Epigenetics and Periconception Environment

Proceedings of the EPICONCEPT Conference 2014

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Editors
Ann Van Soom, Alireza Fazeli, Sofia Engrola

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About the European Co-operation in Science and Technology

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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.

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www.cost-epiconcept-eu

Welcome from the Chairman

Dear Epiconcept Members

This is already our second annual meeting in the sunny south of Europe, in Vilamoura, Portugal. Thanks to Sofia Engrola and her team we can experience exquisite science in this beautiful holiday village. Together with Sofia and the working group leaders, an exciting scientific programme with excellent speakers has been established, and there is also an excursion to visit the coastline of Vilamoura. Laszlo Tecsi our secretary has taken care of all the registrations, the website and the booklet and Alireza Fazeli, as the vice chair, covers our expenses! We are very grateful to COST as well, the organization which enables us to meet and learn. Let's thank them all and let us all enjoy the all aspects of this meeting: epigenetics, people and the environment!

Ann Van Soom
Chair of Epiconcept

Organisers

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Yael Heifetz, Israel

Programme

Tuesday 30 September 2014

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

19:00 – 20:30 **Welcome Reception**

20:30 – 23:00 **Dinner**

Wednesday 01 October 2014

07:45 – 08:45 **Breakfast**

Opening of Meeting

08:45 – 09:00 **Welcome Address**

Working Group 1: Epigenomic Tools

09:00 – 09:35 **Hasan Khatib, University of Wisconsin-Madison, United States**
Genetics and epigenetics of early embryonic development and fertility in cattle.

09:35 – 10:05 **Dawit Tesfaye, University of Bonn, Germany**
Exploring noncoding miRNAs and their role in bovine follicular development: Challenges and potentials.

10:05 – 10:35 **Jose Luis Garcia-Perez, Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research, Spain**
Epigenetic control of retrotransposon expression in embryonic cells.

10:35 – 11:05 **Coffee**

11:05 – 11:20 **Laszlo Tecsi, University of Sheffield, United Kingdom**
Rules of Travel Reimbursements.

Working Group 2: Periconception Environment

11:20 – 11:55 **Kevin Sinclair, University of Nottingham, United Kingdom**
Parental diet, assisted reproductive technology and offspring health: metabolic determinants in the oocyte and early embryo.

11:55 – 12:20 **Karen Lillycrop, University of Southampton, United Kingdom**
Fatty acids and epigenetic programming of offspring health.

12:20 – 12:45 **Jean-Pierre Ozil, French National Institute for Agricultural Research (INRA), France**
Oocyte metabolism and programming of long-term development.

12:45 – 14:30 **Lunch**

12:30 – 14:50 **Pablo Bermejo-Alvarez, Spanish National Institute for Agricultural and Food Research and Technology (INIA), Spain**
Deep sequencing reveals extensive transcriptional reprogramming at imprinting clusters following conversion of mouse SSC into SSCiPSC.

14:50 – 15:10 **Herve Acloque, French National Institute for Agricultural Research (INRA), France**
Sperm DNA methylation analysis in swine reveals conserved and species-specific methylation patterns and highlights an altered methylation at the GNAS locus in infertile boars.

15:10– 15:30 **Dusan Fabian, Institute of Animal Physiology, Slovak Academy of Sciences, Slovakia**
Possible correlation between maternal body condition, embryo quality, somatic development and behaviour of offspring in mice.

15:30 – 15:50 **Anna Kitewska, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Poland**
Transcriptome analysis of the porcine endometrium on day 6 after seminal plasma infusion.

15:50 – 16:20 **Working Group 1 Discussions**

16:20 – 16:50 **Tea**

16:50 – 17:20 **Working Group 2 Discussions**

17:20 – 19:00 **Poster Presentation**

20:00 – 23:00 **Welcome Dinner**

Thursday 02 October 2014

07:45 – 08:45 **Breakfast**

08:45 – 09:15 **Grant Applications in the European Union**

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

09:15 – 09:50 **Peter Hatemi, Pennsylvania State University, United States**
Expression of epigenetic effects in identical twins.

09:50 – 10:10 **Pascale Chavatte-Palmer, French National Institute for Agricultural Research (INRA), France**
The role of the placenta in mediating maternal environment: clones and other models.

10:10 – 10:30 **Charles Long, Texas A&M University, United States**

Are cloned animals in fact identical?

10:30 – 10:50 **Bernard Angers, University of Montreal, Canada**
Epigenetic variation in natural fish populations including parthenogenesis.

10:50 – 11:20 **Coffee**

Working Group 4: Public, Periconception and Epigenome

11:20 – 11:55 **Anna Moles, National Research Council of Italy, Italy**
Epigenetics: Techniques and Strategies for Next-Generation sequencing.

11:55 – 12:25 **Amir Sagi, Ben Gurion University, Israel**
On sexual intervention and safety considerations in the use of RNAi in aquaculture.

12:25 – 12:55 **Heiner Niemann, Friedrich Loeffler Institute, Germany**
Epigenetic studies on bovine pre-implantation embryos.

12:55 – 14:30 **Lunch**

14:30 – 14:50 **Parisa Norouzitallab, Ghent University, Belgium**
Environmental heat stress induces epigenetic inheritance of robustness in Artemia model.

14:50 – 15:10 **Marta De Ruiter-Villani, Utrecht University, Netherlands**
Mother knows best: uterine asynchrony delays conceptus development and alters expression of imprinted genes in an equine model.

15:10 – 15:30 **Sonia Heras-Garcia, Ghent University, Belgium**
Asymmetric H3K9me3 pattern allows to differentiate between maternal and paternal pronuclei in equine zygotes.

15:30 – 15:50 **Elena Ibanez, Autonomous University of Barcelona, Span**
Effect of epigenetic modifiers treatment on cloned mouse embryo morphokinetics.

15:50 – 16:20 **Working Group 3 Discussions**

16:20 – 16:50 **Tea**

16:50 – 17:20 **Working Group 4 Discussions**

Closing of Meeting

17:20 – 17:35 **Farewell Address**

17:35 – 18:35 **Management Committee Meeting**

20:00 – 23:00 **Farewell Dinner**

Friday 03 October 2014

07:45 – 08:45 Breakfast

10:00 – 16:30 Excursion

Abstracts of Presentations

O-1

Oral Presentation

Acloque, Herve

Genetics and Cell Laboratory, French National Institute for Agricultural Research (INRA), France

Congras A, Yerle-Bouissou M, Pinton A, Vignoles F, Foissac S, Lhuillier E, Bouchez O, Riquet J, Ferchaud S, Acloque H

Sperm DNA methylation analysis in swine reveals conserved and species-specific methylation patterns and highlights an altered methylation at the GNAS locus in infertile boars

In the last decade, more and more studies have reported aberrant methylation patterns in sperm DNA of patients with an altered spermogram. It is also admitted that epigenetic reprogramming during germ cell development is a key mechanism for the production of functional gametes and the proper development of the embryo. As poor semen quality is also a key issue for farm animals' productivity, we studied the methylation profile of sperm DNA of fertile and infertile boars.

We first described and compared the methylome and hydroxymethylome in sperm DNA from two fertile boars by combining MeDIP-Seq and Reduced Representation Bisulfite Seqencing. We then focused on the methylation level of imprinted genes in poor-quality semen by MeDIP-qPCR and single base methylation analysis by bisulfite conversion and pyrosequencing.

Methylome analysis and its comparison with mouse and human data revealed the high conservation of the methylation pattern of sperm cells between mammals: sperm DNA is highly methylated with the exception of CpG islands and promoters. We nevertheless observed some discrepancies between species, like in the promoter of the developmental gene POU5F1 which was highly methylated in human sperm only, suggesting a different dynamics of activation of this gene following fertilization.

We first compared global level of sperm DNA methylation between fertile and infertile boars but we did not observed significant differences between these two groups. We then selected 42 loci of interest, most of them known to be imprinted in human or mice and quantified their DNA methylation levels in fertile and infertile boars. Comparison between these two groups of boars revealed that one imprinted region, the GNAS locus, shows an increase in 4 out of 8 infertile boars with low semen quality. This increase in DNA methylation is associated with an altered expression of the genes belonging to the GNAS locus, suggesting a new role for GNAS in the proper formation of functional gametes.

Poster Presentation

Al-Adhami, Hala

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

Al-Adhami H, Tarrade A, Valentino S, Mourier E, Richard C, Kiefer H, Jammes H, Chavatte-Palmer P

Developing a toolbox to study the rabbit methylome and its alteration in IUGR cases during gestation

Epigenome is the essential mediator of the effect of environmental exposures on development. Epigenetic studies show that alterations in DNA methylation marks are associated with developmental reprogramming and linked to environmental exposures. Our objective is to develop different Intra Uterine Growth Restriction (IUGR) rabbit models and to study the alterations occurring in the methylome of the placental unit throughout gestation. The relevance of the rabbit model resides in its placentation similarity with humans. As little is known about rabbit epigenome, we are developing a strategy to establish a toolbox to study the rabbit methylome. First, studying the methylome of the placental unit will be performed by MeDIP-seq on pooled samples to generate a methylation overview in both placenta (fetal compartment) and decidua (maternal compartment) at term. In a second time, a microarray will be designed for individual analysis to establish early epigenetic events related to placenta from IUGR. The array design will integrate methylated sequences from MeDIP-seq data but also promoters of differentially expressed genes obtained by transcriptomic analysis performed on the same samples. Finally, to validate our results, methylated regions of interest will be studied by pyrosequencing after bisulfite treatment. The identification of critical epigenetic marks alterations associated with IUGR will allow a better understanding of the IUGR process and an identification of key actors in placental function. In future studies, these marks could be linked to the establishment of a specific phenotype at adulthood and tested as biomarkers at birth to define the risk of developing an adverse phenotype. In addition, this study will present the first overview of the rabbit methylome and lead to the development of epigenomic tools that can be used systematically.

Poster Presentation

Andronowska, Aneta

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

Andronowska A, Malysz-Cymborska I

Chemokines expression in the porcine endometrium during peri-implantation period

Successful implantation and maintenance of pregnancy requires an appropriate uterine immune response to the embryo. One of crucial factors determining these processes are chemokines, produced by immune cells. The aim of present study was to determine the profile of chemokines and their receptors expression in the porcine endometrium during peri-implantation period .

Endometrium was collected from mature crossbred gilts on days 10, 12 and 14 of estrous cycle and early pregnancy. Examination of mRNA expression of CCL2, CCL5, CCL11, CXCL2, CXCL8, CXCL10 and CXCL12 and CCR2, CCR5, CXCR2, CXCR3, CXCR4 was done using Real-Time PCR. Data were analyzed by two-way ANOVA followed by Tukey's post hoc test. During pregnancy, the CCL2, CCL5, CCL11 and CXCL12 mRNA expression increased on day 14 ($P<0.05$) compared to day 10. Increase in expression of CCR2 and CCR5 ($P<0.05$) strongly corresponded with expression of compatible chemokines. Despite the lack of statistical significance in CXCL2, CXCL8 and CXCL10 mRNA expression, their profile was similar to CXCL12. CXCR2 mRNA expression enhanced on day 12 of the estrous cycle vs. 10 and 14 ($P<0.001$, $P<0.01$ respectively). CXCR3 mRNA expression increased on day 14 of pregnancy compared to day 10 and 12 ($P<0.001$ and $P<0.05$ respectively). Similarly, CXCR4 mRNA expression increased on day 14 vs. 10 of pregnancy ($P<0.001$). There were no significant differences in mRNA expression of most of chemokines and their receptors during estrous cycle. However, the embryo presence on day 14 enhanced chemokines CCL2, CCL5 as well as CXCL11, CXCL12 and their corresponding receptors CXCR3, CXCR4 mRNA expression in comparison with day 14 of the estrous cycle ($P<0.01$ average). In conclusion, our data demonstrate the fact that embryo presence influence chemokines and their receptors expression in the uterine on day 14 of pregnancy that may have significant impact in maintenance of pregnancy.

Oral Presentation

Angers, Bernard
Department of Biological Sciences, University of Montreal, Canada

Angers B, Leung C

Epigenetic variation in natural fish populations

Epigenetic mechanisms enable asexual organisms to cope with environmental fluctuations and survive in different environments. Two contrasting but not mutually exclusive mechanisms are known. Phenotypic plasticity resulting of epigenetic-environment interactions allows phenotypic change in response to the environmental changes. On the other hand, the diversifying bet-hedging resulting of random epigenetic changes is selected to face environmental unpredictability. The objective of this study is to understand the relative importance of these mechanisms when multiple clonal lineages occur sympatrically and allopatrically in different ecosystems. The asexual fish Chrosomus eos-neogaeus was used as biological model. Genetically identical individuals may live in different environmental conditions and display a large phenotypic difference. The different clonal lineages also revealed striking differences in the use of epigenetic mechanisms suggesting they were selected for different ecological purposes.

