 expression progressively decreases in the trophectoderm/trophoblast compartment over the Day4-Day6 period. The four regions were hypomethylated in pluripotent Inner Cell Mass and epiblast. Differences in DNA methylation between pluripotent and differentiated layers occurred as early as Day4 and progressively increased. A principal component analysis segregated pluripotent and highly differentiated samples and identified the CpGs mostly involved in this separation. Interestingly while differentiation-related changes in methylation occurred in the four analyzed regions, they are more pronounced in the region encompassing the proximal enhancer 1A.

Funded by Agence de Biomédecine

P-9

Poster Presentation

Castillo, Catherine

Maternal and Fetal Health Research Centre, University of Manchester, United Kingdom

Castillo C, Horne G, Fitzgerald CT, Johnstone E, Brison DR, Roberts SA

Data mining and modelling the impact of in vitro fertilisation on birth weight outcomes

In vitro fertilisation (IVF) conceived singletons show increased incidence of low birth weight (BW) compared to naturally conceived, raising concerns over offspring long term disease risk. Few causal links between IVF procedures and BW have been established. These two studies aim to explore how IVF procedures may affect BW whilst accounting for patient and pregnancy related influences in two retrospective (1) single-centre and (2) multi-centre UK cohorts. Methods: (1) A dataset was collated from 23 years of data from St. Mary's Hospital (SMH) in Manchester, UK, capturing 2,780 singletons conceived from 1991-2015 from fresh and frozen embryo transfer (ET) cycles. (2) Details of embryo culture systems used for fresh cycles performed from 2011-2013 were collected from 46 clinics using the National Culture Media Questionnaire (NatCMQ), capturing 18,708 singletons, then linked to national patient, IVF procedure and birth outcome data. Associations between IVF treatment factors and BW (adjusted for patient/pregnancy factors) were tested using multiple linear regression models in both datasets. Results: (1) adjusted BW increased by >285 grams in the SMH cohort; frozen ET-conceived singletons remained heavier on average compared to fresh; (2) Unadjusted NatCMQ analyses showed a significant effect of culture medium on adjusted BW. However, this association lost significance when accounting for clinic as a confounder. A patient's increasing duration of infertility and the occurrence of spontaneous fetal reduction were associated with decreased adjusted BW in both cohorts. Conclusions: This consistent historical increase in IVF-conceived singleton BW has not been shown before. The effect of embryo freezing and duration of infertility on adjusted BW suggest that IVF procedures and patient characteristics may have independent yet compounding effects on offspring outcomes. Further investigations into the possible epigenetic and long-term effects of IVF are needed.

P-10

Poster Presentation

Cikos, Stefan

Department of Developmental Physiology, Slovak Academy of Sciences, Slovakia

Cikos S, Janstova Z, Burkus J, Kubandova J, Fabian D, Koppel J

Expression of glucocorticoid receptor subtypes in mouse preimplantation embryos

Maternal stress is characterized by increased levels of glucocorticoids that can reach the developing embryo. Moreover, the increased exposure to glucocorticoids can also occur during the therapeutic administration of synthetic glucocorticoids. Glucocorticoids can act through a large cohort of glucocorticoid receptor isoforms, and expression of different receptor subtypes can lead to different physiological consequences. The aim of this study was to ascertain whether glucocorticoid receptor (GR) is expressed in mouse oocytes and preimplantation embryos. Using RT-PCR with oligonucleotide primers which detect all GR splice variants we found GR mRNA in mouse ovulated oocytes and in vivo preimplantation embryos. We designed primers distinguishing between particular GR splice variants and found two transcripts expressed in oocytes and four splice variants expressed in blastocysts. The identity of PCR products was verified by digestion with restriction enzymes cutting within the sequence specific for a particular splicing variant. Using real-time PCR, we quantified canonical GR transcript (GR alpha) in mouse oocytes and preimplantation embryos at several developmental stages and found significant differences in the mRNA abundance. Expression of glucocorticoid receptor protein was examined by fluorescence immunohistochemistry using the antibody specific to the part of GR protein common for all known isoforms. We detected the GR protein in both examined stages – ovulated oocytes and blastocysts. In summary, our results indicate that several glucocorticoid receptor transcripts are expressed in mouse oocytes and preimplantation embryos, and that GR protein is produced in oocytes as well as in preimplantation embryos.

This work was supported by the Slovak Research and Development Agency under contracts APVV-0815-11, APVV-14-0763, and the Slovak Academy of Sciences under contract VEGA 2/0039/15.

O-11

Oral Presentation

Coy-Fuster, Pilar
Department of Physiology, University of Murcia, Spain

Coy P, Canovas S

Unvealing and palliating the epigenetic cost of assisted reproductive technologies

In vitro fertilization and embryo culture represent artificial intrusions into the natural development that may affect the epigenome of the resulting offspring. Indeed, some specific alterations have been already associated to these assisted reproductive technologies (ART), being the best known those related with abnormal methylation at LIT1, H19 or IGF2R loci. However, genome wide analysis of DNA methylation comparing in vivo and in vitro-derived embryos in mammalians species are not yet available and thus the whole range of consequences of ART are unknown. The suspicion that addition of serum into the in vitro culture media could be the reason for some of the described alterations made most of the laboratories move towards the design of serum-free media, forgetting the possible important role of oviductal proteins on the normal development of zygote and preimplantation embryos. We have got not only first datasets showing whole-genome DNA methylation (Bisulfite-Seq) differences between in vivo and in vitro produced single blastocysts, but also a method to palliate such differences. By using reproductive secretions as additives in the culture media, a significant increase in the yield and quality of the blastocysts produced from a morphological, epigenetic and gene expression point of view was achieved. In addition, the embryos produced with reproductive secretions were more similar to the in vivo specimens than those produced in chemically defined media.

P-12

Poster Presentation

De Ruijter-Villani, Marta
Faculty of Veterinary Medicine, Utrecht University, Netherlands

de Ruijter-Villani M, Gibson C, Bauersachs S, Stout TAE

Effects of asynchronous embryo transfer on the endometrial transcriptome in mares

Conceptus development can be accelerated by a more advanced uterus and retarded by a negatively asynchronous uterus. While embryo-uterine asynchrony is incompatible with embryo survival in most species, the horse embryo can tolerate up to 5 days of negative asynchrony. This study used this ability to try to separate the effects of time after ovulation and embryo developmental stage on the endometrial transcriptome. Day 8 embryos were transferred to recipient mares that either ovulated on the same day as (synch.n=8), or 5 days after (asynch. n=8), the donor mare. Endometrial biopsies were recovered 6 or 11d after ET for RNA extraction. After RNA library, RNA sequencing was performed (IlluminaNS500). Reads were mapped to the equine genome (TopHat2), the number of reads per gene was calculated (QuasR qCount), and statistical analysis was performed (DESeq2). Conceptuses recovered from asynchronous recipients were less developed. On day 6 after ET, 523 genes were classified as differentially expressed in synchronous versus asynchronous endometrium (>1.4-fold,FDR 1%). On day 11 after ET, there were 715 differentially expressed genes (DEGs). Functional groups highly represented among DEGs included growth factors, nutrient transporters, adhesion molecules, ECM components, and inflammatory mediators. Interestingly, when endometrium recovered on day 14 after ovulation (ET+6 synch. and ET+11 asynch.) was compared, only 14 genes were differentially expressed. In conclusion, the changes in the endometrial transcriptome observed appear to be mainly influenced by ovarian steroid priming, with a relatively small number of genes responding to a more advanced conceptus. The genes differentially regulated in the presence of an older conceptus will be interesting targets for examining how the conceptus influences its environment, whereas those that are not induced prematurely by the conceptus will include important regulators responsible for the observed delay in conceptus development.

O-13

Oral Presentation

Diaz, Noelia

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Characterization of the zebrafish 3D genome

The three-dimensional organization of chromatin in the interphase nucleus plays a crucial role in regulating the transcriptional program of the cell. In species ranging from human to worm, the genome adapts a specific topology into compartments and topologically associating domains, which delimit transcriptionally active and inactive states of chromatin. This domain-based organization of the genome seems to be strongly conserved across species. Until now the 3D structure of the zebrafish genome and its conformational changes remain elusive in part due to its genome duplication event and the presence of ohnologous genes scattered throughout its genome. Here, we present the first set of genome-wide chromatin conformation capture (HiC) of the zebrafish genome at a 25 kb resolution to characterize the changes in chromatin topology during mid-embriogenesis. We have characterize the structural features of chromatin topology using interaction maps obtained at 24 and 48 hours post fertilization showing that chromatin in fish embryos has a similar organization than other higher-eukaryotes with conformational features readily apparent at the megabase resolution. Our results provide a first insight into the three dimensional organization of chromatin in zebrafish and open the door to further studies on conformational changes during development.

P-14

Poster Presentation

Dinnyes, Andras
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Dinnyes A

How to continue the epigenetic research beyond Epiconcept: H2020 funding opportunities.

H2020 calls offer an opportunity to continue and expand the research collaborations and ideas initiated by Epiconcept

O-15

Oral Presentation

Dixon, Brian
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Dixon B

Epigenetic control of teleost fish immunity: an ocean of possibilities

Research into fish immunity has advanced to a point where the study of genetic control has become a relevant concern for both basic research and aquaculture. It is also becoming clear that there are large family specific differences in disease resistance that cannot be attributed to genetic content alone. Recently mammalian epigenetic studies have shifted to focus onto processes that require rapid responses to environment shifts such as immune response to pathogens. Mammalian studies on both methylation and miRNA have shown a role for both in both innate and adaptive immunity. Investigation of teleost immunity is only now taking advantage of the recently published genomes and more genomic tools to begin investigating the regulation processes that might control all the newly discovered genes. Many teleost species are polyploidy (for example, salmonids underwent a relatively recent genome duplication that has left the, with multiple copies of many immune genes). There is to date little research on the role of these processes in fish, but given that teleost immune systems are similar, but perhaps even more complicated, to mammalian processes it is not unreasonable to think that epigenetics will play a role here. Teleost immunity, the first steps in studying epigenetics in its regulation and future directions for research will be discussed.

P-16

Poster Presentation

Duranthon, Veronique

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Vitorino-Carvalho A, Jouneau L, Archilla C, Canon E, Laffont L, Ruffini S, Corbin E, Mermillod P, Duranthon V

Effects of BOEC and VERO co-culture systems on bovine early development

Embryo development is known to be impacted by its environment and especially by oviductal secretions in vivo. In cattle, embryo co-culture with bovine oviduct epithelial cells (BOEC) has been developed to mimic the in vivo oviduct/embryo crosstalk. However, whether BOEC had a specific impact on embryo transcriptome hasn't been investigated yet. We thus compared bovine embryos co-cultured with BOEC to embryos co-cultured with VERO cells (a kidney epithelial cell line from monkey). Control embryos were obtained in SOF medium + 5% Fetal Calf Serum (FCS) at 5% O2. Because co-culture systems require 20% O2, embryos cultured at 20% O2 in SOF + 5% FCS were included as an additional control. No impact of co-culture systems was observed on timing and developmental rates. 16-cell stage embryos and day 8 blastocysts' transcriptomes were analyzed on a new customized bovine microarray (GPL21734). Comparing the two co-culture conditions revealed only 14 and 10 differentially expressed transcripts respectively at 16-cell and blastocyst stages suggesting almost no difference induced by the type of co-culture. Nevertheless, BOEC or VERO cells induced differential expression of 83 and 51 transcripts respectively when compared to 5% O2 and 218 and 309 transcripts respectively when compared to 20% O2 in 16-cell embryos and 192 and 229 transcripts respectively when compared to 5% O2 and 542 and 881 transcripts respectively when compared to 20% O2 in blastocysts. A large proportion of the transcripts affected by BOEC presence were also impacted by VERO cells. Several biofunctions relative to cell cycle regulation and lipid metabolism were impacted by both cell types when compared to culture in SOF without feeder cells. Collectively, co-culture systems, using BOEC or VERO cells, induce weak and closely related modifications of embryos transcriptome without improvement of cleavage and blastocyst rates when compared to standard 5% O2 culture conditions.

Supported by EU FP7 KBBE FECUND

P-17

Poster Presentation

Fabian, Dusan

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Embryos originating from overweight mice dams: differences between preimplantation development in vivo and in vitro

To produce females with two different types of body condition (control and fat), a previously established two-generation model was used, based on overfeeding of a portion of laboratory mice during prenatal and early postnatal development. When reaching adulthood, spontaneously ovulating females were naturally fertilized. During preimplantation period, embryos were developing either in vivo (until Day 4 of gestation) or in vitro (from the 2-cell stage until Day 5 of gestation). Similar proportion of embryos developing in reproductive tract of control dams (displaying physiological body weight and fat) and of fat dams (displaying significantly elevated body weight and fat) were able to reach the blastocyst stage (around 80%). However, significantly higher number of degenerated embryos was isolated from fat dams ($P<0.05$). On the other side, when embryos were developing in artificial in vitro conditions, proportion of blastocysts, slowly developing, arrested and degenerated embryos was not significantly different between the groups originating from control and fat dams ($P=0.07$). Neither origin, not developmental conditions did affect the mean number of cells per blastocyst. Anyway, blastocyst isolated from reproductive tract of fat dams showed significantly higher apoptotic index. On the opposite, when embryos were developing in vitro, no difference was recorded between apoptotic indexes in blastocysts originating from control and fat dams. Results suggest that when embryos are transferred from maternal body to standardized in vitro conditions, negative effect of obesity on preimplantation development significantly diminishes.

Work was supported by national grant VEGA 2/0001/14.

O-18

Oral Presentation

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Fazeli A

Eight years of COST: from a dream to the reality

During this lecture I aim to evaluate where we were when we started Epiconcept Action and where we are now; what has happened and how our knowledge has changed and improved during the last few years. I am sure we have not reached to all the goals and objectives that were put forward, however, many of those goals are realised and as the time passed with the new developments, many of our aims needed to be changed to allow us to face the new realities in the field. I will present you the role that Epiconcept played in our field. However, one conclusion I can already make is that the importance of periconception period rather than being diminished during the last few years, thanks to the new developments in the field it has been reinstated. Now we are fully aware of an array of many factors that play a major role during this period of life and can affect or set the epigenetic profile of an individual. We are even starting to have some clues regarding how these events are modulated. The Epiconcept Action played a major role worldwide and within the European Union. It fostered new collaborations and research initiatives, and it was instrumental in building close ties between all of us. I am sure Epiconcept will be remembered in the scientific literature and in our memories forever.

P-19

Poster Presentation

Fernandes, Jorge

Faculty of Biosciences and Aquaculture, Nord University, Norway

Fernandes JMO, Podgorniak T, El-Zaeem S

Expression changes of miRNAs involved in epigenetic regulation are associated with domestication in Nile tilapia (Oreochromis niloticus)

MicroRNAs (miRNAs) are small non-coding RNAs that fine tune expression of their target genes by inhibiting translation or inducing degradation of mRNAs. They are involved in numerous biological processes and are also acknowledged as master epigenetic regulators, as they can influence the expression of key genes involved in epigenetic regulation of gene expression. Nothing is known about their role in animal domestication, but it is plausible that they are associated with adaptation to captivity conditions. In the present study, we used next-generation sequencing to compare the miRNA transcriptomes in fast muscle between one farmed and two wild Nile tilapia populations from lake Edko and river Nile in Egypt. There was a clear separation of miRNA expression profiles between farmed and wild fish and over 20 mature miRNAs and their variants were significantly differentially expressed between groups (i.e., fold-change > 2 and FDR < 0.05). In particular, miR-22 was down-regulated 3-fold in farmed fish compared to their wild counterparts. miR-22 is known to be involved muscle differentiation and to target histone deacetylase, which is a key element in epigenetic regulation. These preliminary data support the hypothesis that miRNAs can play a significant role in fish domestication, perhaps through regulation of chromatin structure.

This work was supported by an ERC Consolidator grant from the European Research Council to J Fernandes (EPIFISH, Ref. 683210).

O-20

Oral Presentation

Fleming, Tom

Centre for Biological Sciences, University of Southampton, United Kingdom

Fleming TP

Embryos: how they are made and what influences their future

Embryos, from fertilisation until implantation, have a busy schedule to complete such as zygotic genome activation, cell cycling resumption, and morphogenesis including cell differentiation and lineage diversification. These internal events are coupled with an external 'awareness' of environmental conditions to optimise their survival chances, a form of developmental plasticity. Several models have now demonstrated that in vivo factors (maternal nutrition, physiology, age, sickness; paternal phenotype) modify embryo development in an enduring way that may programme permanent changes for the lifespan, affecting in particular adult disease risk. Artificial conditions (IVF, embryo culture and transfer etc) also may modulate developmental plasticity with similar consequences for lifetime health. We have identified some of the critical mechanisms affecting embryo potential, particularly in relation to maternal nutrition. These mechanisms straddle epigenetic, metabolic, cellular, signalling and physiological boundaries and will be discussed. They provoke distinct changes in embryonic/ fetal lineages from extra-embryonic/ placental lineages, all apparently designed to promote an optimal phenotype for an anticipated postnatal environment, a conserved evolutionary mechanism now recognised across mammalian species.

Funding: BBSRC, MRC, NICHD, EU-FP7, Rosetrees Trust, Kerkut Trust.

Poster Presentation

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Fouladi-Nashta AA, Hartshorne G, Ghafari F, Gutierrez C, Marei W, Salavati M, Tremaine T

Impact of sperm hyal on sheep blastocyst formation in vitro, viability after cryopreservation and pregnancy rate after embryo transfer

Recent research in our laboratory has reported the presence of members of the HA system including HA synthases and receptors and hyaluronidase in sheep embryos and oviduct. Hyaluronan has a range of physiological functions which seems to be size dependent. We hypothesise that small size HA fragments produced during degradation by hyaluronidases function as a survival factor during preimplantation embryo development. Sheep oocytes were collected from slaughterhouse derived ovaries and matured and fertilized in vitro. Experiment 1: Cleaved embryos were cultured in the absence (control) or presence of increasing concentrations of sperm hyaluronidase PH-20 (10, 30, 300ng/ml) for 6 days when development to blastocyst stage were recorded and the number of hatched blastocysts counted. Significantly higher number of blastocysts were produced in 10ng/ml PH-20 which also resulted in higher number of hatched blastocysts ($P<0.05$). Experiment 2 assessed quality of the blastocysts produced in the presence of PH-20 as compared to control. Embryo quality was assessed based on survival after cryopreservation by vitrification of early blastocyst stage embryos using the Cryoleaf method. Higher percentage of the blastocysts cultured in 10ng/ml PH-20 survived after vitrification as observed by re-expansion and hatching 48h after thawing and culture (76.2% vs. 52.2%, $P<0.05$). Experiment 3 analysed pregnancy rate after transfer of two blastocysts produced in the presence or absence of 10ng/ml PH-20 to each recipient. Pregnancy was assessed by ultrasound scanning on day 35 after embryo transfer. Higher number of pregnancies was observed in the ewes receiving PH-20 treated blastocysts (73%) versus control (55%). In conclusion, sperm hyaluronidase improves blastocyst quality and development rates resulting in higher survival following vitrification. These studies define a new role for sperm as promoter of preimplantation embryo development and survival in the oviduct.

P-22

Poster Presentation

Franczak, Anita

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Franczak A, Zglejc K

Female restricted diet during periconceptional period alters transcriptome profile of the endometrium during peri-implantation period

Females undernutrition during early pregnancy may alter physiological pattern of transcriptome profile in the uterus. We determined if restricted diet applied females during periconceptional period, i.e. from the onset of the estrus until day nine of pregnancy, alters gene expression profiles in the endometrium during the periimplantation period. The restricted diet gilts were fed forage, in which the dose of protein and energy had been reduced by 30% compared to normal diet. Control gilts were fed a normal diet with a standard dose of protein and energy. Microarray analysis revealed that around 4% of transcripts (1690 out of 43 803 probes from The Porcine (V2) Gene Expression Microarray 4□44 (Agilent Technologies, USA) were consistently altered ($P < 0.05$) in the endometrium harvested from pigs fed restricted diet. Out of 796 genes, 284 genes were up-regulated and 512 genes were down-regulated. The proportions of the differentially expressed transcripts were organized into the 6 major categories (the first level) and different subcategories (31) (the second level) containing the 208 different pathways associated with the differentially expressed transcripts. Up-regulated genes: SAL1 (salivary lipocalin; fc = 5.46), ACP5 (type 5 acid phospharase; fc = 4.67), PPAP2C (phosphatidic acid phosphatase type 2C; fc = 2.61), EDNRB (endothelin receptor type B; fc = 1.75), RGS12 (regulator of G-protein signaling 12; fc = 1.67). Down-regulated genes: HMGB2 (high mobility group box 2; fc = −2.3), AQP10 (aquaporin 10; fc = −1.83,), DNMT1 (DNA (cytosine-5-)-methyltransferase;fc = −1.55), HSD17β(4) (hydroxysteroid (17-beta) dehydrogenase 4; fc = −1.55) and TLR3 (toll-like receptor 3; fc = 1.5). These findings suggest that undernutrition during periconceptional period may atler transcriptome of the endometrium during the peri-implantation period.

Funded by grant 528 0206 806 UWM in Olsztyn, Poland

P-23

Poster Presentation

Giller, Katrin

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Giller K, Drews B, Berard J, Schanzenbach C, Kienberger H, Spanier B, Geisslinger G, Ulbrich SE

Dietary rumen-protected omega-3 and omega-6 fatty acids impact differently on histotroph and elongation of preimplantation embryos in heifers

Early embryonic losses in the preimplantation phase are commonly observed in cattle. Before implantation, embryo-maternal communication via factors in the uterine histotroph is crucial for establishment of pregnancy. Dietary fat and especially omega-3 fatty acids (ω3 FA) have been discussed to improve fertility. Therefore, we investigated if rumen-protected dietary ω3 FA modify the fatty acid composition in endometrial tissue and influence the composition of the histotroph and embryo development. Two groups of Angus heifers received either 450 g of a ω3 FA (ω3 group) or ω6 FA (ω6 group) supplement per day. Following artificial insemination, animals were slaughtered at day 15 of gestation. The embryo, uterine fluid and endometrium were sampled. Interestingly, the ω3 group embryos were significantly more elongated than the ω6 group embryos (7.4 vs. 3.4 cm, $p=0.017$). Endometrial arachidonic acid (AA), the precursor for prostaglandins, was reduced in the ω3 group ($p\leq0.001$). Uterine PGF2α and PGE2 increased in both groups with increasing embryo length. However, prostaglandin concentration did not differ between ω3 and ω6 embryos of comparable length. Uterine IFNt also increased with increasing embryo length whereas IFNt induced gene expression of ISG15 and MX1 correlated with embryo length only in the ω3 group. Uterine amino acids and gene expression of specific amino acid transporters (SLC1A5, SLC7A1) correlated significantly with increasing embryo length in the ω3 but not in the ω6 group. Endometrial expression of genes involved in prostaglandin synthesis (PLA2, COX2), PPAR signaling (PPARα, PPARβ, PPARγ) and insulin signaling (IGF1, IGF2, IGF-1R, IGF-2R) was not differentially regulated between diet groups. Until now, it cannot be stated how ω3 FA promote the observed elongation. Effects of ω3 FA on the corpus luteum and on embryonic gene expression are currently under investigation.

P-24

Poster Presentation

Gonzales-Rojo, Silvia

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Gonzalez-Rojo S, Lombo M, Fernandez-Diez C, Herraez MP

Epigenetic effects of BPA during spermatogenesis in adult zebrafish

Bisphenol A (BPA) is a xenoestrogenic endocrine-disrupting compound, widely used in the production of different polycarbonate plastics and epoxy resins. In males, BPA has been described impairing fertility and inducing disruption of spermatogenesis in rats. Our group demonstrated transgenerational inheritance of heart disorders caused by the paternal BPA exposure in adult zebrafish (Lombó et al. 2015) but the changes promoted in germinal cells during spermatogenesis remain still unknown. The present work is aimed to analyze the potential epigenetic effect of BPA on spermatogenesis. Males were exposed to 100 and 2000 ppb of BPA during 14 days, 7 days after clearance testis were extracted. One testicle per male was used for the analysis of the expression of genes related with the epigenetic remodeling machinery. The other testicle was used for the histological evaluation and the analysis of 5-methylcytosine (5mC) and H3K9Ac using immunofluorescence and image quantification. Animals exposed to the vehicle were used as control. Results revealed different level of expression for some of the enzymes involved in epigenetic remodeling in the exposed males. We also showed an alteration of the normal histology of testicle, revealing an increase in acellular areas in those males exposed to BPA, similar to those described by Yang et al., (2014) after exposing to an intermediate dose. A significantly increase of H3K9Ac in spermatogonia and spermatocytes and a significant decrease of 5mC in spermatids and spermatozoa was noticed after BPA exposure. Further number of replicates is necessary to correlate these observations with a direct effect of BPA, but the data suggest that the effects on the epigenetic machinery could deeply affect epigenetic remodeling during spermatogenesis in the adult.