Poster Presentation

Arias-Alvarez, Maria
Veterinary Faculty, Complutense University of Madrid, Spain

Arias-Alvarez M, Arrivas S, Garcia-Garcia RM, Millan P, Rodriguez M, Formoso N, Lorenzo PL, Rebollar PG

Effect of n-3 polyunsaturated fatty acids (PUFA) on endocrine response and fetoplacental development in rabbit

Diets enriched with n-3 PUFA administered during the preconceptional and gestational periods could modulate endocrine response and fetoplacental development. The aim of this work was to study the effects in the rabbit model. A total of 39 rabbit does were fed ad libitum 3 diets supplemented with different fat sources: 7.5g/kg lard (control diet, C); 15g/kg (P15) and 30g/Kg (P30) of a commercial supplement with n-3 PUFA from salmon oil (Optomega 50) from 4 weeks before artificial insemination (AI) and during pregnancy. In all does, plasma LH was determined 30 min before AI and at 0, 30, 60, 90 and 120 min after. Progesterone (P4) concentrations were assessed every 7 days from day of AI until day 26 of pregnancy. Such day, 11 does were euthanized to measure weight, length and thickness of fetal and maternal placentas, and fetal weight and size (crown-rump length, biparietal diameter and transversal thoracic diameter) in a total of 180 fetuses. Ovulation rate (OR), number of viable fetuses, IUGR (intrauterine growth retarded) and reabsortions were determined. Statistical analysis was performed by SAS program. OR, LH peak and serum P4 concentrations were in physiological range in all groups. At day 19 of pregnancy P4 were higher in P15 than in C group (30.7±2.8 vs 15.4±2.4ng/ml; $P<0.02$); P30 showed intermediate concentrations (22.2±2.8ng/ml). This finding could not be associated with significant changes in fetoplacental development since all parameters measured were similar among experimental groups. In conclusion, n-3 PUFA supplementation had no effect on endocrine response of the females and prenatal development of conceptuses in the studied rabbit model. However, we not discarded postnatal changes even in following generations.

We acknowledge CM, FSE and AGL2011-23822 for funding.

Poster Presentation

Barrey, Eric

Animal Genetics and Integrative Biology, National Institute for Agricultural Research (INRA), France

Barrey E, Banrezes B, Sainte-Beuve T, Vaiman A, Ozil J-P

Early differential miRNA expression following in vitro fertilization of mouse eggs incubated in two standard culture medi

At fertilization, the incoming sperm triggers the increase in egg metabolism by causing a series of repetitive Ca2+ signals that stimulate mitochondrial oxidative phosphorylation. Several reports have shown that the culture media impacts egg unctioning and may affect the long-term developmental processes either in animal or in human. According to the recent discovery of mitochondrial function in microRNA pathway, we assumed that early miRNA expression in the egg should be functionally linked with mitochondrial activity during the period of egg activation. The aim of the study was to measure whether the miRNA expressions are differentially regulated by culture media during the initial period of fertilization. Four groups of 30 freshly ovulated oocytes were used as control. Eight groups of 30 mouse oocytes were fertilized by Intra
Cytoplasmic Sperm Injection (ICSI) and incubated for 4 hours in standard M16 or KSOM medium which have the same 10 compounds but at lower concentrations in KSOM (inorganic ions, energy substrates and protein). All the groups were used to isolate
total cells and mitochondria, cytosolic and nucleus fractions by differential centrifugations in specific buffers. RNA extraction kit adapted for small amount of materials was used to extract total RNA from the egg or cell fractions and perform a multiplex RT-qPCR (742 mouse and rodent miRNA). Significant changes of miRNA expressions were recorded 4 hours following ICSI. The total number of miRNA expressed per group
was highly significantly different after fertilization in KSOM (18 miR) versus M16 (26 miR) or in non-fertilized oocytes (20 miR). Moreover, differential miRNA expressions were observed between cyctosolic (15 vs 32 miR), mitochondrial (15 vs 18 miR and nuclear (17 vs 17 miR) fractions in eggs according to the medium (KSOM vs M16). Among the predicted genes targeted by the
more expressed miRNA, the following pathways were significantly identified by miRPath: MAPK signaling, PI3K-Akt, metabolic
pathway, calcium signaling, Jak-STAT and dorso-ventral axis regulation.

Conclusion
We reveal a functional linkage between the culture media formula and the initial miRNA expressions immediately after fertilization. Such miRNA activity could regulate transcription of thousands of maternal mRNA which are further involved in zygotic genome activation. Such functional genomic analysis provides a potential lever to identify the molecular mechanisms involved in phenotype programming following in-vitro fertilization.

P-7

Poster Presentation

Baruah, Kartik

Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Belgium

Baruah K, Mwainge VM, Teshome B, Norouzitallab P, Bossier P

Compound Hspi-P, an inducer of heat shock protein (Hsp) 70, induces Hsp70 gene transcription in Artemia by modification of the histone proteins

The 70 kDa heat shock protein (Hsp70), also known as molecular chaperone, is a highly conserved protein found in all organisms. This protein has been implicated in protecting cells and tissues from various infection and injuries. In our laboratory, we explored whether Hsp70 controls bacterial diseases in aquaculture. In the gnotobiotic (germ-free) Artemia model organism, we unequivocally showed that induction of Hsp70 by exposing Artemia to a non-lethal heat shock of 37°C for 30 min followed by 6 h recovery period (a classical Hsp inducer) was associated with the protection of the animal against pathogenic vibrios. However, laboratory inducer of Hsp70, such as non-lethal heat shock are not safe for application owing to the fact that a slight change in the temperature could be detrimental to the cultured organism. Alternatively, we tested the Hsp70-inducing potential of a plant-based compound named 'Hspi-P' in Artemia and our results showed that Hspi-P can safely enhance Hsp70, both at the transcriptional and translational levels, in Artemia. Furthermore, Hspi-P mediated Hsp70 induction was also shown to generate protective immune responses against experimental bacterial diseases. However, the molecular mechanism(s) underlying the induction of Hsp70 by Hspi-P is unknown. To unravel the underlying mechanism, we carried out the current study based on our assumption that Hspi-P induces hsp70 expression via modifications (acetylation/deacetylation or methylation) of the histone proteins (H3, H4 and H3K4me3), as these epigenetic markers were reported to be associated with chromatin opening, binding of key transcription factors to the hsp70 promoter, and initiation of the hsp70 gene transcription. Our preliminary data indicated that modifications of the histone proteins, mediated by Hspi-P compound, exerted important functions in hsp70 gene transcription in Artemia.

Oral Presentation

Bermejo-Alvarez, Pablo

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

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

Deep sequencing reveals extensive transcriptional reprogramming at imprinting clusters following conversion of mouse SSC into SSCiPSC

Being one of the few exceptions to Mendelian rules, genomic imprinting is an epigenetic mechanism that allows a subset of genes to be expressed only from the paternally or from the maternally inherited allele. The epigenetic marks responsible for genomic imprinting (methylation at imprinting control regions –ICR-) are especially resilient from erasure, as they remain unaltered during preimplantation development, escaping from the global genome demethylation that resets gametes genome. Thus, these marks are only erased in vivo during primordial germ cell formation, prior to the establishment of sex-specific methylation marks. We have developed an in vitro model for imprinting erasure consisting in the reprogramming of spermatogonial stem cells (SSC) to SSC-derived induced pluripotent stem cells (SSC-iPSC) by the expression of Yamanaka factors (Oct4, Sox2, Klf4 and c-Myc). The iPSC were obtained from a mouse model expressing Yamanaka factors under a doxycycline-inducible promoter. SSC were obtained through Gfrα1 sorting of neonatal (6 days old) testis lysate. Methylation levels at H19 ICR, analyzed by sequencing clones of PCR amplified bisulfite treated DNA, were drastically reduced in SSCiPSC (SSC 68 % vs SSCiPSC 0, 0.6 and 2 %). Deep sequencing was performed in SSC, SSCiPSC and fibroblast derived iPSC (fiPSC) (3 samples/group). Total RNA was extracted by miRNeasy MiniKit. Libraries of double stranded DNA and deep sequencing were performed on Illumina platform (TruSeq) The comparison between SSC and SSCiPSC revealed an extensive transcriptional reprogramming affecting 12051 genes (FRD 0.05), whereas SSCiPSC and fiPSC displayed a similar expression pattern, with only 665 genes differentially expressed. A closer look at the 41 known clusters of imprinting genes in the mouse genome revealed that 24 contained imprinting genes whose expression differed between SSC and SSCiPSC. These results suggest that the reprogramming of SSC to SSCiPSC triggers a demethylation wave able to erase several imprinting marks.

P-9

Poster Presentation

Brinkhof, Bas

Farm Animal Health, Utrecht University, Netherlands

Brinkhof B, Groot Koerkamp MJA, Mashayekhi K, Haagsman HH, Roelen BAJ

The mRNA landscape of bovine pluripotent cells

During mammalian pre-implantation development a blastocyst composed of trophectoderm (TE) and an inner cell mass (ICM) that segregates into primitive endoderm (PE) and a pluripotent epiblast is formed. In vitro culture of the ICM can lead to the generation of pluripotent embryonic stem cells, but knowledge how to maintain pluripotency is only available for rodent and primate cells. To identify genes involved in pluripotency of embryos from a large mammal we compared gene expression in bovine embryos using genome wide RNA microarrays representing 43,803 probes.

When comparing morula with blastocyst, expression of 1,895 genes was up regulated, while expression of 2,039 genes was down regulated. Comparing ICM with TE, expression of 419 genes was up regulated, among which those known to be important for pluripotency: NANOG, POU5F1 and SOX2. Additional gene ontology analysis revealed enrichment for genes involved in stem cell development in the ICM. Interestingly, expression of the genes coding for transcription factors MEIS2 and OTX2 was up regulated in ICM cells. These genes are implicated in lineage commitment suggesting that the isolated ICM cells were at a so called primed state. Previous studies indicated that MAPK inhibition by PD0325901 increases the percentage of NANOG expressing cells in the ICM. Indeed, NANOG expression was up regulated in ICM from embryos cultured in the presence of this inhibitor. Other genes, involved upstream in the MAPK pathway, were down regulated in the PD treated group as compared to the control. Genes known to promote differentiation, like VIM and HAS2, were expressed at higher levels in ICM than in TE, but their expression was reduced upon MAPK inhibition. Importantly, expression of genes described to be critical for pluripotency where not up regulated after MAPK-inhibition suggesting that NANOG expression by itself is not sufficient for pluripotency. Altogether these results contribute to a better understanding of pluripotency.

P-10

Poster Presentation

Burkus, Jan

Department of Developmental Physiology, Institute of Animal Physiology, Slovak Academy of Sciences, Slovakia

Burkus J, Cikos S, Kubandova J, Kokosova N, Fabianova K, Fabian D, Koppel J

Why we should not underestimate maternal stress during very early pregnancy

We exposed spontaneously ovulating and naturally fertilized female mice to restraint stress for 30 min three times a day during the preimplantation period of embryo development. Maternal corticosterone was measured using a commercially available EIA kit (Enzo Life Sciences). Blastocysts isolated on day 4 of pregnancy from control and stressed dams were differentially stained using rabbit anti-mouse CDX2 polyclonal antibody, Texas Red-X goat anti-rabbit IgG and Hoechst 33342. Offspring were scanned with EchoMRI for evaluation of the exact amount of body fat deposits. Behavior of delivered mice was tested in open field (60x45x36 cm) for 5 min. Trajectories and actions of animals were collected by CCD camera and evaluated by Ethovision XT 7.0. Blood pressure was measured using CODA tail-cuff blood pressure system. Our results showed that maternal restraint stress significantly decreased maternal body weight ($P<0.001$), increased corticosteron concentration in blood plasma ($P<0.001$), slowed down transition of embryos from oviduct to uterus ($P<0.01$), negatively influenced stage-specific distribution of isolated embryos ($P<0.001$) and decreased cell number in blastocysts ($P<0.001$). Furthermore, reduction of cells in TE was observed, resulting in increased ICM: TE ratio, when compared to control ($P<0.05$). Restraint stress reduced number of implantation sites in uteri ($P<0.05$), significantly delayed eyes opening in delivered mice ($P<0.001$) and altered their behavior in two parameters (scratching on the base of apparatus, $P<0.01$; time spent in central zone, $P<0.05$) as well. Moreover prenatally stressed offspring had significantly lower body weight (females: $P<0.001$; males: $P<0.05$), fat deposits and blood pressure in stressed female was significantly lower ($P<0.05$). Our results indicate that stress exposure during very early pregnancy can negatively influence the developmental capacity of preimplantation embryos, decrease the ability to conceive, influence maturation of nervous system and alter offspring behavior and some physical parameters. Mechanisms behind these changes are not clear; some epigenetic mechanisms may be involved (e.g. predictive adaptive response).

Work was supported by the national grants under contracts APVV–0815–11 and VEGA 2/0029/12.

P-11

Poster Presentation

Canada, Paula

Abel Salazar Biomedical Sciences Institute, University of Porto, Portugal

Canada P, Engrola S, Conceicao LEC, Teodosio R, Mira S, Sousa V, Fernandes JMO, Valente LMP

A high inclusion of fish protein hydrolysate on Senegalese sole larval diet affects growth and is associated with altered expression of DNA methyltransferases

Fish nutrition research has suggested that the partial substitution of dietary native proteins by highly hydrolysed fish meal would result in better larval performances. In the last years, this hypothesis has been studied and different experimental and commercial hydrolysates differing in the raw material have shown remarkably different effects on larval gut maturation and/or growth. However possible transcriptomic effects underlying such response have not been explored so far. To our knowledge, no studies have been published regarding the effect of feed composition and/or nutritional programming on fish larvae metabolic capacity and muscle growth via possible epigenetic mechanim.
In the present study, we hypothesized that a 40% substitution of native proteins by a commercial fish protein hydrolysate (HYDROL diet) delivered from mouth opening onwards would positively influence Senegalese sole larvae capacity to utilize and deposit protein throughout metamorphosis with possible consequences on muscle development and long-term growth. The diet based on native proteins (INTACT) was shown to promote a higher absorption of smaller peptides at the metamorphosis climax (17DAH) and higher expression of the gene encoding for Pepsin (pepsinogen A) after metamorphosis (28DAH), compared to the HYDROL diet. The INTACT diet has also promoted a higher proliferative capacity of myogenic cells at both stages, as it increased the percentage of small fibers (□<5μm). These results suggest that the inclusion of highly hydrolysed protein in the diet does not promote protein utilization or growth at later stages of sole development. However, at 36DAH the HYDROL diet upregulated the transcript levels of Myogenin (myog), which encodes a highly conserved myogenic regulatory factor that is involved in terminal muscle differentiation. This could possibly indicate some attempt of compensatory growth. The group fed the INTACT diet has also lower dnmt3a and dnmt3b mRNA levels compared to the HYDROL group. As these genes encode for DNA methyltransferases essential for de novo methylation, an epigenetic effect at the transcriptional regulation level is suggested as a possible explanation for the higher growth in the INTACT group. Target genes for this epigenetic effect still remain to be identified.

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

Oral Presentation

Chavatte-Palmer, Pascale

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

Chavatte-Palmer P

The role of the placenta in mediating maternal environment: lessons from bovine clones

Maternal and even paternal environment play a role in the development of offspring phenotype through direct effects on gametes and embryos. After implantation, in mammalian species, the placenta, which is of fetal origin but interacts intimately with the maternal endometrium, acts as a mediator between the mother and the fetus, mostly buffering the effects of maternal environment on the fetus. The placenta is involved in materno-fetal exchanges, metabolism, endocrinology and immune pathways and is an active component for fetal growth. Any disruption of placental development may alter its structure and function, notably nutrient supply from the mother to the fetus, affect directly fetal development and induce long term effects in the offspring. The study of placental development and function can therefore provide insight into environmental conditions that really affected the fetus as well as be used as a predictor of putative long term predisposition to disease. Since the first success in cloning sheep, the production of viable animals, with identical nuclear genome, by somatic cell nuclear transfer (SCNT) has developed significantly. Cattle are by far the most successfully cloned species but, despite this, the technique is still associated with a high incidence of pregnancy failure and accompanying placental and fetal pathologies. Pre- and early post-implantation losses can affect up to 70% of the pregnancies. In the surviving pregnancies, placentomegaly and fetal overgrowth are commonly observed, but the incidence varies widely, depending on the genotype of the nuclear donor cell and differences in SCNT procedures. In all cases, the placenta is central to the onset of the pathologies. Although cellular organisation of the SCNT placenta appears normal, placental vascularisation is modified and fetal-to-maternal tissue ratios are slightly increased in the SCNT placentomes. In terms of functionality, steroidogenesis is perturbed and abnormal estrogen production and metabolism probably play an important part in the increased gestation length and lack of preparation for parturition observed in SCNT recipients. Maternal plasma concentrations of pregnancy-associated glycoproteins are increased, mostly due to a reduction in turnover rate rather than increased placental production. Placental glucose transport and fructose synthesis appear to be modified and hyperfructosemia has been observed in neonatal SCNT calves. Gene expression analyses of the bovine SCNT placenta show that multiple pathways and functions are affected. Abnormal epigenetic re-programming appears to be a key component of the observed pathologies, as shown by studies on the expression of imprinted genes in SCNT placenta. The normal phenotype of adult bovine clones is most probably associated with adequate re-programming in the embryo, leading to physiological placental development.

Poster Presentation

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Expression of dopamine receptor transcripts in mouse preimplantation embryos and embryonic stem cells

Catecholamines can play an important role in basic developmental processes regulating cell proliferation, differentiation and migration. Dopamine, noradrenaline and adrenaline were detected in the female reproductive tract, and experimental data indicate that these molecules can act even during the preimplantation period of development. Using RT-PCR with specific oligonucleotide primers distinguishing among all members of dopamine receptor subtypes, we examined expression of dopamine receptor transcripts in mouse ovulated oocytes, preimplantation embryos and embryonic stem cells. We detected all five subtypes of dopamine receptors in undifferentiated as well as spontaneously differentiated mouse embryonic stem cells. D3 dopamine receptor showed higher expression in undifferentiated than in spontaneously differentiated mouse embryonic stem cells. We found several profiles of expression during the preimplantation period. Transcript for D3 dopamine receptor was present at all examined developmental stages (ovulated oocytes, 4-cell embryos, 8- to 16-cell embryos, blastocysts), whereas D1 dopamine receptor transcript was detected only in blastocysts. We found a stage-specific expression in two other dopamine receptor subtypes. The expression profiles of dopamine receptor transcripts suggest different roles of specific receptor subtypes in growth and development of preimplantation embryos and embryonic stem cells. Mechanisms controlling expression of dopamine receptors in preimplantation embryos and embryonic stem cell are unknown, but experimental data indicate the possibility of epigenetic regulation.

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Poster Presentation

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Derivation of porcine iPS-like cells from fibroblast of a translocated azoospermic boar

Chromosomal rearrangements have a crucial impact on the proper proceedings of meiosis and can lead by several mechanisms to the production of unbalanced gametes or to the complete arrest of gametes production. To assess the impact of these rearrangement in the early development of pig germ cells, we proposed to generate a library of stem cells from an azoospermic boar carrying a reciprocal translocation t(Y:14), as a new tool for the development of an in vitro differentiation system from pluripotent stem cells to germ cells.

We report the reprogramming of translocated fibroblast by integrative or non-integrative viral overexpression of Oct4, Sox2, Klf4 and c-Myc. iPS cell lines were characterized for pluripotency, cell cycle and differentiation potential by conventional methods. Genomic stability was analyzed by G-banding karyotype, Comparative Genomic Hybridization and FISH.

The porcine iPS-like cell lines harbored characteristics of ground and naïve pluripotency when cultured in specific media. They expressed several pluripotency genes and harbored an ES-like cell cycle. Nevertheless, contrary to mouse and human iPS, they did not silence the integrated exogenes, leading to a poor differentiation potential. Moreover, cytogenetic analysis revealed a high genomic instability upon passaging which suggest the development of population with an increased selective advantage. We characterized the selected duplications and compared them to those previously described in other species. In contrast, non-integrative reprogrammation system gives us promising results regarding differentiation potential and genomic stability and will bring new insights into the molecular factors controlling and maintaining pluripotency in the pig species.

O-15

Oral Presentation

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De Ruijter-Villani M, Gibson C, Stout TA

Mother knows best: uterine asynchrony delays conceptus development and alters expression of imprinted genes in an equine model

Imprinted genes are expressed in a parent-of-origin manner as a result of epigenetic modifications that dictate paternal or maternal monoallelic expression. It has been proposed that gene imprinting evolved in response to the 'conflict' between parental genomes regarding the allocation of maternal resources, with paternally-expressed genes generally promoting, whereas maternally-imprinted genes suppress, fetal growth. This study examined the effect of asynchronous ET on equine conceptus development and expression of imprinted genes in conceptus membranes. Twenty embryos were transferred to recipient mares that either ovulated on the same day (synchronous; n=10), or 5 days after (asynchronous; n=10) the donor mare. The conceptuses were recovered 6 or 11 days after ET (n=5 per group). The size of the embryonic disk/embryo proper was measured and somites were counted. Bilaminar trophectoderm was used to investigate mRNA expression for a range of imprinted genes (H19, PHLDA2, IGF2R, PEG3, PEG10, SNRPN, IGF2, INSR and INS) by RT-qPCR.

Conceptuses recovered from asynchronous recipients had a smaller (0.5±0.2 versus 1.5±0.2 mm2; $p<0.001$) and less developed embryonic disc. Similarly, embryo length (2.5±0.4 vs 5.1±0.4 mm; $p<0.001$) and somite number (7.5 ± 2.6 versus 20.7 ± 1.9; $p<0.01$) were reduced in conceptuses from asynchronous recipients. mRNA expression for PEG3, PEG10, INSR, IGF2, H19 and PHLDA2 was up-regulated between days 14 and 19 of pregnancy; in addition, expression was higher in synchronous than asynchronous pregnancies at day 19 ($p<0.05$). IGF2R mRNA expression increased from day 14 to 19 in the synchronous pregnancies ($p<0.05$), but did not differ between treatments at day 19. SNRPN expression increased from day 14 to 19, and was unaffected by asynchrony. INS mRNA was not detectable. In conclusion, asynchronous ET delayed conceptus development and altered expression of imprinted genes at day 19. This may be either a consequence of or a contributor to the retarded conceptus development.

P-16

Poster Presentation

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Effects of early glucose stimuli during first-feeding stage on carbohydrate and amino acids metabolism in gilthead seabream juveniles (Sparus aurata)

Nutritional programming begins to arouse interest as a novel tool for fish nutrition research. Since most marine fish species show a low ability to use dietary carbohydrates, the possibility to alter specific metabolic pathways towards a better use of carbohydrates as energy yielding substrates is of great interest. A study relying on 14C-labelled compounds was undertaken to assess the long-term effects of glucose stimuli at first-feeding period (early conditioning) on the carbohydrate and amino acid metabolism of seabream juveniles, challenged with a high carbohydrate diet. Triplicate groups of larvae were submitted to three dietary conditions at the onset of exogenous feeding: CONTROL (CTRL) with a standard feeding condition without any glucose intake; ACUTE (ACU) with a single glucose stimulus delivered by enriched rotifers (3DAH, days after hatching); and RECURRENT (REC) with successive glucose stimuli delivered by enriched rotifers (3DAH), Artemia (20DAH) and inert diet (30DAH). Each stimulus lasted for six days; then all larvae and subsequently juveniles were subjected to a high protein regime. Later, juvenile fish (approx. 9 g) from all treatments were submitted to a dietary challenge with a high carbohydrate diet during 39 days. The metabolic fate of dietary carbohydrates and amino acids in the three treatments was assessed by a force-feeding method, using 14C-Starch and 14C-AA mixture tracers, respectively. Previous results using Artemia labelling technique have revealed that post-larvae (60DAH) from REC group had higher retention in tissues as well lower catabolism of 14C-Glucose in comparison to the CTRL group. Therefore, strong evidences suggest that an intermittent exposure to glucose stimuli can induce the programming of specific pathways related to carbohydrate metabolism. Our data on seabream juveniles can contribute to increase knowledge on the metabolic programming of carbohydrates, and also elucidate if a protein sparing effect can be related to the early glucose conditioning.

O-17

Oral Presentation

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Possible correlation between maternal body condition, embryo quality, somatic development and behaviour of offspring in mice

Our previous studies have shown that fertilized female mice with slightly elevated amount of body fat (8-11%), produced in an experimental model based on overfeeding of outbred mice during intrauterine and early postnatal development, showed increased number of spontaneously ovulated oocytes and elevated fertilization index. On the other hand, mice with highly elevated amount of body fat (>11%) showed increased number of isolated immature oocytes and degenerates, lowered deposits of neutral lipids in the cytoplasm of mature oocytes, lower reduction of DNA cytosine methylation signal in parental pronuclei of zygotes and increased apoptotic index in blastocysts. Females with low amount of body fat (<7%) similarly displayed lower fertilization index, lower percentage of zygotes at pronuclear stage 4 with demethylated DNA cytosine in parental pronuclei and increased apoptotic index in blastocysts. To assess possible effect of observed changes on offspring development, somatic phenotype and behavior were evaluated in delivered mice in present study. Our results showed that highly elevated amount of body fat in mothers was accompanied with lowered weight of newborns and 5 week old offspring, and with several deviations from normal behavior in them: higher locomotion activity, lowered anxiety (represented by higher frequency of entries to central zone) and performance of slightly more comfort behavior (represented by higher resting time). Lower weight of newborns and deviations from normal behavior were recorded in progeny of lean dams as well: depressive-like behavior (represented by higher immobility time during forced swimming test) and performance of less comfort behavior (represented by lowered cleaning duration and frequency). Our results show that peri-conceptional status of maternal body condition might adversely affect not only the quality of oocytes and embryos, but might be correlated with significant changes during postnatal development of offspring as well.