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P-25

Poster Presentation

Gould, Joanna

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Gould JM, Pearson-Farr J, Airey L, Smith PJ, Fleming TP, Willaime-Morawek S

Maternal protein restriction around conception alters the fetal mouse brain by reducing the neural stem cells and increasing neuronal differentiation during gestation, which is associated with the adult offspring behavioural deficits

Maternal malnutrition during pregnancy is detrimental to fetal development and increases the risk of many chronic diseases in later life i.e. neurological consequences such as increased risk of schizophrenia. Previous studies have shown maternal protein malnutrition during pregnancy and lactation compromises brain development in late gestation and after birth, affecting structural, biochemical and pathway dynamics with lasting consequences for motor and cognitive function. However, the importance of nutrition during embryogenesis for early brain development is unknown. We have previously shown maternal low protein diet confined to the preimplantation period (Emb-LPD) in mice is sufficient to induce cardiometabolic and behavioural abnormalities in adult offspring. Using a diet model, female mice were fed different diets from conception to the end of pregnancy: normal protein diet (NPD), low protein diet (LPD) or embryonic LPD (Emb-LPD: LPD for 3.5 days, NPD thereafter). Fetal brains were analysed at three time points in gestation (E12.5, E14.5 & E17.5), with in vivo analysisfor neural stem cell and neuron markers, and in vitro techniques using the neurosphere culture assay. We have also carried out a number of follow up behavioural tests, including short term novel object recognition test in adult offspring. We have shown that Emb-LPD and sustained LPD reduce neural stem cell (NSC) and progenitor cell numbers through suppressed proliferation rates in both ganglionic eminences and cortex of the fetal brain at E14.5 & E17.5($p=0.05$). Moreover, Emb-LPD causes remaining NSCs to upregulate the neuronal differentiation rate in compensation beyond control levels ($p=0.01$). We have also seen a significant deficit in short term memory in the Emb-LPD adult offspring ($p=0.0001$). This study is the first to clearly demonstrate that poor maternal nutrition around conception has adverse effects on early brain development & the adult offspring behavioural deficits.

O-26

Oral Presentation

Guerrero-Bosagna, Carlos

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Guerrero-Bosagna C

Transgenerational epigenetic inheritance: implications from humans to farm animals

Early exposures to environmental toxicants during fetal development are fundamental to explain reproductive impairments and metabolic diseases recently observed in human populations. Some environmental exposures, which include daily practices, occupational exposures and contact with contaminants, are currently known to produce epigenetic changes related to conditions in humans. Interestingly, experiments in animal models have shown that exposure to environmental toxicants can, in addition, induce transgenerational inheritance of some disease phenotypes. The mechanism of transgenerational epigenetic inheritance involves exposure of the germ line to drastic environmental conditions during critical developmental periods. Such exposure generates germ line epigenetic alterations that can be transmitted to future generations and associate with altered phenotypes in the unexposed individuals of subsequent generations. Exposures to environmental toxicants such as fungicides, pesticides or plastic compounds have been shown in rodents to produce abnormal reproductive or metabolic phenotypes that are transgenerationally transmitted. Environmentally-induce and transgenerationally transmitted phenotypes observed in animal models include non-communicable diseases of increasing incidence in human populations such as of obesity, polycystic ovary syndrome (PCOS), pregnancy defects or fertility impairments. This presentation summarizes recent findings showing that early developmental exposures to a variety of environmental toxicants can induce epigenetic transgenerational inheritance of phenotypes associated with non-communicable diseases of common incidence. I will also discuss the implications of environmental epigenetics for farm animals.

O-27

Oral Presentation

Gutierrez-Adan, Alfonso

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Impaired folliculogenesis function in defective Zrsr2 mutant mice

Mammalian oocytes are arrested at prophase I. During puberty germinal vesicle breakdown marks resumption of meiosis I and progression to meiosis II. All events controlling the progression of folliculogenesis requires a tight regulation of gene expression to produce functional gametes. Among other processes, epigenetic events control splicing of mRNA, a post-transcriptional modification, should modulates the expression of some essential genes involved in follicle maturation. ZRSR2 is a splicing factor necessary for the recognition of 3' splice site in unprocessed mRNA. Interestingly, ZRSR2 is located in the X-chromosome in all mammals. To study the function of ZRSR2 in folliculogenesis, we generated mutant mice containing nonsense mutations in the RNA-recognition motif (RRM) using CRISPR-nCas9 technology. Three lines of homozygous mutant females exhibited severe defects in growing oocytes, mice did not respond to the standard superovulation ultimately leading to female sterility. Quantitative PCR analysis revealed that ZRSR2 is overexpressed in primary follicles, decreasing its expression in mature oocyte. Hematoxylin-eosin histological sections showed evident defects during follicle maturation in mutant mice. At 3 months of age, ovaries were smaller than control ovaries, with the presence of less advanced-stage follicles, abnormal antral follicles, and small number of fully-grown oocytes, suggesting that oocyte development was reduced in the presence of mutant ZRSR2. Also, a drastic decrease of ZRSR2 mutant in oocytes at the antral follicle stage, when meiotic competence is acquired, likely contributes to the reduction in meiotic resumption. Our findings uncover a functional link between ZRSR2 and splicing governing meiotic progression during foliculogenesis.

P-28

Poster Presentation

Hankele, Anna-Katharina

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The process of estrogen glucuronidation is sensitively regulated across the estrous cycle in pigs

The formation of conjugated estrogens during estrogen metabolism inhibits estrogen receptor binding and thus inactivates estrogens. In humans, the most abundant circulating estrogen conjugates are the sulphates, followed by the glucuronides. Based on our study in gilts where estradiol-17β (E2) was orally applied (1000 µg E2/kg body weight/day) and estrogen metabolites were analyzed in plasma, bile, heart muscle and endometrium by GC-MS, we have good evidence that the glucuronides are the most abundant conjugates in pigs, while sulphates are less dominant. Therefore, we analyzed the mRNA expression changes for enzymes (UGTs, GUSB) and transporters (MRPs, OATPs) possibly involved in glucuronide metabolism in the endometrium across the estrous cycle in pigs (day 0, 3, 6, 10, 12, 14, and 18, respectively, following estrous). The orally administered E2 was relocated as conjugated estrogens in plasma, as free and conjugated estrogens in bile, as free estrogens and estrone glucuronide in the heart muscle and exclusively as free estrogens in the endometrium. The mRNA expression of the influx-transporter OATP1A2 significantly increased from day 0 to 6 and decreased again by day 10, while efflux-transporters (MRP1, MRP2, MDR1) displayed minimal expression at day 3 and/or day 6. The mRNA expression of the UDP-glucuronsyltransferases UGT1A1, UGT1A6 and UGT1A10 followed a similar pattern, with minimal expression found at day 6. Glucuronidase-β in turn showed highest expression at estrous, minimal expression during diestrous and increasing expression towards proestrous, suggesting an inhibition by progesterone. Taken together, the endometrial metabolism and transport of estrogen glucuronides is precisely regulated across the estrous cycle in pigs and might exert a yet unknown role in the local tissue-specific estrogen availability.

P-29

Poster Presentation

Heifetz, Yael

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Controlling reproduction through microRNAs

Mating induces a rapid change in the Drosophila female's physiology and behavior. The female increases her oviposition rate and avoids other males, and the morphology of her reproductive tissues changes rapidly. Mating-induced physiological responses are modulated by major changes in the expression profile of the female reproductive tissues. In the lower female reproductive tract (lower RT) miRNAs are involved in the regulation of this rapid response to mating. It has been demonstrated that miRNAs also regulate reproductive processes in mammals, such as mouse, pig and human. In human, for example, miRNAs regulate responses to gametes and modulate communication between the embryo and the maternal tract during implantation and pregnancy. miRNAs are highly conserved across species and thus might play a role in regulating the basic modules of reproduction which are broadly conserved. To better understand the role of miRNAs in female reproductive success, we used interdisciplinary approach using Drosophila genetics and focused on intercellular communication that involves intercellular transfer of extracellular vesicles (EVs). We will present results, highlight and discuss current experimental limitations that need to be resolved and possible functions of EVs and miRNAs in setting female fertility.

P-30

Poster Presentation

Hulinska, Pavlina

Department of Genetics and Reproduction, Veterinary Research Institute, Czech Republic

Hulinska P, Hanzalova K, Knitlova D, Jeseta M, Nemcova L, Kanka J, Machatkova M

The maturation with L-carnitine influences development and gene expression in bovine embryos derived from oocytes with different meiotic competence

Supplementation of maturation media by metabolism regulators improves development and influences expression of metabolism regulating genes in mammalian embryos. The study was designed to characterize the effect of L-carnitine during maturation on development and gene expression in bovine embryos derived from oocytes with different meiotic competence. Meiotically more competent (MMC) and meiotically less competent (MLC) oocytes were collected separately. Both oocyte subpopulations matured with or without 2.5 mM L-carnitine were fertilized and cultured into blastocysts. The blastocyst rate and kinetics of blastocyst expansion were assessed. To analyse the expression of some genes playing roles in apoptosis, embryonal development and mitochondria biogenesis, real RT-PCR was carried out at the expanded blastocyst stage. Significantly more MLC oocytes matured with L-carnitine developed into D7 early blastocysts and D8 expanded blastocysts in comparison with the control oocytes (31.7% vs. 23.1% and 33.3% vs. 25.8%, respectively). On the other hand, a significantly higher proportion of D8 expanded blastocysts was obtained in MMC oocytes matured with L-carnitine compared with the control oocytes (72.7% vs. 59.3%). The ATP5C1, BCL2 and GJB5 mRNA levels were increased in blastocysts developed from MLC oocytes and from both MLC and MMC oocytes, respectively, after they were matured with L-carnitine. No differences were found in BAX and GJA1 mRNa levels between blastocysts developed from oocytes matured with or without L-carnitine. It can be concluded that L-carnitine presence during maturation enhances production of bovine embryos from less competent oocytes, accelerates expansion of blastocysts from more competent oocytes and influences gene expression in both oocyte subpopulations.

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P-31

Poster Presentation

Ibanez, Elena

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Pique L, Mallol A, Santalo J, Ibanez E

Histone deacetylase inhibitors render somatic cell nuclear transfer mouse embryos more resistant to subtle alterations in culture conditions

Somatic cell nuclear transfer (SCNT) embryos show poor development and are extremely sensitive to external perturbations. Subtle alterations in culture conditions may cause their developmental arrest, while not affecting the viability of the more robust fertilized or parthenogenetic embryos. Treatment of SCNT embryos with histone deacetylase inhibitors (HDACi) improves nuclear reprogramming and embryo development. Our preliminary observations suggest that HDACi treatments may also allow more consistent results across experiments, by rendering SCNT embryos more resistant to imperceptible alterations in culture conditions. To confirm these observations, we retrospectively examined the developmental rates of SCNT embryos generated over the course of three years using similar experimental conditions: B6CBAF1 oocytes reconstructed with a cumulus cell nucleus, activated during 6h in 10 mM SrCl2 and 5 µM latrunculin A, and either non-treated or treated for 16 or 24 h with 10 µM psammaplin A (PsA) or 1 µM suberanilohydroxamic acid (SAHA). Only experiments in which blastocyst rates of control parthenogenetic embryos were >90% were considered. In global, non-treated embryos (n=701) showed significantly lower blastocyst rates (31.8%) than PsA- (n=366; 39.7%) or SAHA-treated (n= 375; 47.6%) ones. When experiments were divided into optimal and suboptimal according to the blastocyst rates in the non-treated group (>25% in optimal experiments), blastocyst rates were significantly lower in non-treated (7.7%) than in PsA- and SAHA-treated embryos (36.5% and 45.8%) in suboptimal experiments, but no differences were detected in optimal experiments (41.8%-48.9%). Blastocyst rates significantly differed between suboptimal and optimal experiments only for non-treated embryos (7.7% vs 48.1%). In conclusion, HDACi treatments improve in vitro development of SCNT embryos and result in more consistent blastocyst rates across experiments.