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P-18

Poster Presentation

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The potential for de novo methylation in porcine endometrium harvested on days 10-11, 12-13 and 15-16 of pregnancy and the estrous cycle – the effect of reproductive status and intrauterine steroids level

Environmental and intrauterine factors affecting maternal organism at the earliest stages of embryos development have a direct impact on success of pregnancy. The mechanisms of gene expression regulation in the uterus, based on hypermethylation-induced attenuation of gene activity, can be important for modulation of endometrial activity during the most important periods of early pregnancy. We assumed that physiological status of female, as well as intrauterine concentration of steroid hormones, contribute to changes in expression of genes encoding methyltransferases in the endometrium. We focused on the potential for de novo DNA methylation in endometrium, defined as changes in the expression of gene encoding methyltransferase 3 (DNMT3), during days 10 to 11, 12 to 13 and 15 to 16 of pregnancy. Expression of DNMT3 in pregnant females was compared with that expression in the tissue harvested from pigs on respective days of the estrous cycle. We found that endometrial DNMT3 expression did not changed during selected days of pregnancy. In gravid pigs on days 15 to 16 it was statistically higher than in non-gravid females ($P \leq 0.05$). Observed differences in DNMT3 expression between gravid and non-gravid endometrium on days 15 to 16 coincide with decreased intrauterine concentrations of progesterone (P4) and estradiol 17β (E2). In cyclic pigs, the expression of DNMT3 decreased from day 10 to day 16, similarly as the intrauterine level of E2. Thus, between days 10 and 13 of pregnancy the potential for de novo methylation in the endometrium does not depend on the presence of embryos in the uterus. On days 15 to 16 of pregnancy and the estrous cycle, expression of DNMT3 depends on female reproductive status and intrauterine levels of P4 and E2. Ability for de novo methylation may be the most important for determination of endometrial genes activity mainly on days 15 to 16 of pregnancy and the estrous cycle.

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O-19

Oral Presentation

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Epigenetic control of LINE-1 retrotransposons

More than 40% of the human genome is composed of repeated DNA, and some types can be mobilized within the genome (e.g., Transposable Elements (TE)). Long Interspersed Element-1 (LINE-1 or L1) is the only autonomous retrotransposon (a class of TE) in the human genome, with more than 600,000 copies per genome (~17% of the genome). Furthermore, L1 is able to mobilize other non-autonomous TEs (Alu, SVA, U6) and cellular mRNAs (giving rise to processed pseudogenes). Overall, L1 is responsible for a third of the human genome.

Although most L1 elements in the genome are inactive, each human genome contains ~100 active L1 retrotransposons. Its mobility has resulted in a variety of human diseases, and its activity has shaped the human genome during evolution. L1 insertions within genes can alter their function, can modulate their expression at both splicing and poly-adenylation steps, and can provide regulatory sequences to genes. In addition, L1 insertions can be accompanied by genomic instability processes (deletions, translocations, etc), adding to the myriad of ways they shape our genome. Despite their abundance, little is known about human cell types that can accommodate the mobility of such repeated DNA sequences or its regulation.

P-20

Poster Presentation

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Goncalves A, McEvoy K, Kobolak J, Roberts SA, Dinnyes A, Brison D

Analysis of time-lapse parameters as predictors of pregnancy outcome in human embryos

In Assisted Reproductive Treatments (ART) the usual method used to select the best embryos to transfer is still based on morphological grading. To improve take-home baby rate more than one embryo is often transferred. However, multiple transfers often result in multiple gestation pregnancy and subsequent neonatal complications. Therefore there is a need to improve the technique to identify the embryos with the highest development potential resulting in positive outcomes and consequently in live births. Although still based on morphology, time-lapse imaging technology offers a non-invasive tool and provides a wider range of morphological parameters over time that can be used to select embryos without interfering with embryonic development. In the present study, embryo kinetic analysis of 1512 human embryos from 233 patients following In Vitro Fertilization (IVF) or Intracytoplasmic Sperm Injection (ICSI) using the time-lapse images from EmbryoScope was performed. The morphokinetic parameters that were significantly correlated with pregnancy outcome were the second cell cycle (CC2), the second synchrony (S2), the time of 5-cell stage (t5) and the third cell cycle (CC3). These parameters showed no significant correlation with the traditional morphological grades. Finally, a putative algorithm for embryo selection combining these measures was developed. Although time-lapse technology has improved over the years, large multicenter studies are still needed to validate individual predictors and develop and validate robust algorithms that can determine optimal embryo selection.

O-21

Oral Presentation

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Hatemi P

Expression of epigenetic effects in identical twins

Following Fisher's mathematical theory that reconciled Mendel's work with Galton's and Pearson's correlations, for the past century, relying on twins, researchers have been able to estimate the relative extent to which certain traits are inherited and "learned". With their near identical chromosomal sequence, monozygotic twins are also the best means to explore epigenetic differences in humans that accumulate from differences in environmental effects, and offer researchers the ability to explore epigenetic variation as a dynamic quantitative trait. Here I review the import of twins in epigenetic research focusing on differences between twins that accumulate over the life-span, including traumatic life events, and propose large-scale epigenetic studies of twins can provide a more comprehensive understanding of not just which specific genetic pathways affects complex traits, but what environments alter the expression of those pathways and molecular networks and possibility our best means for clinical intervention.

O-22

Oral Presentation

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Asymmetric H3K9me3 pattern allows to differentiate between maternal and paternal pronuclei in equine zygotes

The traditional method to distinguish between maternal and paternal pronuclei was based on their relative size and position with respect to the polar body. Recently, the asymmetric pattern of the histone 3 lysine 9 trimethylation (H3K9me3) in pronuclei of several species was discovered. In this species, H3K9me3 is present in the maternal while absent in the paternal pronucleus, constituting reliable method to identify the parent of origin of the pronuclei. But although this asymmetry is present in mouse, human and rabbit, it is only present in the initial pronuclear stages of bovine and pig zygotes. The aim of this study was to determine if H3K9me3 presents an asymmetric pattern in equine zygotes and can be used to differentiate between maternal and paternal pronuclei. Equine oocytes were matured for 28 h in a DMEM/F12 - based medium. Half of the matured oocytes were fertilized by means of piezo drill-assisted ICSI, and the other half was parthenogenetically activated by exposition to 5µM Ionomycin for 5min followed by incubation in 2mM 6DAMP. Resulting zygotes of both groups were cultured in DMEM/F12 with 10% FCS for 22-24h, fixed with 4% paraformaldehyde and permeabilized. After permeabilization, zygotes were blocked in PBS containing 2% BSA, incubated with the primary antibody (rabbit anti-H3K9me3) and subsequently incubated with the secondary antibody (goat anti-rabbit FITC). DNA was counterstained with EthD-2. In the parthenogenetically activated zygotes, H3K9me3 was present in both pronuclei, as expected since both are of maternal origin. In zygotes produced after ICSI, H3K9me3 was strongly present in the maternal pronucleus while it was absent or practically absent in the paternal pronucleus .
In conclusion, the H3K9me3 epigenetic modification presents a parent of origin asymmetric pattern in equine zygotes, being present in the maternal but absent in the paternal pronucleus.

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P-23

Poster Presentation

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Effect of L-carnitine on intracellular lipid status of porcine oocytes with different meiotic competence during their in vitro maturation

Meiotic and developmental competences of porcine oocytes closely correlate with the size of follicles from which the oocytes have been recovered. Recently, it was described that beta-oxidation of lipids is essential for expression of oocyte developmental competence. L- carnitine (LC) has a pivotal role in mitochondrial oxidation of long-chain fatty acids which increase energy supply to oocytes. The aim of this study was to characterize the effect of L- carnitine on intracellular lipid status of porcine oocytes with different meiotic competence during their maturation. Cyclic sows, examined for ovarian status, were used as oocyte donors. To optimize the LC concentration and pH of medium, in the first experiment, the oocytes without regard to their meiotic competence matured with 0, 5, 10, 15 and 20 mM of LC. In the second experiment, the oocytes matured without or with 10 mM LC at pH 7.47 or pH 7.12 in the same way. In the third experiment, meiotically more competent (MMC) and less competent (MLC) oocytes were isolated from medium (6-9 mm) and small follicles (<5 mm) and matured with 10 mM LC at pH 7.12 for 44 hours, using a standard protocol. For visualization of chromatin and lipid droplets in oocytes, confocal microscopy was used. The proportion of mature oocytes did not differ significantly in oocytes matured without LC (85.0 %) in comparison with oocytes matured with 5, 10, and 15 mM LC (87.2, 91.6 and 78.6 %) except those matured with 20 mM LC (28.5 %). The proportion of mature oocytes was significantly higher in oocytes matured with 10 mM LC at pH 7.12 (89.1 %) than in those matured without LC (78.8 %) and oocytes matured with 10 mM LC at pH 7.47 (74.7 %). The proportion of lipids did not differ in MMC oocytes matured with LC compared with those matured without LC. However, it was significantly higher in MLC oocytes matured with LC (37.2 %) in comparison with those matured without LC (29.2 %). The mean number of lipid droplets was significantly higher in both MMC (521.6) and MLC (353.8) oocytes matured with LC than in MMC (292.2) and MLC (294.4) matured without LC. The ratio of small and large droplets was significantly higher in MMC matured with LC compared to all oocyte categories. In can be concluded that supplementation of medium with L-carnitine during maturation positively influenced the lipid supply in meiotically less competent porcine oocytes. In the cytoplasm of oocytes matured in L-carnitine presence, more lipid droplets are found independently of meiotic competence of oocytes, but in meiotically more competent oocytes, small lipid droplets prevail as an available energy source.

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O-24

Oral Presentation

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Effect of epigenetic modifiers treatment on cloned mouse embryo morphokinetics

Embryo morphokinetics is a powerful tool to predict developmental success. The application of time-lapse techonology to cloned embryos, with low developmental potential, may help to increase birth rates and derivation of embryonic stem cells (ESC). On the other hand, cloned embryos are often treated with epigenetic modifiers to improve cloning efficiency and the effect of these treatments on embryo morphokinetics is unknown.
In the present study, time-lapse development of fertilized embryos (n=68) and somatic cell cloned embryos non-treated (n=83) or treated with the epigenetic modifiers psammaplin A (PsA; n=75) or vitamin C (VitC; n=93) was assessed, and the ability of the resulting blastocysts to give rise to ESC lines was determined. Blastocyst rates were similar among cloned PsA-treated (58.3%), VitC-treated (60.9%) and ICSI embryos (70.6%), and lower for cloned non-treated embryos (45.8%). Rates of ESC derivation were higher in the two groups of treated cloned embryos (31% and 40%) than in non-treated clones (15.2%) or ICSI embryos (17.9%). Time-lapse analysis showed that all cloned embryos had a delayed development from 2nd division compared to ICSI ones, and that PsA treatment increased this delay whereas ViC-treated embryos were the fastest. Slow-cleaving embryos had inferior developmental potential in all groups. Several parameters were significantly correlated with developmental success, such as cleavage times, number of cells at compaction or presence of fragmentation. The time of completion of the 3rd division alone, or combined with the duration of compaction and/or the presence of fragmentation, had a strong predictive value for blastocyst formation in all groups of embryos analyzed, with an accuracy of 65% (VitC) to 91% (ICSI). In contrast, we failed to predict ESC establishment from embryo morphokinetics.

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Poster Presentation

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Effects of different cell lines on the SCNT blastocyst yield and pregnancy rates in bovine

Somatic cell nuclear transfer (SCNT) is currently the most used technique to produce transgenic cattle. The aim of this study was to determine the influence of different cell lines on the blastocyst development and pregnancy rates using SCNT.

The primary fibroblast cell lines were made from the skin of the slaughterhouse derived 2-3 months old female fetuses. Two cell lines which gave the best blastocysts rates were transfected with the vectors containing human insulin (INS), growth hormone (GH) or erythropoietin (EPO) cDNA sequences under β-casein promoter, using Lonza Amaxa Basic Nucleofector Kit for Primary Mammalian Fibroblast. The selected cell lines were used for SCNT. The embryos were cultured till blastocyst stage and transferred to virgin heifers. From the cell line L2-GH, a GH-transgenic calf June was born whose fibroblasts were also used for SCNT.

Altogether 12 different cell lines were created and 409 cloned blastocysts produced in 102 experiments. The SCNT blastocyst rate varied from 8 to 19.9% between the cell lines. The highest rate was achieved with L3 (19.9%), however it gave no pregnancies. The cell line from transgenic calf June yielded 8.6% blastocysts. As blastocysts derived from non-transgenic L2 were able to initiate and maintain pregnancy, L2 was used to make EPO, INS and GH transgenic cell lines. Due to the limited number of embryo transfers, no significant differences between the pregnancy rates between the transgenic cell lines were detected. The non-transgenic cell lines gave more pregnancies than transgenic, indicating that cell line modifications alter SCNT embryo survival.

In conclusion, our data suggest that different cell lines influenced the blastocyst yield and the in vivo development potential of bovine SCNT embryo

This study was supported by Project EU29023 of Enterprise Estonia, Project 3.2.0701.12-0036 of SA Archimedes, CCRMB and grant P8001 from the Estonian University of Life Sciences.

P-26

Poster Presentation

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The influence of maternal stress on preimplantation development – modulation by maternal metabolic status

Maternal restraint stress and maternal obesity are negative factors which can have significant effect on preimplantation embryo development and reproduction. In our study, we compared the effect of maternal restraint stress on preimplantation embryos developed in mothers with different amount of body fat. We used the mouse model of diet-induced obesity, and divided female mice of the F1 generation into four groups, according to the percentage of body fat. Spontaneously ovulating females of all four groups were mated with males and half of them were exposed to restraint stress for 30 minutes three times a day during the four-day preimplantation period. Microscopic evaluation of collected blastocysts showed that the amount of maternal body fat did not affect significantly the number of cells in blastocyst. The percentage of blastocysts containing no dead cell was higher in stressed females with elevated body fat than in stressed females with normal body fat amount. Interestingly, the proportion of dead cells (in blastocysts containing at least one dead cell) was also higher in stressed females with elevated body fat than in stressed females with normal body fat amount. The analysis of apoptosis occurrence in blastocysts showed almost identical results, indicating that cells died almost exclusively by the apoptotic way. These results suggest potentially protective effect of increased maternal body fat amount to the action of stress on preimplantation embryos. The underlying mechanisms are unknown, and we suppose that an epigenetic regulation reflecting the maternal environment acting on the oocyte and early embryo can be involved.

This work was supported by the national grants under contracts APVV–0815–11 and VEGA 2/0029/12

O-27

Oral Presentation

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Khatib H

Genetics and epigenetics of early embryonic development and fertility in cattle

In recent years it has become evident that genetic selection to improve milk production has resulted in a decline in dairy cattle fertility. The greatest degree of embryonic loss in cattle occurs before day 16 with even greater loss before day 8 in the high producing dairy cow. With high demand for agricultural products, assisted reproductive technologies have been used to better cope with the economic pressure and the decreasing fertility. As genetics underlies biological processes such as fertility, there is a need for further identification and characterization of genesand pathways that affect fertility and development within both the parental gametes and embryo. Indeed, several strategies have been employed in investigating the genetic components of fertility and subsequent embryonic development. Potential predictors can be derived from the study of the genotypic, transcriptomic and epigenetic profile of both the parental gametes and developing embryo. The presentation will provide an overview of studies relating to genetic markers at the DNA level, parental and embryonic gene expression, and the effects of epigenetics on embryonic development. Specifically, imprinted genes and microRNAs will be discussed as potential biomarkers for embryonic development and fertility success.

P-28

Poster Presentation

Khurana, Pooja

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Derivation and use of mouse embryonic stem cell lines for mechanistic analysis of periconceptional developmental programming

The Developmental Origins of Health and Disease concept proposes that maternal environment during pregnancy influences offspring health and predisposes chronic disease risk in later life. We have shown using mouse models that maternal low protein diet (LPD) in vivo and embryo culture in vitro (as in assisted reproductive treatment, ART) programme the preimplantation embryo to adult disease, notably cardiometabolic dysfunction. To understand the mechanistic basis of periconceptional programming, we derived mESC lines from blastocysts of LPD and control normal protein diet (NPD) fed mothers. Derivation efficiency of LPD ESC lines was reduced (~20% blastocyst/mother) compared to NPD ESC lines (49%; $P<0.0155$), reflecting a reduced ratio of Nanog:Oct4 expressing cells in LPD outgrowths as ESC lines first form ($P<0.05$). Cell proliferation, % S-phase cells and cell cycle phase durations in synchronised cell populations was unchanged between treatments. However, LPD ESC clones had increased dead and apoptotic cells($P<0.05$). Cell signalling activity was similar between treatments for Akt, Stat3 and p38 pathways but ERK 1/2 was reduced in activity in LPD lines($P<0.05$), suggesting reduced MAPK survival signalling may contribute to LPD ESC enhanced apoptosis. In a second ES model, to assess the effect of later conception as in ART, we have derived ESC clones from blastocysts from aged (8 month) and young (8 week) mothers. These new clones show similar male:female ratio and % normal karyotype across treatments and are being characterised for proliferation, signalling and gene expression profiles. Our study shows firstly the suitability of mESC models for analysis of periconceptional developmental programming mechanisms, thereby reducing the numbers of animals used in research. Second, we have shown using the mESC LPD model that altered expression of pluripotency genes, increased apoptosis and deficient ERK 1/2 survival signalling may contribute to the adverse programming induced in embryonic lineages, culminating in increased adult disease risk.

Oral Presentation

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Kitewska A, Kaczmarek MM, Moza-Jalali B, Wasielak M, Bogacki M

Transcriptome analysis of the porcine endometrium on day 6 after seminal plasma infusion

The application of artificial insemination shows that pregnancy can be maintained even when highly diluted seminal fluid is deposited into the uterus/cervix. However, reduction of early fetal loss was found to be achieved by exposure of uterine tissues to biologically active constituents of seminal plasma (SP). In the present study we hypothesized that effects of SP are reflected in transcriptome changes in the porcine endometrium on day 6 after infusion. Differentially expressed genes (DEGs) were analyzed in synchronized gilts (750 IU of PMSG and 500 IU of hCG 72 h later) randomly assigned into two groups (n=5 per group): SP- and PBS-infused (control). Transcriptome comparative analysis between SP- and PBS-infused animals was performed with Pig Microarrays 4x44k, v. 2 and Gene Spring v. 12.1 software (moderated T test; FDR $p < 0.05$; FC ≥ +/-1.5). DAVID database was used for the biological process and pathways analysis. Validation of microarrays was performed using qRT-PCR for CCR3, CXCL11, TGFA, IL18, SPAG1, S100A8 and S100A12 genes. Comparative analysis identified 246 DEGs, 114 down- and 132 up-regulated, classified by biological processes as participating in: ion transport, cell adhesion, response to wounding, inflammatory response, steroid metabolic processes ad reproductive developmental processes. Additionally, we found arginine and proline metabolism, gap junction, cell adhesion molecules, Wnt-signaling and hedgehog signaling pathways to be altered in KEGG Database. qRT-PCR analysis revealed that TGFA was up-regulated and CCR3, CXCL11, IL18, SPAG1, S100A8 and S100A12 were down-regulated in SP-exposed endometrium.

Summarizing, our results confirm that endometrial response to seminal plasma constituents persist until day 6 after infusion at the transcriptome level. Identified DEGs appear to be involved in processes and pathways recognized as an important for the establishment and maintenance of pregnancy in pigs.

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P-30

Poster Presentation

Kubandova, Janka

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Kubandova J, Stroebech L, Cikos S, Fabian D, Burkus J, Janstova Z, Koppel J, Hyttel P

Identification of glucocorticoid receptor protein in bovine preimplantation embryos

Maternal organism is frequently exposed to negative influences of the surrounding environment, which can induce stress responses characterized by increased levels of several hormones, including glucocorticoids. The information about expression of glucocorticoid receptor (GR) protein in bovine preimplantation embryos is needed because of only insufficient information on GR expression is available. Authors of previous studies did not examine whether the mRNA is translated into functional protein. The aim of the study was to find whether GR protein is produced in bovine oocytes and preimplantation embryos. For the experiment, in vitro maturated bovine oocytes and in vitro derived preimplantation embryos were used. Immunostaining using antibody against selected receptor was performed in bovine oocytes and preimplantation embryos at various stages of development. Embryos were evaluated using a confocal microscope. The immunohistochemical (IHC) study proved the presence of the GR protein in bovine preimplantation embryos. In embryos at cleavage stage, we observed IHC signal of high density and high intensity located predominantly in nuclear area. In these embryos, signal in cytoplasm had significantly lower density and intensity. In zygotes and blastocysts we detected signal of equal intensity within nuclear area and the cytoplasm; however, in the cytoplasm signal displayed lower density. In nucleoli and in condensed chromatin (nuclei undergoing mitosis or apoptosis), there was no IHC signal. In conclusion, we confirmed the presence of GR protein in in vitro derived bovine zygotes and preimplantation embryos.

Work was supported by national grants APVV-0815-11 and VEGA 2/0029/12.

P-31

Poster Presentation

Lazzari, Giovanna

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Colleoni S, Galli C, Lazzari G

Human embryonic stem cells for toxicological testing: protocols for the detection of teratogenic effects on neural precursors and neurotoxic effects on mature neurons

The recent European legislation REACH (Regulation, Evaluation Authorisation and Restriction of Chemicals) has encouraged the development of in vitro toxicological tests based on human cell lines as alternative to conventional animal testing for the screening of chemicals and drugs. In this context several human embryonic stem cells (hESCs) lines have been intensively used to develop protocols suitable for the screening of teratogenic effects, but also of tissue specific toxic effects, given their ability to differentiate in virtually all cell types present in the human organism. In particular, since hESCs efficiently undergo neuronal differentiation and many protocols are available for the derivation of neural precursors and regionalised neuronal derivatives, the area of neurotoxicity has been intensively explored by several research teams. In our laboratory we have developed two protocols to investigate chemical and drug toxicity on early neural precursors and on mature differentiated neurons. The first protocol takes advantage of the propensity of hESCs to undergo neural differentiation organising in radial shape as neural rosettes. These peculiar structures display several biological features of the developing neural tube in vivo. For this reason they are suitable to detect teratogenic effects. We will present gene expression and morphological data to show that toxic effects of drugs such as retinoic acid and valproic acid can be reproduced during the neural differentiation of hESCs. Extending the protocol of neural differentiation for a few weeks it is possible to design toxicological tests capable of revealing adverse effects on mature neurons. Data on the toxicity of the known neurotoxicant rotenone will be shown highlighting the remarkable correspondence with reported in vivo effects following human exposure.

This work was supported by European FP7 projects ESNATS, n°201619 and SCR&TOX, n°266753.

O-32

Oral Presentation

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Lillycrop KA

Nutritional programming in health and disease

There is now substantial evidence from both human epidemiological studies and animal models that an adverse intrauterine environment induced by a variety of environmental and maternal factors such as diet, body composition or endocrine factors can induce a phenotype in the offspring that is characterized by an increased risk of developing chronic non-communicable diseases in later life. The mechanism by which cues about nutrient availability in the postnatal environment are transmitted to the fetus and the process by which different, stable phenotypes are induced are beginning to be understood and involve the epigenetic regulation of specific genes. Epigenetic processes induce heritable change in gene expression without altering gene sequence. The major epigenetic mechanisms include DNA methylation, histone modification and non coding RNAs.

There is now growing evidence that a range of environmental factors including diet can alter the epigenome. The epigenetic changes induced in response to nutritional cues from the mother may allow the fetus to adjust its developmental programme in order to be better adapted to the future environment, while inappropriate adaptations may predispose an individual to increased risk of a range of non-communicable diseases.

This talk will describe how changes in macronutrient and micronutrient intake can induce persistent changes in DNA methylation and phenotype. Moreover detection of such altered epigenetic marks may also allow the identification of individuals at increased risk of disease. Further understanding of how the environment can induce such epigenetic changes and what makes one CpG stable another susceptible to environmental influences will allow the development of new preventative and intervention strategies to combat the rapid rise in non-communicable diseases seen worldwide.

Oral Presentation

Long, Charles
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Long C, Westhusin M, Golding M

Are cloned animals in fact identical?

Successful development of somatic cell nuclear transfer (cloning) transformed the fields of developmental biology, cellular reprogramming and regenerative medicine. The practical application and adaptation of these cloning technologies has allowed the production of viable offspring in over 20 different species, proving the technology is robust, albeit very inefficient and prone to developmental anomalies. However, the offspring from cloned animals demonstrate none of the abnormalities of their parents, suggesting the low efficiency and high developmental mortality are epigenetic in origin. The epigenetic barriers to reprogramming somatic cells into a totipotent embryo, capable of developing into a viable conceptus and subsequently a live offspring, are significant and varied. Despite their intimate relationship, chromatin structure and transcription are often not uniformly reprogramed after nuclear transfer and many cloned embryos develop gene expression profiles that are hybrids between the donor cell and an embryonic blastomere. Failure to completely reset the somatic chromatin to an embryonic epigenotype, and with it the appropriate transcriptional state, can result in a range of outcomes ranging from embryonic and fetal mortality, placental and fetal abnormalities or undetectable anomalies that approximates “normal”.
This overview will summarize the data and published reports relevant to the transformation of a somatic cell into an embryo capable of full term development. It is clear that resetting somatic transcription and associated epigenetic marks are absolute requirements for proper development of SCNT embryos; however, life does not demand the SCNT embryo get it perfect.