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O-32

Oral Presentation

Jhamat, Naveed

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Jhamat N, Guo Y, Niazi A, Ivanova E, Kelsey G , Bongcam-Rudloff E, Andersson G, Humblot P

Identification of differentially methylated regions in the genome of bovine endometrial epithelial cells (bEEC) challenged by E. coli LPS and its effect on transcription

Lipopolysaccharide (LPS) from Gram-negative bacteria induces the activation of pro-inflammatory pathways in the endometrium and such reactions potentially affect fertility and more specifically the implantation process. Gene expression studies based on RNA sequencing [Guo et al., Epiconcept Conference 2015, Abstract O-23, 34p] have shown that more than 2000 genes were differentially expressed (DEGs) in bEEC exposed to LPS. This study aimed to identify epigenetic correlates of these DEGs by detecting Differentially Methylated Regions (DMRs) in same cells. bEEC were exposed in vitro to 0, 2, and 8 µg/ml LPS for 24 hours. DNA libraries were prepared at The Babraham Institute, UK for reduced representation bisulfite sequencing (RRBS) using MspI digestion followed by end-repair/A-tailing and 5mC adaptor ligation and bisulfite conversion plus PCR. Sequences were analyzed using BS-Seeker2 for alignment of reads and MethylKit v0.9.5 for finding significant DMRs. AliBaba2 was used to predict potential transcription factor binding sites (TFBS) at DMRs using the TRANSFAC database. Analysis of RRBS data revealed 78 DMRs with q-value<0.05 between controls and 2 µg/ml LPS samples (34 hyper- and 44 hypo-methylated) and 109 DMRs between controls and 8 µg/ml LPS samples (47 hyper- and 62 hypo-methylated). 17 common DMRs (in 2 µg/ml and 8µg/ml LPS groups vs. controls) were identified. A reciprocal relationship was found between methylation and gene expression data for a sub-set of loci known to be key regulators of endometrial function. For example, LIF (Leukemia Inhibitory Factor) gene was found to contain a hypo-methylated DMR and its expression was up-regulated. Predicted binding sites in this DMR have 100% conservation and gene synteny in the human genome. These analyses allow the identification of regions harbouring candidates for key regulatory elements of endometrial function, thus contributing to the understanding of LPS-induced deregulation that may impact implantation.

O-33

Oral Presentation

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Teperek M, Simeone A, Gaggioli V, Miyamoto K, Allen GE, Erkek S, Kwon T, Marcotte EM, Zegerman P, Bradshaw CR, Peters AHFM, Gurdon JB, Jullien J

Sperm is epigenetically programmed to regulate gene transcription in embryos

For a long time it has been assumed that the only role of sperm at fertilization is to introduce the male genome into the egg. Recently, ideas have emerged that the epigenetic state of the sperm nucleus could influence transcription in the embryo. However, conflicting reports have challenged the existence of epigenetic marks on sperm genes, and there are no functional tests supporting the role of sperm epigenetic marking on embryonic gene expression. Here we show that sperm is epigenetically programmed to regulate embryonic gene expression. By comparing the development of sperm- and spermatid-derived frog embryos we show that the programming of sperm for successful development relates to its ability to regulate transcription of a set of developmentally important genes. During spermatid maturation into sperm, these genes lose H3K4me2/3 and retain H3K27me3 marks. Experimental removal of these epigenetic marks at fertilization deregulates gene expression in the resulting embryos in a paternal chromatin dependent manner. This demonstrates that epigenetic instructions delivered by the sperm at fertilization are required for correct regulation of gene expression in the future embryos. The epigenetic mechanisms of developmental programming revealed here are likely to relate to the mechanisms involved in transgenerational transmission of acquired traits. Understanding how parental experience can influence development of the progeny has broad potential for improving human health.

P-34

Poster Presentation

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Identification of regulatory effects of PGF2a-PTGFR signaling in porcine conceptus cells

Introduction: Prostaglandins (PGs) are key factors regulating interactions between conceptus and uterus during early pregnancy. Our recent study indicates that expression of prostaglandin F2α receptor (PTGFR) increases in porcine endometrium and conceptus during implantation period. Thus the aim of the present study was to identify the regulatory effects of PGF2a in porcine conceptus cells using global transcriptome profiling. Methods: Cells were isolated from porcine conceptuses collected from gilts (n=5) on day 14 of pregnancy. Confluent cells were treated with PGF2α (100 nM, 1 μM) or vehicle for 24 h at 37 °C in a humidified atmosphere (95% air and 5% CO2). Cells were then lysed with Fenozol and total RNA was isolated for microarray analysis. Agilent Sus scrofa 4x44k microarrays were used. Statistical analyses were conducted using LIMMA package in BioConductor software. To identify the regulatory effects of PGF2a on porcine conceptus cells bioinformatic analysis was performed using Ingenuity Pathway Analysis (IPA) software. Results: We found 58 differentially expressed genes (DEGS) - 19 down-regulated and 39 up-regulated ($p<0.05$; FDR=0.1). We found that these genes were involved in following networks: 1) Post-Translational Modification, Lipid Metabolism, Molecular Transport (14 DEGs / 30 genes in the network); 2) Cellular Assembly and Organization, Cell Death and Survival, Cellular Compromise (15 DEGs / 28 genes in the network); 3) Cell Death and Survival, Cell-To-Cell Signaling and Interaction, Developmental Disorder (13 DEGs / 27 genes in the network); and 4) Cell Morphology, Cellular Compromise, Cell Cycle (10 DEGs / 19 genes in the network). Summary: Our results indicate that PGF2a-PTGFR signaling is involved in conceptus growth and development as well as in embryo-maternal communication.

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O-35

Oral Presentation

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Negative effect of valproate on embryonic growth in vitro can be compensated by subsequent transplantation in vivo

Our aim was to investigate the unkown impact of valproate, the epigenetic therapeutic and histone deacetylase inhibitor (HDI), on the embryo-proper (three germ-layers) in an original ex vivo/in vivo model. 9.5-day-old Fisher rat embryos were microsurgically isolated from deciduas, cut and divided at the amnion level. Embryos-proper were cultivated in Eagle's MEM and rat serum (1:1) at the air-liquid interface for two weeks. Embryos-proper were cultivated with valproate (1 mM or 2mM). Cultures without additions served as controls. After 14 days, explants were transplanted under the kidney capsule. To follow overall growth, major and minor diameters were measured every other day in culture and transplants were weighted after additional 2 weeks in vivo. Embryos cultivated with valproate have shown dose dependent reduction of growth, being smaller when cultivated with the higher dose of 2 mM. After transplantation, no teratomas developed when previously cultivated with the higher dose. However, embryos cultivated with the dose of 1 mM showed no difference in weight in comparison to controls. It seems that the negative effect of the valproate on growth can be compensated in vivo, in a dose dependent manner.

P-36

Poster Presentation

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Mouse embryonic stem cell lines as models for periconceptional developmental programming

Developmental Origins of Health and Disease proposes maternal environment during pregnancy influences adult offspring disease risk. Programming may occur within the preimplantation embryo. Maternal low protein diet (LPD) and assisted reproductive technology (ART) factors (advanced maternal age (AMA); superovulation with IVF and culture) programmes the mouse embryo to altered postnatal growth and cardiometabolic dysfunction. Our aim was to derive mouse embryonic stem cell (mESC) lines from LPD and ART models, and assess mESCs for early mechanisms in programming. The mESCs were derived from blastocysts of mothers fed LPD (9% casein) and normal protein diet (NPD,18% casein); and AMA (7-8 months) and young (7-8 weeks) dams. Cell lines were characterised for derivation efficiency, karyotype, gender and normal lines were assessed for developmental programming mechanisms. The mESC lines show similar derivation efficiency per embryo (eg, 45% AMA; 36% young) resulting in 28 AMA and 10 young mESC lines with 80% and 90% being male. AMA lines showed higher aneuploidy (28.5%) than young (16.6%). Lines are being analysed for apoptosis, cell cycling, gene/protein expression and metabolic pathways. Early data shows AMA lines have reduced viability and increased cell death. LPD lines preserve cellular and epigenetic programming changes inherited from embryos. Metabolomics of LPD vs NPD lines (with Metabolon Inc) has identified changes in glucose metabolism, fatty acid and ascorbate utilization. LPD lines show increased glucose 6-phosphate and fructose 6-phosphate with downstream metabolites reduced, implicating alteration of enzyme expression such as phosphofructokinase, contributing to LPD programming. The mESCs models mimic the inaccessible embryo in vivo, reducing animal numbers used.

P-37

Poster Presentation

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Changes of the BMP15 expression in rabbit' ovaries, provoked by supplementing does with Spirulina platensis, inherit in F1 offspring

Now there are many evidences that nutrition, as an environmental factor, affects folliculogenesis. The metabolic changes in the environment surrounding oocytes can alter the genes expression by oocytes, with consequences for both immediate and longer-term development. Epigenetic processes can occur at critical periods for the germ cells, such as folliculogenesis during the sexual maturation. Our previous results shown that dietary supplementation of Spirulina platensis (SP) to female rabbits during the sexual maturation lead to an increase of the BMP-15 mRNA level in the oocytes and cumulus cells and the GDF-9 mRNA level in the oocytes. Should these changed expressions of genes be heritable? The research was conducted with 14 female white New Zealand rabbits, F1 offspring of the control and treated with SP (350 mg/kg) mother-rabbits. They were reared until puberty onset under the same as mothers conditions and received the same diet, but without feed additive. The RT-PCR analysis of the BMP15 and GDF9 genes expression in the oocytes and cumulus cells from ovaries of the F1 female offspring after slaughtering was performed. The increased level of bmp15 mRNA, observed in treated mothers' ovaries, was inherited in the oocytes and cumulus cells of the F1 offspring born to mothers consumed the SP. Their bmp15 mRNA level was about two times higher than in F1 offspring born to control group. There were no significant differences of the gdf9 mRNA levels in the oocytes and cumulus cells of daughters born to control and to treated with SP mothers. The results confirm that including the biological active substance in rabbit feed during the sexual maturation can have a transgenerational effect on the expression of the oocytes-specific genes.

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P-38

Poster Presentation

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Development of alternative and effective strategies for induction full nuclear reprogramming of somatic nuclei introduced into enucleated MII oocytes

The current cloning efficiency is low, only 1-6% of the offspring are delivered to term. Many reconstructed embryos die probably due to the improper reprogramming of the transplanted somatic nuclei. Given that spermatozoa are the best nuclear transfer devices, our hypothesis is that the forced expression of testis specific nuclear remodeling factors in somatic cells might improve nuclear reprogramming. The aim of the project was to induce nuclear remodeling of mouse somatic cells by the heterologous expression of mice Bromo Domain Testis-specific (BRDT) protein, and to investigate the role of this factor in the development of efficient in vitro “pre-reprogramming” of somatic cells prior to nuclear transfer. Mouse and sheep fibroblasts were cultured in DMEM with 10% FBS and transfected with BRDT-GFP (BRDT tagged with Green Fluorescent Protein plasmid and treated with Trichostatin A (TSA) (100ng/mL) for 16h. BRDT positive cells were used as donors for somatic cell nuclear transfer (SCNT) using mouse metaphase II oocytes under standard conditions. Non transfected fibroblasts were used as control. The successfully reconstructed embryos were cultured in KSOM medium till blastocyst stage. BRDT-GFP was expressed in 60% of transfected cells and condensation of chromatin was observed in 50% of those cells. Moreover, oocytes reconstructed with BRDT positive fibroblast developed to blastocysts stage in higher proportion compare to control ones (9,7% vs 3.6%, respectively). BRDT expressing fibroblasts can be used for SCNT, and the chromatin reorganization induced by BRDT result in more extensive nuclear reprogramming, and better development of preimplantation embryos. Therefore, BRDT protein creates a better physiological remodeling and improves nuclear reprogramming of somatic nuclei till blastocyst stage.

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P-39

Poster Presentation

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The effect of neonicotinoids and pyretroids on mouse preimplantation embryo development in vitro

The aim of our study was to evaluate the potential toxicity of active ingredients of two types of insecticides on preimplantation embryos. Insecticides are substances used for extermination of insects damaging crop or acting as vectors of various pathogens. They are used in prevention and therapy of ectoparasitic diseases in livestock and domestic animals as well. We have analysed the influence of 4 neonicotinoids (Thiamethoxam, Clothianidin, Acetamiprid and Thiacloprid) and 4 pyretroids (Deltamethrin, Fenvalerate, λ-Cyhalothrin and Permethrin) at various concentrations (1 – 1000□M) on developmental and qualitative parameters of mouse preimplantation embryos cultured in vitro from the 2-cells stage to the blastocysts stage. Our data showed that the treatment of embryos with chemicals at concentration 1000 □M during preimplantation period had negative effect on embryo developmental abilities in the case of all used neonicotinoids and pyretroids. Such concentration also significantly decreased average cell numbers and elevated incidence of cell death in blastocysts in case of all used neonicotinoids and 2 pyretroids (λ-Cyhalothrin, Fenvalerate). Some neonicotinoids and one pyretroid showed negative effect even at the concentration 100μM: Thiamethoxam, Clothianidin, Deltamethrin (developmental abilities) and Acetamiprid, Thiacloprid (quality of blastocysts). The concentrations lower than 100μM had no effect on embryo development or blastocysts quality. In summary, our results demonstrated that acute poisoning (1000μM) presents the high risk in terms of reproductive health in the case of all used neonicotinoids and pyretroids. The chronic intake of acceptable concentrations of residues (1μM) presents no risk for reproductive health.

This work was supported by the Slovak Research and Development Agency under contract APVV-14-0763.

P-40

Poster Presentation

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Impact of peroxisome proliferator-activated receptor (PPAR) ligands on pro-inflammatory cytokines synthesis in the porcine endometrium on days 10-12 of pregnancy or the estrous cycle

The involvement of cytokines in the regulation of female reproductive functions is strongly emphasized. They are important during embryo implantation, the process crucial for establishing the proper course and maintenance of pregnancy in mammals. The aim of the present study was to investigate in vitro effect of PPARs selected ligands (agonists and/or antagonists) on mRNA expression of pro-inflammatory cytokines (IL-1β, IL-6 and IL-8) in the porcine endometrium during early pregnancy (days 10-12 present maternal recognition of pregnancy) or the estrous cycle (days 10-12 present mid-luteal phase of the cycle), determined by real-time PCR. During the estrous cycle (days 10-12), a significant increase of IL-1β and IL-8 mRNA abundance in the endometrium was noted in the presence of PGJ2 (10 μM, PPARγ natural agonist) or rosiglitazone (1 μM, PPARγ synthetic agonist). An activation of PPARβ by L-165,041 (1 μM and 10 μM) enhanced IL-8 or tended to increase of IL-1β mRNA level. The blocking of MK-886 (10 μM, PPARα antagonist) augmented the expression of both cytokines. Any of the tested PPAR ligands did not change the expression of IL-6. During early pregnancy (days 10-12), all tested factors (α, β, γ isoform ligands) did not affect mRNA level of IL-1β, IL-6 and IL-8. Our results indicate that PPARs are involved in pro-inflammatory cytokines synthesis (Il-1β and IL-8) in the porcine endometrium. This effect depended on the physiological status of gilts (pregnancy vs. the estrous cycle) and is probably connected with a different receptivity of the tissue during analyzed reproductive stages. Such changes in the cytokines expression would be also related with different activity of immune system during early pregnancy and corresponding days of the estrous cycle.

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P-41

Poster Presentation

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Addition of insulin during oocyte maturation impacts the epigenome of Day 8 bovine blastocysts

Metabolic disorders such as obesity or diabetes are associated with impaired fertility and changes in insulin signaling. Bovine in vitro oocyte maturation is a good model to study the impact of metabolic imbalance on subsequent embryonic development. Effects of elevated insulin during oocyte maturation on the epigenome of Day 8 bovine blastocyst (BC8) were investigated. Oocytes (n=882) were in vitro matured with two different insulin concentrations, INS10 (10µg/ml) and INS0.1 (0.1 µg/ml) or as control, INS0. Pools of BC8 were used for gDNA extraction and epigenome data of DNA-methylation were obtained from an EDMA oligo-array (EmbryoGENE). A bioinformatics pipeline was developed to analyze the differentially methylated regions (DMR) when compared to controls by quantification of methylation measurements based on M values, former described by Shojaei Saadi et al., 2014. The analysis showed that 7632 and 3914 regions were hypo-methylated in the INS0.1 and INS10 groups versus INS0 whereas 6026 and 8504 regions were hyper-methylated in INS0.1 and INS10 groups versus INS0. Further investigation of the localization of DMR in genes showed that the conservation odds (methylation) were in general higher in coding regions and CpG islands compared with noncoding regions. We observed a large overlap of DMRs in the insulin groups (3233 common DMRs). These numerous changes illustrate that insulin added during the preconception period alters the methylation pattern of the early embryo. More investigations about gene groups/pathways corresponding to highly DMR will be performed and compared with the gene expression data obtained from the same samples. These findings confirm the wide range of changes induced by insulin on gene expression of Day 8 embryos [Laskowski et al., http://dx.doi.org/10.1071/RD15315] and contribute to a better understanding of mechanisms by which metabolic disorders can affect embryonic development and subsequent health of the offspring.

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P-70

Poster Presentation

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Effect of nanoceria on in vitro embryo production derived from adult and prepubertal ovine oocytes

Recently increasing attention has been focused on the use and potential benefits of antioxidants during in vitro culture of gametes and assisted reproduction outcomes. The present study aim to evaluate whether supplementation of Cerium Oxide nanoparticles (CeO2ENPs), known to have an antioxidant activity, during in vitro maturation (IVM) of adult and prepubertal ovine oocytes, influences their subsequent embryonic development in vitro. Cumulus - oocyte complexes derived from the ovaries of slaughtered adult and 4 weeks old prepubertal ovine underwent IVM in standard condition for 24h. CeO2 ENPs were added during the whole IVM culture at the concentration of 0 µM (control), 44 µM, and 220 µM. Matured oocytes were fertilized with frozen thawed ram semen for 22h and zygotes were cultured in vitro for 8 days. Cleavage, blastocyst rates and total cell count were analyzed by Chi square and ANOVA test. The treatment with CeO2 ENPs did not affect cleavage rate, irrespective to the dose used, both in adult and prepubertal oocytes. However, while in adult oocytes the percentages of blastocysts were not significantly different between control (49,2%) and 44 µM (54,5%) and 220 µM (64,5%), in the prepubertal oocytes matured with 44µM we observed a significantly (P≤0.05) increased of the blastocyst yield/cleaved embryos (35.8%) compared to control (14.8%), and 220 µM (9.09%) groups. The total cell number of blastocyst derived from prepubertal oocytes showed a statistical increase in the group of 44 with a total cell number (105.21±2.15, mean±s.e.m) compared to control (81.09±4.4 4), and 220 µM (67±7.98) groups while no statistically differences were observed in the blastocysts derived from the adult oocytes irrespective the doses of CeO2 ENPs used. In conclusion, our results indicated that the presence of low doses of CeO2 ENPs benefits the in vitro embryo production of poor developmental competent oocytes increasing the blastocyst yield and their quality.

P-42

Poster Presentation

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Bisphenol A embryo exposure induces changes in zebrafish epigenetic profile

Bisphenol A (BPA) is an endocrine disruptor widespread used in manufacturing of plastic devices, resulting in an ubiquitous presence in the environment linked to human infertility, obesity or cardiovascular diseases. Much evidence supports in mammals that both direct and in utero exposure during pregnancy induce changes at genetic, transcriptomic and epigenetic level, leading to a wide range of disorders. Dolinoy and colleagues firstly reported the epigenetic toxicity of BPA showing that maternal exposure during pregnancy in agouti mouse model led to a change in coat coloration pattern of the next generation due to a sharp decrease in IAP particles upstream agouti gene (Dolinoy et al., 2008). Afterwards, some studies have associated the modification of epigenetic pattern of gonads when exposing breeders to this toxic with an altered reproductive capacity. However, effects of BPA exposure during germ cell migration (a window of heritable epigenetic damage) remain still unknown.
In our study zebrafish embryo were exposed to BPA (100, 2000 and 4000 µg/L) during the first 24 hours after fertilization (when germ cell migration occurs). To evaluate the epigenetic profile of control and exposed embryos, global DNA methylation (5mC by UPLC-MS and whole mount immunofluorescence) and histone acetylation (H3AcK9 by whole mount immunofluorescence) were assessed at different stages of development and specifically in genital ridges. Fluorescence emitted by cells in prophase and interphase was quantified using the imageJ software. Immunofluorescence allowed the detection of such a significant decrease of 5-methylcytosine in early embryos, whereas no similar effects were noticed by UPLC-MS, demonstrating a higher accuracy of methods based on single cell analysis. Results showed a sharp increase in malformations of exposed embryo, mainly cardiac failures that could be related to changes in the global epigenetic profile at early stages of development.

O-43

Oral Presentation

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Determining the role of placental endocrine zone Igf2 in the 'tug of war' over resources between mother and fetus in mice

Pregnancy success depends on a co-operative interaction between mother and fetus over resources. Failure to appropriately allocate resources can cause pregnancy complications with long-term consequences for both mother and fetus. The placenta is central to this “tug of war” over resources as it is responsible for nutrient exchange and secretes hormones which are thought to adapt maternal metabolism to favour nutrient delivery to the fetus. Insulin growth factor (Igf2) is a gene that promotes fetal growth and may adapt maternal metabolism in response to pregnancy. Global inactivation of the paternal copy of Igf2 in the mouse results in fetal growth restriction. Whereas overexpression Igf2 by deleting maternal H19 copy, results in overgrowth. However, the effects of manipulating Igf2 expression only in the endocrine cells of the placenta on maternal body composition, metabolic status and conceptus growth remain unclear. To address this aim, transgenic mice were crossed to produce entire litters with complete deletion or overexpression of Igf2 in the placental endocrine zone (Jz) by using the Jz-specific Cre line (TpbpaCre) and Igf2-floxed and H19-floxed lines, respectively. On day 16 of pregnancy, deletion of Igf2 in the Jz reduced maternal kidneys and liver weights and plasma lipid concentrations [cholesterol, triglycerides and non-esterified fatty acid (NEFA)]. However, over-expression of Igf2 in the Jz increased maternal heart weight and circulating glucose concentration, but reduced NEFA and triglycerides concentrations. In both models, placental weight was heavier and related to an increase in the Jz or the transport zone when Igf2 was over-expressed or deleted in the Jz, respectively. Only the deletion of Igf2 in the Jz, reduced fetal weight. In conclusion, Igf2 in the Jz is important in determining maternal metabolic profile in pregnancy, which affects resource allocation and potentially fetal growth.

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Poster Presentation

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Phosphoproteome analysis of endocrine regulation on the cow oviduct

The oviduct is a dynamic organ which is the site of fertilization and contact with the early embryo and contributes important factors to its development. The interaction with gametes and embryo occurs under the influence of sex hormones that circulate during the fertile stage of the female cycle in vivo. Protein post-translational modifications regulate their function playing a pivotal role in main cellular processes, being phosphorylation the best characterized. To gain insight into the hormonal regulated oviductal environment that interacts with gametes and embryo we analyse the phosphoproteome in relation to endocrine control. Bovine oviductal cell extracts from luteal and follicular phases (LP, FP) were classified according to ovary characteristics and assayed by 2D electrophoresis followed by Western blot analysis for phosphorylation. No changes were detected when anti-PKC substrate and anti-phosphotyrosine antibodies were used. Instead, with anti-PKA substrate antibodies 32 changes between LP and FP were detected, 26 spots were positive exclusively in FP and 6 in LP. When oviducts were treated with the cAMP analogue dibutyryl cyclic-AMP, 7 exclusive spots and 1 shared with LP were detected, indicating a probable cAMP independent pathway. When pregnant cow oviducts were analysed most phosphorylated spots from both phases became undetectable. Upon primary cultures analysis to interrogate their use as model for phosphorylation studies the pattern was completely loose. Seventeen phase exclusive spots were identified by LC-MS/MS followed by bioinformatics analysis, showing involvement in response to hypoxia, endoplasmic reticulum signalling and apoptotic pathways. In conclusion, PKA produces changes in the phosphorylation state of oviductal cell proteins in relation to the female cycle. Further study of the identified proteins will help to get insight over the complex nature of the maternal environment that surrounds the gametes and early embryo.

P-45

Poster Presentation

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Porcine IVF efficiency is improved by increasing pH of culture medium

In pigs the efficiency of in vitro embryo production is very low because of the high incidence of polyspermy in the in vitro fertilization (IVF) (Grupen et al., 2014). Although the pH in the oviductal ampulla is close to 8 during the periovulatory phase (Rodriguez-Martinez, 2007), most of laboratories use IVF media at pH 7.4. The objective of this study was to compare porcine IVF output at both pH 7.4 and 8. In vitro matured cumulus-oocytes complexes (COCs) were inseminated with 1x105 spermatozoa/ml in TALP medium at pH 7.4 vs 8. After 18 h coculture, putative zygotes were fixed and stained with Hoechst. Percentages of penetration (from total oocytes used, n=485), monospermy (from the penetrated oocytes) and efficiency [final putative zygotes (monospermic) per 100 penetrated oocytes] was evaluated. The results (mean ± SEM) showed that pH 8 produce a lower % penetration (pH7.4: 76.7 ± 2.6; pH8: 54.3 ± 3.4; $p<0.05$) and a higher % monospermy (pH7.4: 24.6 ± 3.0; pH8: 65.8 ± 4.5; $p<0.05$), providing a higher % IVF efficiency (pH7.4: 18.9 ± 2.4; pH8 35.7 ± 3.3, $p<0.05$). The pH could be acting at three levels over gametes, non-mutually exclusive, that reduce multiple penetrations: i) decreasing the number of capacitated or partially acrosome-reacted spermatozoa surrounding oocytes at fertilization time (Funahashi and Nagai, 2000), ii) slowing down the activity of the acrosomal enzymes of spermatozoa over zona pellucida minimizing the number of spermatozoa that completely dissolve it and/or iii) optimizing the cortical granules releasing to the perivitelline space in oocytes and improving conformational changes of ZP proteins that correctly blocks the polyspermy. The system proposed in this work, more similar to in vivo conditions, increases the potentially viable zygotes obtaining and optimizes IVF in porcine.