Poster Presentation

Lopera-Vasquez, Ricaurte
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Lopera R, Hamdi M, Maillo V, Lloreda V, Nunez C, Gutierrez-Adan A, Bermejo-Alvarez P, Yanez-Mo M, Ramirez MA, Rizos D

Effect of extracellular vesicles of bovine oviductal fluid on in vitro embryo development and quality

Intercellular exchange of proteins, lipids and mRNA through extracellular vesicles (EV) represents a new paradigm in oviductal environment where fertilization and early embryonic development takes place. The aim of the present study was to evaluate the developmental capacity of bovine zygotes and the quality of the produced embryos when cultured in vitro with previously purified EVs recovered of Ampulla and Isthmus under different centrifugal forces from oviducts of slaughtered heifers in early luteal phase. EVs were quantified with the use of Nanosight and their integrity and size assessed by electron microscopy. In vitro produced zygotes were cultured in SOF+3mg/ml BSA (C-), C- with 3x105 EV/ml from the Ampulla (A) and the Isthmus (I) previously purified at 1x103 (10) and at 1x105 xg (100), and compared with SOF+5% FCS (C+). Cleavage rate was assessed on day 2 and blastocyst development on day 7, 8, and 9 (D0: day of fertilization). Representative number of blastocysts on days 7/8 was used for quality evaluation through survival rates after vitrification/warming. No differences were found between groups for cleavage rate or blastocyst yield on Day 7-9 (range: 88.0±1.1 - 89.6±1.3 and 29.6±1.9 - 30.8±4.5 for cleavage rate and blastosyst yield on day 9 respectively). After vitrification/warming, significantly more embryos survived at 24 hours for both Isthmus groups (I10-100) (range: 83.6±4.7 - 91.3±3.8) than C+ (48.3±9.1). At 72 hours, the difference was even higher for A100 and both I groups (range: 66.8±7.6 - 80.1±3.9) when compared with C+ (34.5±8.0). Moreover, during all post warming period (24 to 72 h), I100 embryos (range: 80.1±3.9 - 91.3±3.8) survived significantly higher than C- (range: 50.5±8.1 – 71.0±6.6). In conclusion, in vitro culture with extracellular vesicles of oviductal fluid, especially from isthmus area, has a positive effect on the development and quality of bovine embryos.

P-35

Poster Presentation

Lopez-Cardona, Angela

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Altered mouse sperm histone methylation affect offspring development and health

It has been suggested that epigenetic information passed on by sperm allows for the transmission of information, however there is a lack of mechanistic evidence to support epigenomic transmission via the sperm. Although no functional role has been assigned to histones retained in sperm, recently H3K4 and H3K9 methylation in human and mouse sperm were localized to the promoters of genes critical for development indicating that paternal epigenetic contribution in sperm may serve as a route to altered offspring phenotypes. The aim of this work was to modify the sperm epigenome by spermatocyte/spermatid specific over-expression of the lysine-specific histone demethylase 1 (LSD1), and to characterize the consequences of altered sperm epigenome for offspring development and health. LSD1 act demetylating on mono- and di-methylated H3K4. The expression of the transgene was restricted to spermatocytes by using a 908 nt of the Heat shock protein 2 gene promoter (Hspa2) to control the expression of LSD1). We produced six transgenic lines and the phenotype was analyzed in two of them by scrotal heat stress of the transgenic males. The results indicated that only after scrotal heat stress, the spermatocyte expresses LSD1 and the sperm has more hypo-methylated histone H3 at lysine 4 and 9. Examination of 150+ pups sired by transgenics in comparison to 200+ pups sired by non-transgenics showed that 35% of offspring sired by scrotal heat stress transgenics are abnormal, and that 25% die before postnatal day 21. Analysis of fetus and animals after birth revealed severe abnormalities included severe morphological defects like eye defects, neuromuscular defects, dropping forelimbs and abnormal body curvature, absence of milk in the stomach, lack of movement coordination, neonatal loss of weight, etc. Paternal sperm epigenome, specifically the effect of LSD1 oxidase in spermatids has significant effects for offspring development and health.

O-36

Oral Presentation

Moles, Anna

Limited, Genomnia, Italy

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Epigenetics : Techniques and Strategies for Next Generation Sequencing

Epigenetics is the study of reversible, heritable mechanisms that regulate gene expression without altering the DNA sequence . The development of epigenetics biomarkers is an exciting and rapidly evolving area of research that holds promise for potential applications in diverse clinical settings. DNA methylation is one of the most well studied epigenetic mechanisms in mammals. It refers to the addition of a methyl group to the fifth carbon of a cytosine that precedes a guanine (CpG). Frequently, but not exclusively, CpG dinucleotides occur in CG-rich DNA stretches known as CpG islands. Moreover, histone marks, such as acetylation and methylation of lysine residues of histone H3, play an important role in regulating transcription. Combinations of these marks create an “epigenetic code” that defines the transcriptional status of genes. There are still many challenges to the effective implementation of laboratory protocols and bioinformatics analysis of epigenetic signals. Emerging innovative strategies are focused on addressing these gaps in this field; In this presentation we will review the most relevant issues faced when tackling epigenetic analysis in our laboratory.

P-37

Poster Presentation

Nainiene, Rasa

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Fatty acid profiles in testes of bulls fed with rapeseed or linseed cake supplementation

Dietary fatty acids in particular the ratio of omega-6 (n-6) to omega-3 (n-3) polyunsaturated fatty acids play an important role in animal health, production and reproduction. The aim of the study was to examine the effect of diet with rapeseed or linseed cake on the fatty acids profiles in bull testes. A trial was carried out with 12-17 month-old Lithuanian Black and White bulls allocated in 2 groups. Bulls received the same amount of maize silage, green feed and compound feed, however the composition of the latter was different. Bulls of I group (RC) received compound feed with 17% rapeseed cake and the II group (LC) was provided with compound feed with 20% linseed cake. Trial lasted 178 days. Testes of bulls were obtained at the time of slaughter in the abattoir and freed from surrounding tissue. The extraction of lipids for fatty acid analysis was performed with chloroform/methanol. Fatty acid methyl esters were analyzed using gas liquid chromatography. Feeding diets containing RC versus LC have no significant effects on saturated (SFA) and monounsaturated (MUFA) fatty acid profile in testes of bulls. SFA represented 36.5%, while MUFA – 21% of the total fatty acids. The main SFA were palmitic (C16:0) and stearic acids (C18:0) that both together represented 97.6% and 96.4% of the total SFA in RC and LC groups, respectively. Feeding diets, containing LC versus RC significantly increased polyunsaturated fatty acids (PUFA) proportions in testes of bulls (35.8% vs. 34.4%): docosahexaenoic (DHA, C 22:6n-3), eicosopentaenoic (C20:5n-3), eicosadienoic (C20:2n-6), α-linolenic (18:3n-3) acids. The main PUFA in testes of bulls was arachidonic acid (C20:4n-6), which contributed from 35.5% to 37% of total PUFA in RC and LC groups respectively. The percentage of DHA was higher in testes of bulls, fed with linseed cake supplementation (10.14% vs. 8.61% $P<0.05$) and contributed from 25% to 28% of the total PUFA. The concentrations of n-3 fatty acids differed between groups with higher concentration in LC group (12.4% vs. 10.8%, $P<0.05$), whereas n-6 fatty acids values were similar.

Oral Presentation

Niemann, Heiner
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Niemann H

Epigenetic studies on bovine preimplantation embryos

Normal development critically depends on a precise sequence of changes in the configuration of the chromatin which are primarily related to the acetylation and methylation status of the genomic DNA. These epigenetic modifications control the precise tissue specific expression of genes and play an essential role in regulation of mammalian embryo and fetal development. We have investigated various epigenetic hallmarks, including DNA methylation, differentially methylated regions (DMR), global DNA methylation, and the role of gene-specific histone modifications in bovine oocytes and embryos. To better understand ART (assisted reproductive techniques) induced epigenetic alterations and the reprogramming which allows somatic cell nuclear transfer (SCNT), we have screened a panel of 25 developmentally important genes (1079 amplicons) to identify discriminative methylation patterns for embryos of different origin, i.e. in vitro production, SCNT or in vivo. The methylation status of imprinted loci PEG3, PEG10, PEG11, IGF2 and IGF2R revealed distinct patterns typical for the respective embryo groups, indicating sensitivity to external factors (Niemann 2010). The identification and analysis of imprinting control regions (ICRs) and DMRs in the bovine genome has been the focus of another study. We have analyzed three different imprinted genes (H19, SNRPN and PEG3), POU5F1 and two methyltransferases (DNMT3A and DNMT3Lo) with regard to DNA methylation status (El Hajj 2011; Heinzmann 2011) and found only little changes related to the in vitro maturation protocol. Furthermore, global histone modifications and methylation status of in vitro matured oocytes derived from two different follicle sizes were determined by immunohistochemistry. In contrast to the signal of the α-H4K12ac antibody signal which decreased after GVBD, the signals for α-H3K9me2 and methylation remained present during the complete maturation process until the Metaphase II stage (Racedo 2009). In a recent study, four different histone modifications, H3K4me3 and H3K9ac as transcriptionally permissive and H3K27me3 and H3K9me3 as transcriptionally repressive epigenetic marks were investigated via ChIP analysis in the inner cell mass (ICM) and the trophectoderm of bovine blastocysts (Herrmann 2013). Analysis of the promoters for POU5F1, NANOG, INFT, SLC2A3, IGF1 and the housekeeping gene GAPDH revealed that histone modifications are consistent with the gene expression profiles analyzed in parallel. Epigenetic research in the bovine preimplantation embryo model is promising to gain a better understanding of embryo development with significant benefit for ARTs and in vivo development failures.

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Poster Presentation

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The amino acid composition as developmental indicator in IVP culture media

The use of assisted reproductive technologies (ART) has been increasing steadily within the past decade. There is a great need for non-invasive embryo quality assessment methods for selecting the best embryos for the transfer. Several methods have been proposed to evaluate the developmental potential of IVF embryos whereas search for metabolic biomarkers has been quite promising during last years. In this study, we analysed the changes of essential and non-essential amino acids in embryo culture media. Single bovine embryos were cultured in 60µl SOF+0.4%BSA droplets under mineral oil. 20 µl of culture media was removed at day 2, 5 and 8 post-fertilization. The collected samples were divided into three groups: culture media droplets from blastocyst stage embryos, droplets from embryos which development was arrested in the morula stage and cultured droplets without a zygote served as the control. A total of 45 samples were analysed using liquid chromatography-mass spectrometry (Q-Trap 3200). Data were analysed with the statistical software R. Our results showed that there were significant differences in concentrations of some amino acids between the groups. The concentrations of leucine and isoleucine were significantly lower and the concentration of phenylalanine was significantly higher in culture media were the embryos developed into blastocysts. The concentration of threonine was lower in samples were embryo development was arrested in morula stage. In conclusion, we detected significant changes in some amino acid concentrations in culture media between the embryos with different developmental capacity. These findings when further validated could offer a possibility to predict the viability of embryos by analysing amino acids in the culture media.

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O-40

Oral Presentation

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Environmental heat stress induces epigenetic inheritance of robustness in Artemia model

Living organisms are constantly challenged by different environmental stressors to which they react with a battery of responses. Recent evidences have suggested that environmental factors influence the emergence and inheritance of phenotypic traits. This phenomenon of non-Mendelian trans-generational epigenetic inheritance (i.e. the inheritance of traits that are not determined by the DNA sequence), however, has largely been debated. In our study, by using parthenogenetic Artemia (originating from one single female) as a model organism, we provided clear evidences by means of a common garden experiment that, upon exposure to non-lethal heat shocks, the parental population of Artemia experiences an increase in the levels of Hsp70 production, resistance towards lethal heat stress, and pathogenic Vibrio campbellii. Interestingly, these acquired phenotypes were transmitted to three successive generations, none of which was exposed to the parental stressor. This transgenerational inheritance of the acquired traits was associated with altered levels of global DNA methylation and acetylated histones H3 and H4 in the heat shocked group compared to the control group, where both the parental and its successive generations were reared at standard temperature. Next, we extended the above experiment with a view to determine the impact of selection on epigenetic modifications. To this end, we conducted another experiment using a wild population of bisexual Artemia franciscana as model organism. These animals were given a lethal heat stress for once in their life, with the selection pressure of 1%. This process was continued for 12 generations. Similar to the results of the above experiment, thermally-selected Artemia exhibited increased resistance towards lethal heat stress and V. campbellii challenge, and this acquired phenotypes was associated with altered levels of H3 and H4 acetylation. These results suggest that environmental experiences can influence the development and heritability of phenotypes by means of both epigenetic and selection.