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P-46

Poster Presentation

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Effects of lipopolysaccharide on the expression of cytokines in porcine granulosa cells

Ovarian granulosa cells have been recognized to participate in the innate immunity of ovarian follicles. The aim of the present study was to examine whether initiation of immune response in porcine granulose cells, following detection of the most common PAMP, the component of the cell wall of Gram-negative bacteria lipopolysaccharide (LPS) is mediated by cytokines. Granulosa cells from small (<3mm) and large (>3mm) follicles were cultured in vitro and were stimulated with 1μg/ml LPS at different time courses (0, 6, 12 and 24 h), in order to study the early response of the cells treated with LPS. Quantitative Real-Time PCR analysis revealed a significant up-regulation in the expression of several cytokines, in RNA extracted from LPS treated granulosa cells, from both small and large ovarian follicles. These data suggest that porcine granulosa cells initiate an innate immune response to LPS via the cytokine pathway, which probably participates in the protection of ovarian tissues from invasive pathogens.

P-47

Poster Presentation

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The effect of dietary selenium to fatty acid profiles in blood serum and semen of rams

Mammalian spermatozoa are characterized by high proportion of polyunsaturated fatty acids (PUFA) which play a crucial role in fertilization. Some micro-minerals may have a role in maintaining semen quality. The objective of the study was to determine influence of different levels of selenium in diets on fatty acid profiles in ram's serum and semen. A trial was carried out with 24 Lithuanian Native Coarsewooled rams allocated in 3 analogous groups and lasted 218 days. Rams received the same nutritional background, except I group (RO) received oats (0,04mg/kg Se); II group (RC) – compound feed (0,11mg/kg Se) and the III group (RN) was provided with oat supplemented with mineral-vitamin premix with sodium selenite (0.49 mg/kg Se). Whole blood Se values were below the normal range (31.18–73.87 µg/l) and did not differ among treatment groups at the initiation of the experiment. Se concentration increased till 213.73 µg/l and 244.56 µg/l in RC and RN groups respectively, but declined to 18.35 µg/l in group without Se supplementation during the experiment. The concentration of Se in sperm was significantly different between groups: Feeding diets without Se supplementation resulted in the lowest Se concentration in whole blood and semen of rams in RO group. The fatty acid composition in serum is different from semen composition. In serum saturated fatty acid (SFA) represented 44.12%, monounsaturated (MUFA) 30.4% and (PUFA) 24.62% of the total fatty acids. In sperm these proportions were 25.18% for SFA, 9.98% for MUFA and 41.47% for PUFA. Feeding diets, with Se supplementation significantly influenced fatty acids composition in serum: decreased SFA and MUFA to 37.85% and 26.8% respectively.

O-48

Oral Presentation

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Evidence for developmental programming in dairy cattle

In dairy cattle gestations occur while the dam is still growing or is still producing large amounts of milk. We hypothesized that lactation during gestation in modern dairy cows affects growth and development of the calf, potentially affecting its health in later life. In a first study, in which 1.594 Holstein Fresian calves born out of primi- (n=540) and multiparous (n=1.054) dams were included, we demonstrated that multiparous dams produced on average 6.193,1 (±1.352,8) kg milk during gestation. Weight of the newborn calf was in first parity mothers shown to be significantly associated with the dam's age while with gestational milk yield in multiparous cows. Calves born following a higher gestational yield tended to be more insulin resistant. Besides gestational milk yield level, also the length of the dry period had a significant effect, with calves born after a longer dry period being havier and more insulin resistant. Gender of the calf was associated with most of the above mentioned parameters, female calves being lighter and more insulin resistant. In a second study, placentas were collected to count and measure cotyledons as a proxy for surface availability in terms of exchange between mother and calf. Growth and lactation during gestation increased the number of cotyledons, while total cotyledonary surface seemed to be positively influenced by the final nutrient demand of the calf. Remarkably, season of calving was significantly associated with most of the traits examined. Calves born during the warmer months were lighter and less insulin resistant, while placentas expelled in the warmer months had a significantly larger cotyledonary surface. The latter suggests season of birth potentially being an important characteristic in terms of health and production for dairy cattle.

P-49

Poster Presentation

Ortiz-Escribano, Nerea

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The vitrification process triggers the opening of hemichannels in in vitro produced bovine blastocysts

The vitrification process causes cellular stress impairing the embryo development, and inducing epigenetic changes. In cells, it is known that non-junctional hemichannels can open under stressful conditions, but no data are available yet in embryos. In this work, we study if the vitrification procedure triggers the opening of hemichannels in bovine blastocysts and if we are able to prevent this by adding a mimetic connexin hemichannel peptide, Gap26. Blastocysts were produced in vitro and divided in three groups; non-vitrified (n = 47), vitrified (n = 44) and vitrified with Gap26 (n = 46). On day 8, blastocysts were vitrified in 15% ethylene glycol ,15% dimethyl sulphoxide and 0.5M sucrose. During the dye uptake study, Gap26 was supplemented at a final concentration of 200 µM. Dye uptake studies were performed to assess the hemichannel opening by exposing the blastocysts to propidium iodide (1mM) and dextran-fluorescein dye (1Mm) for 25 min at room temperature. Cells with open hemichannels were positive for PI, whereas dextran-fluorescence was used to distinguish between dye uptake and cell dead, since it is impermeable to hemichannels. Nuclei were counterstained with DAPI (10 µg/ml) for 10 min and visualized using Leica TCS-SP8 X confocal microscope. The percentage of PI-positive and dextran-negative cells from vitrified blastocysts was significantly higher compared to non-vitrified specimen ($p<0.05$). In addition, the supplementation of Gap26 significantly lowered the uptake of PI in vitrified blastocysts ($p<0.05$). From these data, we can conclude that vitrification triggers a stress response that leads to the opening of hemichannels in the cells of the blastocysts and this response can be reduced with the inclusion of Gap 26. Further experiments will be necessary to study the stress proteins involved and the effects of vitrification on methylation patterns in the embryo.

O-50

Oral Presentation

Palazzese, Luca
Faculty of Veterinary Medicine, University of Teramo, Italy

Palazzese L, Iuso D, Czernik M, Loi P

Starvation improves sheep fibroblast chromatin remodeling in spermatide-like structure

We have recently demonstrated that the chromatin of somatic cells can be converted into spermatid-like structures, by the transient expression of human protamine 1 gene Prm1. Here we have further advanced our protocol, by mimicking the nuclear remodelling taking place in spermatids. Since nuclear maturation in spermatids occurs in G0, our first aim was to test if protaminization of somatic nucleus increases when G0-stage fibroblasts are trasfected with Prm1 gene. Protamine deposition on DNA is anticipated by a genome-wide histone acethylation. Thus, our second aim was to induce a genome hyper-acethylation of G0 cells, by optimizing timing of exposure/concentration of Histone De-Acethylase Inhibitor (HDAI) Tricostatin A. Results: G0-stage fibroblasts cultured for 24h pre-transfection with medium containing 0.5% FBS showed an higher proportion of spermatid-like cells, compared to the control (CTR: 10% FBS) ($p<0.05$). Furthermore, Bromodeoxyuridine incorporation demonstrated that starved somatic cells were effectively in G0-stage during protaminization ($p<0.0001$). Finally, we have found a greater number of spermatid-like cells with TSA concentration between 25 and 50 nM, comparing to 100 nM ($p<0.05$). To conclude, we have demonstrated that G0 stage, and the open nuclear structure conferred by TSA resulted in a more efficient Prm1-mediated conversion of somatic nuclei into spermatid-like structures.

P-51

Poster Presentation

Penailillo-Escarate, Reyna
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Penailillo-Escarate RS, Burton MA, Burdge GC, Eckert JJ, Fleming TP, Lillycrop KA

The effect of folic acid supplementation during the juvenile pubertal period or adulthood on gene expression in the ovary

Introduction: Women with mutations in BRCA1 gene have an increased lifetime risk of ovarian cancer. Many studies but not all have shown that an adequate folate intake is protective against many cancers including ovarian, but recent studies have shown that high levels of folic acid (FA) supplementation can promote cancer risk. The aim of this study is to determine FA impact on the expression of cancer related genes in the ovary such as OCT4 a pluripotency factor, BRCA1 related to DNA repair and the chromatin modifying enzyme EZH2. Methods: Juvenile (4 wks old) or adult (10 wks old) female C57BL/6 mice were fed for four weeks with normal (1 mg/Kg), high (5 mg/Kg) or supramaximal (20 mg/kg) doses of FA and then kept on maintenance diet until four or twelve weeks after supplementation. Quantitative RT-PCR was performed to determine the mRNA levels. Results and Discussion: FA supplementation during the juvenile period led to an increase in Oct4, Brca1 and Ezh2 expression immediately after the treatment. However, those effects did not persist after the end of supplementation. In contrast, FA during adulthood led to an increase in Oct4, Brca1 and Ezh2, which persisted 4 weeks after the end of supplementation. These results show that FA affects the expression of cancer related genes in the ovary, but the effects are dependent on the dose and time of supplementation. Future work is needed to identify histological alterations on ovaries and if FA could modify the quality and/or quantity of ovarian follicles.

O-52

Oral Presentation

Pendzialek, Mareike

Department of Anatomy and Cell Biology, Martin Luther University Faculty of Medicine, Germany

Pendzialek SM, Grybel KJ, Gurke J, Schindler M, Seeling T, Fischer B, Navarrete-Santos A

Maternal diabetes mellitus type 1 downregulates the embryonic microRNA biogenesis in trophoblast cells

MicroRNAs (miRs), a class of highly conserved small (19-24 nucleotides) non-coding RNAs, are involved in various biological processes such as development and embryogenesis. Their expression is associated with physiological and pathological conditions and might therefore represent a new class of biological markers. In a diabetic pregnancy both maternal and embryonic microRNA expression were affected as early as during the preimplantation period. To elucidate the regulatory pathways 6 day old blastocysts were exposed to factors known to be regulated by diabetes (insulin, glucose, IGF2) and to be important for implantation (LIF) for 2, 4 or 5 hours in vitro. In vitro culture with insulin (17nM), IGF2 (13nM) and LIF (10ng/ml) induced an upregulation of miR-27b, -141 and -191 in trophoblast cells, whereas embryoblast cells were not amenable to hormonal stimuli. MiR amounts in blastocyst cavity fluid were not affected neither by insulin nor glucose. Glucose (10mM, 25mM) effectively stimulated miR-191 expression in both embryoblast and trophoblast cells. A short pulse exposure of insulin, IGF2 and LIF led to a distinct upregulation of trophoblastic microRNAs, demonstrating that endocrine and paracrine factors are potent regulators of trophoblastic microRNA expression. Maternal disorders and endocrine dysfunctions have consequences for trophoblastic microRNA biogenesis and regulation.

O-53

Oral Presentation

Piferrer, Francesc
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Genetic, epigenetic and transcriptomic studies aimed at improving the breeding and control of reproduction in the european sea bass and the turbot

The European sea bass (Dicentrarchus labrax) and the turbot (Scophthalmus maximus) are two of the most important species for marine aquaculture in Europe, with a production in 2014 of 157,000 and 72,000 tonnes, respectively. Both species are gonochoristic and while the sea bass has a polygenic sex determination system the turbot has a chromosomal system of the ZW/ZZ type. For different reasons, in both of them there is much interest in knowing about sex determination and differentiation in order to produce monosex all-female stocks. Regarding the sea bass, under farming conditions most fish develop as males, which grow less than females and mature earlier, one third of them precociously during the first year. On the other hand, the turbot exhibits the largest sexual growth dimorphism recorded in farmed fish in Europe. Thus, understanding sex determination and differentiation in these species is essential to bring sex under our control. In this talk, we will first discuss essential aspects of sex determination and differentiation in fish, with particular emphasis on the fishes sex ratio response to temperature. Next, we will discuss our research both in sea bass and turbot. In the sea bass, we will explain how by integrating different lines of evidence we ended up with a change of paradigm as regards to the temperature-resistant fish. We will also show effects of temperature on sex differentiation and gonad gene expression. In the turbot, we will explain the use of a genetic marker to produce WW “superfemales”, which are now being used in the industry. Finally, we will discuss our work on epigenetics and how we try to integrate environmental and genetic information for sea bass broodstock management and juvenile production.

P-54

Poster Presentation

Pokharel, Kisun
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What makes Finnsheep a highly prolific breed? Transcriptomic study of Corpus Luteum (CL) cells and Endometrium identifies genetic aspects of fecundity in sheep (Ovis aries)

Ovulation rate and litter size in sheep are complex traits affected by endocrinological, genetic and environmental conditions. We have explored structural and functional variations in its genome as well as relevant factors affecting fecundity of Finnsheep . A total of 31 ewes representing two pure breeds showing high (Finnsheep) versus low (Texel) litter sizes and their F1-crossbred ewes are included in the study. Experiments are focused on two different time points during the establishment of pregnancy: follicular growth phase (first phase) and early pregnancy prior to implantation (second phase). In the first phase, one ovary from each ewe was surgically removed during follicular growth phase of the estrus cycle for transcriptomic study. In the second phase, the sheep were mated and slaughtered during early pregnancy to collect another set of tissue samples. Here we present results from the second phase of our experiment that includes transcriptome (mRNA and miRNA) profiles of corpus luteum (CL) and endometrium from 21 ewes (7 samples from each breed group). On average, 84.75 million mRNA-seq reads from the CL samples represented 16,067 ovine genes and 1,759 novel transcripts. Similarly, 84.63 million mRNA-seq reads from the endometrial samples represented 15,457 genes and 1,895 novel transcripts. Contrastingly, the number of alternative splicing events were higher in endometrium (n=31278) than CL (n=28472). A total of 10 and 76 genes were differentially expressed between the pure breeds from endometrium and CL, respectively. Up to 103 known and 562 novel ovine miRNAs were expressed in our samples with few miRNAs differentially expressed between breed groups. Biological pathways such as circadian rhythm, steroid hormone biosynthesis and PPAR signaling pathway were enriched among differentially expressed genes. These results will improve the knowledge of important fertility traits in sheep, providing an invaluable new data for genomic research.

P-55

Poster Presentation

Schindler, Maria

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Schindler M, Dannenberger D, Pendzialek SM, Grybel K, Seeling T, Fischer B, Navarrete-Santos A

Distinct fatty acid metabolism in embryoblast and trophoblast cells in response to a maternal diabetes mellitus

An insulin-dependent diabetes mellitus during early pregnancy causes a maternal hyperlipidaemia and leads to a non-physiological high amount of intracellular lipid vesicles in trophoblast and embryoblast cells in rabbit blastocysts (Schindler et al. 2014). The rabbit blastocyst is able to take up fatty acids from the uterine environment. These fatty acids can either be metabolised or stored in intracellular lipid vesicles. Our aim was to analyse fatty acid profiles and determine potential mechanisms of intracellular fatty acid accumulation and metabolism in rabbit blastocysts, separately in embryoblast and trophoblast cells. Fatty acid profiles of individual embryoblast and trophoblast samples were analysed by gas chromatography with flame ionization detection (GC/FID). Expression of relevant marker molecules involved in fatty acid metabolism were analysed in the embryoblast and trophoblast by qRT-PCR and Western Blot. Fatty acid profile was different in embryoblast and trophoblast cells, especially for saturated fatty acids (C16:0 and C18:0) and polyunsaturated fatty acids (C18:2n6, C22:5n3 and C22:6n3). Furthermore, maternal diabetes mellitus affects fatty acid profile in both cell lineages, embryoblast and trophoblast, and blastocyst cavity fluid. In embryoblast cells expression of genes encoding for fatty acid uptake (FATP4) and binding (FABP4), as well as beta-oxidation (CPT1) was increased, whereas no significant changes were observed in trophoblast cells due to maternal diabetes. These differences emphasize the need to study embryoblast and trophoblast cells separately in relation to fatty acid metabolism and show that both cell lineages store intracellular different fatty acids which can be influenced by maternal diabetes.

O-56

Oral Presentation

Schoen, Jennifer

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Modelling the embryo-maternal contact zone in vitro: an air-liquid interphase approach to mimic the oviduct milieu

Early embryonic mortality is one of the major causes for reproductive failure in mammals. To understand underlying mechanisms we need to disclose the dynamics of early embryo-maternal interactions. The first ‘contact zone’ between the female reproductive tract and the early embryo is the luminal epithelium of the oviduct. Epithelia are structurally and functionally defined by polarized distribution of organelles and specific proteins: function, growth and survival of epithelial cells correlate with their degree of polarity. Oviduct epithelial cells (OEC) contact the early embryo with their apical membrane while their basolateral cell compartment receives nutrients and signals from the subjacent maternal tissue and circulation. Maintenance of OEC polarity is therefore a prerequisite for in vitro studies regarding oviduct physiology and embryo-maternal interactions. Recently, air-liquid interphase (ALI) culture was applied for OEC, which resulted in the formation of highly differentiated OEC in vitro models. We established a two-step ALI-OEC culture procedure applicable for model species frequently used in mammalian reproductive biology (mouse, pig and cattle). ALI-OEC cultures showed morphological and functional similarity to their in vivo counterparts and produced an oviduct fluid surrogate. Mass spectrometry analysis of this fluid identified >1000 proteins in each species. Western blot revealed the abundance of several glycosylated forms of OVGP1. In co-culture experiments ALI-OEC supported embryo development, passing of the developmental block and even blastocyst formation in all three species without additional IVC medium supply. In conclusion, the ALI culture system allows long-term co-culture of differentiated OEC with developing embryos. Therefore, it is a powerful tool to investigate early embryonic development and its fine-tuned interaction with the maternal epithelium.

O-57

Oral Presentation

Skinner, Michael

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Skinner MK

Environmentally induced epigenetic transgenerational inheritance of disease: ancestral ghosts in your genome

Transgenerational effects of environmental toxicants, nutrition or stress significantly amplify the impact and health hazards of these exposures. One of the most sensitive periods to exposure is during fetal gonadal sex determination when the germ line is undergoing epigenetic programming and DNA re-methylation occurs. Previous studies have shown that endocrine disruptors can cause an increase in adult onset disease such as infertility, prostate, ovary and kidney disease, cancers and obesity. Interestingly, this effect is transgenerational (F1, F2, F3 and F4 generations) and hypothesized to be due to a permanent (imprinted) altered DNA methylation of the germ-line. The transgenerational epigenetic mechanism appears to involve the actions of an environmental compound at the time of sex determination to permanently alter the epigenetic (e.g. DNA methylation) programming of the germ line that then alters the transcriptomes of developing organs to induce disease susceptibility and development transgenerationally. In addition to DNA methylation, alterations in sperm ncRNAs have also been observed. A variety of different environmental compounds have been shown to induce this epigenetic transgenerational inheritance of disease including: fungicide vinclozolin, plastics BPA and phthalates, pesticides, DDT, dioxin and hydrocarbons. Interestingly, exposure specific epigenetic alterations were observed between the specific toxicants. The suggestion that environmental factors can reprogram the germ line to induce epigenetic transgenerational inheritance of disease and phenotypic variation is a new paradigm in disease etiology that is also relevant to other areas of biology such as evolution.

O-58

Oral Presentation

Soen, Yoav

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Knafo M, Elgart M, Soen Y

Adaptation by natural improvisation

During the lifetime of a developing organism, every individual encounters many combinations of diverse changes in its somatic genome, epigenome and gut microbiome. This gives rise to unimaginable number of novel combinations of internal perturbations which are unique to each individual. How any individual can tolerate this high load of new, individual-specific perturbations is not clear. We have recently proposed a conceptual solution to this problem. It explains how (biased) random variation of any kind can safely and rapidly confer a wide range of individual-specific adaptations beyond the existing outcomes of natural selection. We also provided examples for adaptive changes that can be transmitted to the offspring, allowing rapid improvement and assimilation in a few generations. We evaluate this adaptation theory by exposing flies to conditions of stress that cannot be addressed solely by pre-evolved responses. Our findings provide initial support for the ability to acquire heritable adaptations by improvisation in a single generation. I will describe this Lamarckian theory of adaptation and the supportive evidence.

P-59

Poster Presentation

Staicu, Florentin-Daniel

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Staicu FD, Lopez-Ubeda R, Matas C

Localization of Nitric Oxide Synthase (NOS) isoforms in boar spermatozoa

Extensive in vitro studies were performed to expand the current knowledge about the nitric oxide's role in the male gamete physiology. There are three different Nitric Oxide Synthases (NOS) responsible for its synthesis: the neuronal (nNOS), the endothelial (eNOS) and the inducible NOS (iNOS), which have been identified in different mammalian spermatozoa, such as mouse, bull, human and boar. Since the NOS localization has not been described in porcine ejaculated spermatozoa, the objective of this study was to determine their site by using the immunofluorescence technique. Sperm obtained from fertile boars were permeabilized with 100% ethanol at 4°C for 30 min, fixed in 2% p-formaldehyde/PBS (v/v) for 60 min at 4°C and blocked overnight at 4°C with 5% (w/v) BSA–PBS. After, the samples were centrifuged, resuspended in PBS and smeared on microscope slides. The sperm were incubated for 1 h at 4°C with mouse monoclonal anti-NOS antibodies (nNOS: N-2280; eNOS: N-9532; iNOS: N-9657 from Sigma Chemical, Missouri, USA) at 1:200 dilution in 0.1% BSA-PBS. The slides were incubated for an additional 1 h at 4°C with a FITC-labelled goat anti-mouse antibody (Bio-Rad Laboratories) at 1:300 dilution in 0.1% BSA-PBS. The nNOS related fluorescence was localized in the sperm head region, with a maximum fluorescent signal in the equatorial and postacrosomal segment, as well as in the principal and end piece of the flagellum. The eNOS was identified in the acrosomal region, although a weak fluorescent signal was also registered in the principal and end piece of the flagellum. Moreover, immunofluorescent iNOS-staining was spread over the acrosomal, postacrosomal and neck region, but also in the principal and end piece of the tail. In this study, we provide further evidence of the NOS isoforms presence in boar spermatozoa. The methodological approach we applied allowed us to identify their distribution, which could be related to some functional aspects of the spermatozoa.

P-60

Poster Presentation

Strauss, Carmit

Department of Animal Sciences, Hebrew University, Israel

Strauss C, Schlesinger S

Characterizing bovine embryonic stem cells

Embryonic stem (ES) cells are undifferentiated cells with the ability of unlimited self-renewal and the capability to differentiate into all cell types of the body. The derivation of bovine pluripotent ES (bES) cells is an important goal, both for agricultural and research means. For example, a true naïve, pluripotent embryonic cell culture system could promote the understanding of environmental stress on the genetic and epigenetic substance in early stages of embryonic development in cattle. We culture bES that were taken from the inner cell mass of a hatched blastocysts. The cells were characterized as stem cells by morphology and expression of embryonic markers. Different culturing conditions were examined to determine optimal growth conditions for bES to maintain undifferentiated state and self-renewal capacity. Next, we plan to follow the molecular pathway that makes ES cell more susceptible to heat shock and to oxidative stress, since the genetic changes in the cell following induced stress will be easily evident in the cell culture. Moreover, established bES cell line will open a door to a better and easier molecular research on domesticated animals in many other aspects and directions.

P-61

Poster Presentation

Terova, Genciana

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Histone modifications and the expression of genes related to epigenetic regulatory mechanisms in european sea bass (Dicentrarchus labrax) fed a diet supplemented with sodium butyrate

The short-chain fatty acid butyrate has positive effects on the health of the intestinal tract and peripheral tissues. The mechanisms of action of butyrate are related to its potent regulatory effect on gene expression since butyrate is a histone deacetylase inhibitor. We investigated in sea bass the effects of butyrate used as a feed additive on fish epigenetics as well as its regulatory role on gene expression. Seven target genes related to inflammatory response and reinforcement of the epithelial defense barrier (tnf interleukin 1beta, il-6, il-8, il-10, muc2) and five target genes related to epigenetic modifications (dicer1, ehmt2, pcgf2, hdac11, jarid2a) were analyzed in fish intestine and liver. We also investigated the effect of dietary butyrate on histone acetylation, by performing an immunoblotting analysis on liver core histone extracts. Results of the 8-week-long feeding trial showed no significant differences in weight gain of sea bass that received 0.2% Na-butyrate in the diet in comparison to control fish that received a diet without Na-butyrate. Butyrate led to a threefold increase in the acetylation level of histone H4 at lysine 8, but showed no effect on the histone H3 at Lys9. Moreover, two different isoforms of histone H3 that might correspond to the H3.1 and H3.2 isoforms found in terrestrial animals were separated on the immunoblots. The expression of four out of seven analyzed genes related to mucosal protection and inflammatory response was significantly different between the two analyzed tissues but only il10 showed differences in expression due to the interaction between tissue and butyrate treatment. In addition, butyrate caused significant changes in vivo in the expression of genes related to epigenetic regulatory mechanisms such as hdac11, ehmt2, and dicer1. Statistical analysis by 2-way ANOVA for these genes showed significant differences due to butyrate treatment, and to the interaction between tissue and treatment.