P-41

Poster Presentation

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Nuclear architecture of blastomeres in bovine preimplantation embryos – study of selected genes controlling cell differentiation

A set of pluripotency markers including Oct-4, Cdx-2 and Nanog genes has been described in the mouse embryos. Transcripts for OCT4, CDX2, NANOG genes have also been detected in bovine preattachment embryos. It was shown that gene expression is related to chromosome position within the nucleus. Chromosomes are nonrandomly positioned within the nuclear space of a somatic cell. The localization of chromosome territories (CT) depends on the chromosome size (chromosome size theory) or/and gene density on chromosome (chromosome gene density theory). According to the current state of knowledge, blastomere differentiation in preimplantation embryos is correlated with defined changes in the nuclear architecture. Moreover, in the bovine embryo changes in the position of chromosome territories (CT) have been related to transcriptional activity and to the embryonic genome activation at the 8-16 cells stage. The aim of this study was to describe the three-dimensional nuclear position of three chromosomes (5, 12, 23) harboring genes controlling cell differentiation (NANOG, CDX2, OCT4). Bovine embryos produced in vitro from zygote to blastocyst stage were subjected to 3D-FISH and analyzed using confocal microscope (Zeiss). Series of optical sections were collected with a z-step of 0.4 to 0.5 µm and the three-dimensional images were processed by the NEMO software. Up to now analysis of the CT23 and OCT4 gene has been accomplished whereas investigation of the remaining two chromosomes is in progress. Preliminary results for CT12 and CDX2 indicates peripheral localization in nuclei of early stages of embryo development (zygote and 2-4-bl stage). Similarly CT23 and OCT4 dominated in the peripheral localization in zygote and 2-4 bl stage however, their nuclear organization altered at further stages of embryo development. The changes first occurred at the 8-16-bl stage with CT23 approaching the nuclear center. At the 8-16-bl, morula and blastocyst stages CT23 were observed more often in intermediate and interior localization than in zygote and 2-4-bl stages. With regard to the position of OCT4 locus within the CT23, the surface or outside localization were significantly more often observed in the blastocysts than in the zygotes and the 2-4-bl embryos. We suggest that the changes in CT23 and OCT4 positions observed from the 8-16-bl stage onwards coincides with the onset of embryonic genome activation and consecutive changes in the gene expression patterns within the bovine embryos.

This study was financed by National Science Center Poland; grant UMO-2011/01/N/NZ9/00792. I am a scholarship holder within the project “Scholarship support for PH.D. students specializing in majors strategic for Wielkopolska’s development”, Sub-measure 8.2.2 Human Capital Operational Programme, co-financed by European Union under the European Social Fund.

Poster Presentation

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O'Shea LC, Hensey C, Fair T

Regulation of expression of the epigenetic regulator, ATRX, in mammalian and amphibian oocytes during maturation

In humans, mutation of the ATRX gene leads to improper methylation of repetitive DNA sequences. In this case failure to establish the necessary epigenetic modifications essential for inactive X chromosome results in genomic and chromosome instability and leads to X-linked α-thalassaemia with mental retardation (ATRX) syndrome. The aim of the present study was to determine the regulation of ATRX expression, in both Xenopus laevis oocytes and the bovine cumulus oocyte complex (COC). ATRX protein was found to be conserved during oogenesis and to be dramatically downregulated during oocyte maturation in both model systems. We have previously shown that inhibition of progesterone signalling during bovine in vitro oocyte maturation has a detremental affect on subsequent embryonic development. Here we show that this is characterized by an increase in ATRX expression and a parallel increase in the expression of a known marker of apoptosis (active Caspase-3) during oocyte maturation, in both oocytes and cumulus cells. Our findings suggest that ATRX expression may be linked to the apoptotic pathway; playing a role in progesterone regulation of oocyte quality. Mechanical fractioning of germinal vesicle (GV) stage Xenopus oocytes revealed ATRX to be nucler localized.Immunohistochemistry studies performed on bovine oocytes at different stages of meiotic maturation showed ATRX to be localized to the chromosomal area of GV oocytes, but undetectable in MII oocytes. Inhibition of progesterone signalling during maturation resulted in the stabilization of ATRX in bovine oocytes, with ATRX remaining localized to the chromosomal area of mature MII oocyte. In conclusion, ATRX protein expression and localization appears to be progesterone regulated and associated with oocyte quality. However, further work is needed to determine how the regulation of ATRX corresponds to specific epigenetic regulations and subsequent developmental competence.

Oral Presentation

Ozil, Jean-Pierre

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Ozil J-P, Banrezes B, Sainte-Beuve T, Vaiman A, Berteaux L, Barrey E

Culture media modulates the regime of Ca2+ signaling, miRNA expression at fertilization and the post-natal growth in the mice

Several reports have shown that incubation media used for in-vitro fertilization impacts adult phenotype. However, it is still difficult to identify the critical composition of the culture media that modulates the development. Here we develop a new method that makes it possible to establish functional linkage between the composition of the medium, the regime of Ca2+ signaling and mitochondrial activity during fertilization, the miRNA expression and the post-natal growth. Mouse eggs were fertilized by ICSI, incubated 4h in M16 or KSOM and transferred to recipients. With a specific algorithm, we used Ca2+ signaling following ICSI as a detector of changes of egg functioning. Pools of 30 eggs incubated in each condition were used to screen the miRNA expression 4h after ICSI. The results show that the females issued from egg cultured for 4 hours in KSOM are significantly smaller at the 8th week (-15%, $p<0,05$) than those cultured in M16. In KSOM, eggs displayed an average of 15 (+/- 5) Ca2+ impulses versus 9 (+/-2) in M16. Differential miRNA expression ($Cp<37$) were found between eggs incubated in M16 (26 miR) and KSOM (18 miR). Among the predicted gene targets by the more expressed miRNA we found: MAPK signaling, PI3K-Akt,metabolic pathway, calcium signaling, Jak-STAT and dorsoventral axis regulation. Therefore, since the M16 and KSOM formula are known, it becomes possible to establish a precise functional linkage between the parameters of the culture media, the modulation of egg metabolism, mRNA regulation and the post-natal consequences.

Poster Presentation

Pendzialek, Mareike

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Determination of pregnancy-associated microRNAs in maternal serum, placenta and rabbit preimplantation embryos

Maternal diabetes mellitus is linked to placental dysfunction with long-lasting effects on offspring's health. MicroRNAs (miRNA) may play a critical role in prenatal programming of trophoblast differentiation and placentation with a relationship between aberrant microRNA expression during pregnancy and placental insufficiency. Moreover, miRNAs have been detected in body fluids like blood plasma making them strong candidates as biological markers. Therefore we have analyzed the expression of specific pregnancy-associated miRNAs in preimplantation embryos, blastocoel fluid, maternal serum and placenta, using the rabbit as experimental model. Diabetes was induced by alloxan treated in females 2 weeks prior to mating. In contrast to miR-498, miR-141, -433 and -494 were expressed in maternal serum, placental tissue and in embryoblast and trophoblast cells and blastocoel fluid. The non-pregnancy associated miRNAs miR-27b, -191 and -222 could also be identified in embryoblast and trophoblast cells, placenta and blastocoel fluid, but not in maternal serum. In blastocoel fluid of diabetic embryos the amounts of miR-141, -191 and -222 were decreased. Additionally, miRNAs miR-27b, which is considered to play a role in placental angiogenesis, and -191 were differentially expressed in blastocysts from control and diabetic rabbits. We conclude that pregnancy-associated miRNAs (141, 433, 494) are expressed as early as during the preimplantation period and can be detected in maternal serum, too. Furthermore, we demonstrate that maternal diabetes mellitus leads to distinct changes of miR-27b and -191 expression in blastocysts. Overall, our results show for the first time the incidence of miRNAs in blastocoel fluid, indicating that the blastocoel fluid might serve as communicator between embryoblast and trophoblast cells.

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Poster Presentation

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Epigenetic related- changes of cell plasticity modulate expression of gamma-H2AX and RAD51

Starting from an haploid gamete from each parent, it is possible to obtain the over 200 different cell types that compose an adult organism. Development is achieved through a specification process that is regulated by epigenetic mechanisms. Tissue differentiation is thus the results of restrictions and differential gene expression that gradually decrease cell differentiative potency to a more phenotype-related expression pattern. This process drives cells along different plasticity states is modulated by complex mechanisms still to be fully understood. In the present work, we used a protocol recently set up in our laboratory where a mature adult cell can be reversed to a higher plasticity state and then induced to acquire a different phenotype though the use of a demethylating agent. We investigated whether the demethylation events that lead to the acquisition of such permissive condition are accompanied by stress-related DNA damage and repair mechanisms. Two molecules involved in DNA damage and repairing, gamma-H2AX and RAD51, were analyzed in human and murine skin fibroblasts subjected to the following protocol: exposure to 5-aza-CR for 18h, recovery in embryonic stem cell (ESC) medium for 3h and differentiation induction for 24h. No statistically significant differences for gamma-H2AX and RAD51 levels were detected between untreated fibroblasts and fibroblasts exposed to 5-aza-CR. By contrast we could find a significant increase of the two molecules after 3h of culture in ESC medium. These levels significantly decreased after 24 h of differentiation, returning to values comparable to those of the starting populations. The data obtained suggest that plasticity and methylation changes in cells may involve DNA rearrangements and stress-related response signals. Interestingly enough both gamma-H2AX and RAD51 were highly increased in ESC medium. This might be the results of a delayed response to the demethylation process. Alternatively it might suggest that exposure to a medium designed for supporting self-renewal, rather than cell differentiation, after a demethylating stimulus, has a direct effect on DNA, activating damage and repair mechanisms.

Oral Presentation

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Sagi A

On sexual intervention and safety considerations in the use of RNAi in aquaculture

Monosex is a desirable practice in aquaculture. Differences between males and females of the same species, in growth rate, alimentary needs and behavioral patterns, dictate the need to establish management procedures specifically adjusted to one sex or the other. In one of the most economically important cultured freshwater species, the prawn Macrobrachium rosenbergii, males grow faster and reach larger size at harvest than females thus all-male culture is desired.
Temporal knockdown of an insulin like androgenic gland encoding gene, using RNA interference (RNAi), in M. rosenbergii has recently enabled the alteration of the phenotypic sex of genetic males into functional 'neo-females' capable of producing an all-male progeny. This is the first instance of an aquaculture commercial use of a monosex population derived from a single gene silencing-induced sex reversal.
Safety considerations to the use of RNAi in commercial aquaculture practices were demonstrated through clearance of non-specific exogenous dsRNA and recovery of Mr-IAG expression following dsMr-IAG injection in a time dependent manner. dsRNA is present in the prawn hemolymph and cells up to three days post administration and is fully cleared thereafter. Mr-IAG transcript level was reduced by 97.4% three days post administration and was almost fully recovered (87% of control) 28 days post administration.
The neo-females generated through Mr-IAG silencing show normal female reproductive behavior and reproductive development as compared to a female fraction of a normal mix population.
The generated all-male progeny populations showed similar social structure compared to a males fraction of a normal mix populations. As presented in a two-phase time lined scheme, the RNAi employed at specific short time during the sexual differentiation occur at the post larval stages, dsRNA is cleared during a short time and the all-male production for commercial culture starts 9-10 months after RNAi administration. Based on the above, RNAi-based biotechnologies represent a secure and environmentally safe methods eliminating the need for exogenous hormones or other chemicals and does not pose genetic modifications (non-GMO). Thus, several applications for both aquacultural and environmental use of RNAi-based biotechnologies are suggested.

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Poster Presentation

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Designing molecular beacons for microRNA detection in the periconceptional environment

Background: Evidence suggests that periconceptional environment dictates the epigenetic profile of the embryo and influences its future health. A number of factors like hormones and growth factors mediate the maternal-embryonic communication. microRNAs (miRs) have been found to have a key role in this communication. The implanting blastocyst might rely on miRs to prime the maternal tract for pregnancy, due to the low energy required to produce them and the high impact on the maternal transcriptomic profile.
Aim: To develop miR-sensors that can be transfected to mammalian cells in vitro for detection of miRs produced by trophoblasts and blastocysts.
Methods: A miRNA sensor is a reporter gene with a 3' UTR that contain binding sites of a specific miRNA thus showing a reduced fluorescence signal in the miRNA presence. A miR-sensor carrying reporter proteins was constructed based on the work by Amendola et al. (Nat Biotechnol 23, 108–116; 2004) and optimised for conventional transfection to mammalian cells. To test the feasibility of the miR-sensor system, a number of miR-sensors were generated based on miRs that were found differentially expressed in endometrial epithelial cells in response to trophoblasts, and in the Drosophila reproductive tract post-mating. The functionality of the miR-sensors was verified in the endometrial (RL95-2) cells.
Results: Four miR-sensors were generated for conventional transfection of mammalian cells. Their functionality was examined and confirmed in endometrial cells by fluorescence microscopy. For example, our system detected that let-7a is constitutively expressed as the fluorescence of the sensor was reduced in most of the cells in accordance with the microarray data.
Conclusion: The miR-sensor is a powerful high throughput-tool to monitor the cross-talk of the mother-embryo in mammals.