P-62

Poster Presentation

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Reproductive Biology, Ghent University, Belgium

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Platelet-activating factor (PAF) and platelet-activating factor receptor (PAF-R) revisited: clues from bovine and human oocyte maturation on a subcellular signaling mechanism

Mammalian embryos both produce the lipid messenger platelet-activating factor (PAF) and express the PAF receptor (PAF-R). It is known that PAF levels are correlated with pregnancy potential, blastocyst quality and viability. However, no data are available concerning the expression and subcellular localization of PAF and PAF-R during oocyte maturation. Bovine oocytes from slaughtered cows were matured in serum-free conditions and collected according to maturation stage (GV, MI and MII). The residual human oocytes (immature after ovarian stimulation or in vivo matured oocytes with aggregates of smooth endoplasmic reticulum SER-MII) that were used in this study, were donated after fertility treatment. After zona digestion using 0.25% pronase treatment (from Streptomyces griseus), the oocytes were left to recover in hepes-buffered TCM199 with FBS for 1h. Subsequently, they were fixed in 2% paraformaldehyde solution for 30 min and immediately subjected to immunofluorescence staining. The subcellular localization of PAF and PAF-R is highly dynamic and cell cycle-dependent during oocyte maturation. In bovine oocytes, we demonstrated that PAF spreads homogeneously throughout the nucleoplasm during the germinal vesicle (GV) stage. After germinal vesicle breakdown (GVBD), PAF redistributed to the cytoplasm. We observed a similar pattern for the bovine PAF-R. In human oocytes, PAF is associated with the nucleoplasm of the GV, similar to bovine, but co-localizes with the spindle in MI and MII oocytes. Also, PAF-R accumulated around the chromatin in the GV with redistribution along the putative spindle during meiosis in human. In this study, a nuclear localization of both PAF and its receptor in mammalian oocytes was described for the first time, as well as its dynamic changes through meiosis.

O-63

Oral Presentation

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Diddens J, Steyaert S, Frankl C, Ter-Haar S, Galle J, Van Criekinge W, Gahr M, Balthazart J, Van der Linden A, De Meyer T, Vanden-Berghe W

Does epigenetic control of zebrafinch birdsong reveals secrets of human speech and language development?

Scientists believe that birds may hold the key to resolve how human language evolved. As Darwin noted, young birds learn their songs from adults by imitation, and develop them into a song or repertoire of their own. Human infants demonstrate exactly the same process of vocal learning, first by babbling and then developing this into words and sentences. Interestingly, humans and birds share similar brain structures and more than 50 genes connected to speech and vocal learning. Remarkably, although DNA methylation has been traditionally viewed as a highly stable epigenetic mark in differentiated cells, recent findings demonstrate that postnatal brains appear to show dynamic stimulus-induced (de)methylation changes during learning and memory formation. Therefore, we studied the brain methylome dynamics in the zebrafinch songbird, in which learning of song is limited to the first year. Neuronal circuits in many regions of the developing brain, undergo defined phases of enhanced plasticity, called critical periods during which auditory memory is formed and and song becomes “crystallized”. The mechanisms underlying the opening and closing of this critical period are unknown. We investigated the possible involvement of DNA (hydroxy)metylation in the regulation of the critical period, using Reduced Representation Bisulfite sequencing (RRBS) of telencephalon samples at critical timepoints in vocal development. We detected methylation changes in many genes involved in neurogenesis and nervous system development. Interestingly, we found an enrichment of both estrogen and androgen response elements in this gene list, suggesting an interplay between sex hormones and DNA methylation regulation of song behaviour. Altogether, we believe that DNA (hydroxy)methylation control of neuroplasticity may underly song learning in zebra finch and speech and language development in men.

P-64

Poster Presentation

Vaquerizas, Juanma

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Enriquez-Gasca R, Ishiuchi T, Torres-Padilla ME, Vaquerizas JM

Genomic analyses of repetitive elements in the context of early mouse development

The presence of retroviral particles in human placenta was first recorded in the early 1970's. Since then it has been shown that transposable elements play a key role during pre-implantation development and to have mediated the recruitment of genes throughout the evolution of pregnancy in mammals. In particular, previous analyses have reported a temporal de-repression of murine endogenous retrovirus with leucine tRNA primer (MERVL) and major satellite repeats during the 2-cell stage of mouse development. Furthermore, it has been proposed that de-repression of these repetitive elements could have a functional role in reprogramming of the embryonic genome. Here, we perform a genome-wide analysis of the relationship between repeat elements and genes found to be up-regulated in 2-cell embryos. Our results demonstrate a clear association between different types of repeats and genes expressed specifically at this point in development, suggesting an interconnected regulatory mechanism for both elements involved in early mouse development.

P-65

Poster Presentation

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Prostaglandin F2α regulates adhesion and proliferation of the human trophoblast-derived HTR-8/SVneo cell line

Due to luteolytic action of prostaglandin F2α (PGF2α), exogenous administration of this hormone decreases pregnancy rates or even terminates early pregnancy in many species such as humans and domestic animals. On the other hand, elevated amounts of PGF2α in the uterine lumen and/or its increased endometrial synthesis are observed during the implantation period in various mammals with different types of placentation. This arises the question if PGF2α could be involved during pregnancy establishment. Aims of our study were to determine if PGF2α is involved in trophoblast cell adhesion and proliferation. Human trophoblast cell line (HTR8/SV-neo) was incubated with vehicle (0.1% ethanol) or PGF2α (100 nM; 1 μM) with/without PTGFR antagonist (AL8810, 50 μM) and for 30 min at 37°C in humidified atmosphere of 95% air and 5% CO2. Subsequently, adhesion of HTR8/SV-neo cells was determined using Millipore ECM101 kit accordingly to the manufacturer's protocol. To study effect of PGF2α on cell proliferation, the trophoblast cells were seeded onto 96-well plate and incubated overnight. Afterwards, the cells were cultured with RPMI containing vehicle (0.1% ethanol), PGF2α (100 nM; 1 μM) with/without PTGFR antagonist (AL8810, 50 μM) for 48 h. Cell proliferation was measured by using CellTiter 96-AQueous solution. Adhesion of HTR8/SV-neo cells to extracellular matrix proteins was stimulated by PGF2α ($p<0.05$). This effect was decreased by PGF2α receptor inhibitor (AL8810). PGF2α (100 nM and 1 μM) significantly increased cell proliferation ($p<0.05$). This effect was diminished by AL8810. Summarizing, our results indicated that PGF2α regulated proliferation human trophoblast cells and increased their capacity to adhere to ECM. These results suggest that PGF2α is involved in implantation process.

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P-66

Poster Presentation

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Warzych E, Madeja Z, Pawlak P, Lechniak-Cieslak D

Quality of bovine blastocysts produced in vitro with the 3i/2i inhibitor systems

Novel approach to ESC derivation relies on preserving the ground state of pluripotency, by altering the innate signaling. Prevention of cell death in the epiblast cells is crucial for successful ESC derivation. Thus understanding of the role of the specific signaling pathways in the early embryo is crucial to provide stable ESC culture conditions. The results of our studies indicate that prolonged exposition of early bovine embryos to WNT signaling (by CHIR99021) resulted in reduced potential to form primary ESC colonies. Consequently we anticipate, that commonly used 3i/2i culture systems may affect embryo development and quality. Bovine embryos were cultured in vitro (SOF) up to the blastocyst stage with specific inhibitors: 3i (CHIR99021, PD184352, FGFR SU5402) and 2i (CHIR99021, PD0325901). The apoptotic index (AI) and the total cell count were estimated by TUNEL staining. The highest AI was noted in SOF+2i group (7.55). The AI values for SOF+3i (4.79) and SOF (5.56) did not differ. The lowest total cell count was noted for SOF+3i. According to the literature the AI for good quality bovine IVP embryos varies from 3 to 22, thus all of the observed values are within the published range. The higher AI in the 2i system may result from the fact that PD03259 is a much more potent MEK/ERK signaling inhibitor than SU5402 used in the 3i system. Besides, MEK inhibition results in dramatic increase of apoptosis in cancer cell lines and our studies (unpublished) show that the 2i system alone is not sufficient to maintain bovine ESC derivation. In the 3i system blocking of the FGFR is not restricted only to the MEK/ERK cascade, but may also affect phosphatidylinositol-3 kinase and other downstream pathways crucial for pluripotency maintenance, what may result in the observed lower total cell count in the 3i system.

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P-67

Poster Presentation

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Weyrich A

Changes in diet altered paternal epigenetic transmission in male wild guinea pigs

We investigated the paternal epigenetic response to a temporal exposure to a low protein diet in wild guinea pigs. Epigenetic modifications, such as DNA methylation is a mechanism conveying environmental information through generations via parental germ lines. The paternal role in transgenerational transmission of epigenetic information received little attention. In most wild mammal species, males are the dispersing sex and have to cope with differing habitats. Thus, in comparison to phylopatric females, males have to cope more rapidly with environmental changes, such as nutrition. To address the paternal impact on the offspring's adaptability to changes in nutrition composition, we investigated if nutritional changes in males caused epigenetic alteration in sons they sired afterwards. We fed male Wild guinea pigs with a low-protein diet and analysed DNA methylation changes in two organs of their sons (F1): in the liver as the main metabolic and thermoregulation organ, and in testes for potential transmission to the F2 generation. Reduced representation bisulfite sequencing revealed differentially methylated regions (DMRs) in annotated regions ('annotated DMRs') in sons sired before and after the low-protein diet. 'Annotated DMRs' of sons sired by low protein diet-treated fathers were partly shared between organs and groups. Among the shared 'annotated DMRs' we detected changes in promoter-methylation in 18 genes of three main GO-pathways: 1) metabolism, cell and body structure 2) gene regulation, and 3) reproduction and hormone metabolism. Our results indicated a 'heritable epigenetic response' to the diet change of the fathers, which was transmitted paternally to the F1 and potentially even to the F2 generation. Epigenetic mechanisms, allowing immediate and inherited adaptability might be important for the survival of species in the context of a globally changing environment.

O-68

Oral Presentation

Whitelaw, Christopher Bruce

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Genome editing technology

Genome editing although only a few years old is now widespread in both academic and private/business research and development. Recent technological developments, in particular CRISPR Cas9, have increased the volume and range of applications in this technique. Genome editing technology is simply to use, efficient and precise. As each week goes by, new applications are demonstrated. In my own research genome editing technology is opening up numerous new avenues for research into the use of livestock in agriculture and biomedicine. In human medicine, genome editors other new and exciting opportunities. In basic research they are unparalleled in their ability to advance knowledge. This presents huge opportunities to the life sciences community as well as challenges – should we progress unrestrained with this technology or be more cautious in how we embrace it – to ensure this science is used responsibly to advance society.

P-69

Poster Presentation

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Use of CRISPR/Cas9 to edit OCT4 gene and investigate high plasticity of epigenetically erased fibroblasts

Epigenetic erasing is a promising approach that allows terminally differentiated cells to acquire a transient high plasticity state, without the involvement of retroviral vectors. Although this methodology has been successfully applied (1-5), limited information are available on the role of pluripotency-related transcription factors in the control of the high plasticity state acquired. The experiments here described were carried out to investigate the specific role played by the OCT4 (POU51) gene in the process. We selected CRISPR/Cas9 to edit the genome for the complete deletion of this important plurypotency-related gene from mouse primary fibroblast cell lines (6). We first identified the most promising murine sequences, designing four sgRNAs to target the gene. To generate the CRISPR sgRNA vector, synthesized sgRNA spacer oligos were annealed in vitro and cloned in the all-in-one CRISPR/Cas9 vector (PX330_pSPCas9(BB)-2A-Puro). These vectors were transformed into E.Coli competent cells and, then, transfected into the cells using a Lipofectamine Transfection kit. Puromycin was applied in order to select the OCT4 KO cells. We have derived independent clones to generate pure cell lines. Twelve clones were obtained altogether, with varying yields. PCR screening demonstrated 2 clones, in particular, displaying highly homogeneous population for OCT4 deletion and we derived a cell line from them. KO cell line and wild type fibroblasts were exposed to the epigenetic modifier 5-Azacytidine-CR. A rate of 65% of cells expressing OCT4 was observed in KO cell line after treatment. On the other hand, 86% of wild type treated fibroblasts were positive for OCT4 expression. Although still preliminary, these results support the possibility to establish CRISPR/Cas9 mediated OCT4 gene knock out models. Further experiments will be needed to optimize genome editing efficiency and to determine how OCT4 deletion may interfere with the acquisition of 5-Aza-CR induced high plasticity.

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