Oral Presentation

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Depletion of insulin and branched-chain amino acids during mouse in vitro preimplantation development can alter body weight and cardiovascular function during postnatal life

Maternal depletion of insulin in serum and branched-chain amino acids (BCAA) in uterine luminal fluid during the preimplantation period has been linked with altered phenotypes (i.e. high blood pressure) in offspring born to female mice subjected to protein undernutrition (9% casein). This study aimed to determine the effects of insulin and BCAA depletion during in vitro culture of murine preimplantation embryos on blastocyst cell allocation and postnatal phenotype. Two-cell embryos were cultured to the blastocyst stage in KSOM medium supplemented with insulin and BCAA at normal (N, 100%) or low (L, 50%) concentrations: N-Insulin+N-bcaa, L-Insulin+N-bcaa, N-Insulin+L-bcaa, and L-Insulin+L-bcaa. Control medium (N-Insulin+N-bcaa) was supplemented with serum insulin (1 ng/ml) and uterine luminal fluid concentrations of 19 amino acids, including BCAA (valine[0.46 mM], isoleucine[0.21 mM], leucine[0.32 mM]), found in well-fed mice (18% casein). Blastocysts were either subjected to differential cell staining or transferred to pseudopregnant recipients. Cell allocation in blastocyst was not significantly affected by treatment. Compared to control counterparts, the L-Insulin+L-bcaa group showed an increased birth weight and higher body weight at weeks 5-8 and 4-6 in males and females respectively. Mean blood pressure measured at weeks 9, 15 and 21 was not affected in females. However, males from the L-Insulin+L-bcaa group showed a higher mean blood pressure than controls at week 9. Intraperitoneal glucose tolerance tests did not reveal any significant differences between the groups. Although no clear cell allocation phenotype was detected during early embryo development, this experimental model indicates that both insulin and BCAA can substantially alter offspring growth and cardiovascular physiology during postnatal life. Our data supports the notion that insulin and BCAA possess a significant potential for the induction of adverse developmental programming during periconceptional maternal protein restriction.

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P-49

Poster Presentation

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Insufficient B-vitamin levels in the feed for zebrafish increases the lipid accumulation in the liver of the next generation

In the aquaculture industry the feed raw materials have changed from containing high levels of fishmeal to high levels of plant based alternatives. One consequence is a diet with altered naturally sources of folate, amino acids, and vitamin B12, all of which are important methyl donors (1-C donors) vital for many biological processes including gene regulation through DNA methylation. Negative effects of plant based ingredients on growth performance and flesh quality for fish have been observed, but how the fish feed affect the gene transcription for the following generations remains an important unexplored field. To study this we made one feed that was just below the requirements for methyl donors (low 1-C feed) and one feed that was enriched with methyl donors (high 1-C feed). These feeds were given to F0 generation of zebrafish from 27 days post fertilization until maturity, while both groups of the next generation (F1) were given the same type of feed enriched with methyl donors. Embryo and mature livers were sampled for transcriptome analyses. Surprisingly, both genes related to redox regulation and lipid metabolism, but also genes related to 1-C metabolism, were significantly differentially regulated in both embryo and liver samples from F1. The changes in lipid metabolism were confirmed with a higher inclusion of lipids in the hepatocytes of F1 mature livers of the low 1-C feed group.

P-50

Poster Presentation

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Expression of aquaporin 1 and 5 and theirs regulation by ovarian hormones, arachidonic acid, forskolin and cAMP during embryo implantation in pigs: in vitro study

Aquaporins are small membrane proteins that facilitate transepithelial fluid transport and are involved in a variety of physiological and cellular functions. To assess their role in implantation, an in vitro experiment was used to elucidate whether the steroid hormones, progesterone (P4) and estradiol (E2), and other factors: oxytocine (OT), arachidonic acid (AA; substrate for prostaglandins synthesis) as well as forskolin (FSK; adenylate cyclase activator) and (cAMP; second messenger cyclic adenosine monophosphate) may impact on the AQPs expression in porcine uterine tissue.

Tissue samples were recovered from mature cross-bred gilts (Large White x Polish Landrace) on Days 14-16 (n=5) of gestation. The tissue explants were incubated for 3 and 24 hours. The expression of AQP1 and 5 mRNA and protein was determined by Real-Time PCR as well as immunocytochemistry and Western blot analyses.

By immunohistochemistry, AQP1 was associated with uterine endothelial cells without changes in localization within the cells after 3- and 24-h incubation with all examined factors. AQP5 was associated with uterine epithelial cells and smooth muscle cells. Treatment with P4, E2, AA, FSK and cAMP caused movement of AQP5 to the apical and basolateral plasma membranes of epithelial cells. AQP1 mRNA expression decreased after treatment with E2, oxytocin, AA and cAMP. In turn, significant increase in AQP1 mRNA expression was seen after longer treatment with oxytocin, AA, cAMP and FSK. Treatment of uterine explants with E2, OT and AA resulted in decreased AQP5 expression. cAMP caused an increase in uterine AQP5 expression, but only after 24-h. A stimulatory effect on AQP1 at the protein level was observed after treatment of explants with E2 and P4. AQP5 protein expression significantly increased after treatment with P4, E2, FSK and cAMP.

Collectively, these results suggest that uterine expression of AQP1 and AQP5 are involved in uterine fluid homeostasis and potentially remains under control of steroid hormones and AA-derived compounds.

Poster Presentation

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Progesterone, estradiol, arachidonic acid, forskolin and cAMP influence on aquaporin 1 and 5 expression in porcine uterus during placentation: in vitro study

Aquaporins (AQPs) are water channel proteins responsible for water homeostasis and important for proper functioning of all body systems, including reproductive structures. AQP 1 and 5 are water selective and belong to the classical AQP family. In previous studies, we have established the presence of AQP1 and 5 in porcine uterus. In this study, an in vitro system was used to investigate the effect of progesterone (P4), estradiol (E2), oxytocin (OT), arachidonic acid (AA), forskolin (FSK; adenylate cyclase activator) and cAMP on AQP1 and AQP5 mRNAs and protein levels in porcine uterine tissue from (placentation) pigs. Tissue samples were recovered from mature cross-bred gilts (Large White × Polish Landrace) on Days 30-32 (n=5) of gestation (placentation).The tissue explants were incubated for 3 and 24 hours. The expression of AQP 1 and 5 mRNA and protein was determined by Real-Time PCR as well as immunocytochemistry and Western blot analyses. AQP1 mRNA expression in uterine explants increased significantly only after treatment with E2. In turn, AQP5 mRNA expression significantly increased following treatments with E2, OT, AA, FSK and cAMP. At the protein level, AQP1 expression was up-regulated by P4, E2 and AA, but down-regulated by FSK and cAMP. In turn, increased expression of AQP5 protein was observed in response to all treatments, except OT. Moreover, it was demonstrated by immunohistochemistry that treatment with P4, E2, AA, FSK and cAMP induced significant translocation of AQP5 from the apical to the basolateral plasma membrane of epithelial cells suggesting the transcellular route of the water movement between uterine lumen and blood vessels. Concluding, the present study suggests that uterine expression of AQP1 and AQP5 potentially remains under control of steroid hormones and AA-derived compounds (eg. prostaglandins). It might be also concluded that the adenylate cyclase/cAMP pathway participates in the regulation of AQPs expression in the uterine tissue of pregnant pigs.

Poster Presentation

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Szymanska M, Blitek A

Endometrial and conceptus response to P4 supplementation in early pregnant gilts

Hormonal induction of estrus with PMSG/hCG in prepubertal gilts results in lower P4 level in blood plasma and decreased endometrial expression of genes crucial for proper embryo-uterine interactions. Thus, the present study aimed to examine the effect of P4 supplementation during early pregnancy on conceptus development and uterine preparation for implantation. Prepubertal gilts were induced to ovulate with 750 IU PMSG/500 IU hCG, and inseminated. On days 3 to 10 of pregnancy, gilts received daily i.m. injections of corn oil vehicle (control; n=6) or P4 (50 mg/100 kg; n=6). Animals were slaughtered on day 12 of pregnancy and blood samples were collected to examine P4 concentrations using RIA. Uterine horns were weighted and flushed with PBS to obtain conceptuses and uterine luminal flushing (ULF). Endometrial and/or conceptus expression of P450arom, PTGS2, PGIS, LIF, IL6 and TGFβ1 mRNA was determined using qPCR. ULF was examined for E2, PGE2, 6-ketoPGF1α (a PGI2 metabolite), LIF, IL6 and TGFβ1 concentrations with RIA/EIA.
P4-treated gilts demonstrated higher P4 level in blood plasma and had heavier uteri after slaughter ($P<0.05$). The expression of P450arom mRNA tended to increase ($P=0.07$) in conceptuses after P4 supplementation, what was accompanied by a greater concentration of E2 in ULF ($P<0.05$). PTGS2 mRNA expression decreased in the endometrium, while increased in conceptuses of P4-treated animals ($P<0.05$). PGIS mRNA expression tended to increase in conceptuses ($P=0.06$), but decrease in the endometrium ($P=0.07$) of supplemented gilts. The concentration of 6-keto PGF1α in ULF did not differ between both groups. In contrast, P4-treated gilts showed lower concentration of PGE2 ($P<0.01$). Among cytokines, greater amount of LIF transcripts were detected in the endometrium of supplemented gilts ($P<0.05$).
In summary, P4 supplementation differentially affects the expression of genes involved in PGE2 and PGI2 synthesis in the endometrium and conceptuses of early pregnant gilts.

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Oral Presentation

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Exploring noncoding miRNAs and their role in bovine follicular development: Challenges and potentials

The bovine follicular development is characterized by recruitment of a group of follicles in 2 or 3 follicular waves though high rate of growth is initiated only in one of the follicles which later on succeed to be dominant over the others and becomes ovulatory. The programmed and complex transition of the primordial follicle into large sized antral follicle is mainly initiated by morphological transformation and functional differentiation of follicular cells. Several studies in mammalian species have evidenced that the various steps in folliculogenesis to give rise to ovulation of developmentally competent oocyte is accompanied by the spatio temporal expression of several classes of genes. Accordingly, it is postulated that these genes are either transcriptionally or post-transcriptionally regulated in time and tissue specific manner. MicroRNAs as short classes of noncoding RNAs are recently emerged as post-transcriptional regulators of genes involved in various physiological processes and diseases. Besides the cellular miRNAs recently much attention is given to the circulatory miRNAs in the body fluids. As follicular environment involves a coordinated interaction of the gamete and the surrounding cells which is mediated by the follicular fluid, investigation of regulatory miRNAs enrichment and degradation in various follicular cells at different time points of follicular development would provide a deeper insight into the molecular regulatory mechanisms associated with follicular recruitment, selection and dominance. We have recently explored the enrichment and degradation of noncoding miRNAs in bovine granulosa cells collected from dominant and subordinate follicles at days 3, 7 and 19 of the oestrus cycle and we could identify differential expression of miRNAs which are potentially targeting genes involved in various pathways related to follicular growth. Moreover, we have recently shown the presence of extracellular miRNAs in bovine follicular fluid and their relative abundance varied depending on the growth status of the oocyte. In the same study we have evidenced an exosome mediated transfer of miRNAs between the follicular cells, which is crucial for the cell-to-cell communication during follicular development. Despite their enormous potential in regulation of several biological processes the way from identification to the association of those miRNAs to the true targets remains the challenge for researchers in the field. There are several web-based tools which differ in their species coverage and criterion of ranking predicted target genes. Despite all the challenges and complexities, in the last decade the number of studies aiming at identification, expression profiling and functional analysis of miRNAs are increasing rapidly and several miRNAs have been identified as potential biomarkers for various physiological processes including development and cancer.

Poster Presentation

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Platelet-activating Factor Receptor is present and differentially expressed throughout bovine embryo development in vitro

Growth factors play a predominant role in many mammalian tissues, including the reproductive tract. In vivo, a broad range of growth factors, originating from paracrine secretions from the mammalian reproductive tract, have been proven to positively interact with the embryo. Also, in vitro data suggests that the beneficial effect of culturing embryos in group, is due to autocrine growth factors, secreted by the embryos themselves. A possible candidate for this paracrine/autocrine interaction, is platelet-activating factor (PAF, 1-o-alkyl-2-acetyl-sn-glyceryl-3-phosphocholine). Platelet-activating factor binds to a specific trans-membrane receptor, which belongs to the G-protein coupled (GPCR) superfamily. To date, there is no information available on the PAF-receptor (PAFR) in the bovine embryo. In this study, we 1) compared the mRNA expression of the PAF-receptor from the 4-cell stage to blastocyst formation, by RT-qPCR, complying with the MIQE-guidelines, and 2) confirmed the presence of the protein by immunofluorescence microscopy. Embryos were collected at different time points according to their development (4 – 8 – 16 cells – compacted morula – expanded blastocyst). PAF-receptor transcripts and proteins were detected in all stages collected. Expression levels were rising until the 16-cell stage ($P>0.05$) and then dropped significantly in the post-compaction stages ($P<0.01$). These results may suggest a role of PAF in the compaction process of bovine embryos. In the intestines, PAF is known to increase tyrosine phosphorylation of the E-cadherin protein (Tan et al., 2000). Phosphorylation of E-cadherin is strongly associated with compaction in the mouse embryo (Sefton, 1992). Further research will be necessary to elucidate the exact mechanism behind PAF induced compaction.

P-55

Poster Presentation

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Epigenetics and reproductive health: linking nutritional biochemistry to long-erm development

The long-term 'programming' effects of specific dietary nutrients on key epigenetic processes during gametogenesis and pre-implantation development are the focus of ongoing investigations at Nottingham. We demonstrated previously in sheep that physiologically relevant reductions in the dietary supply of specific B-vitamins (i.e. vitamin B12, folate) and methionine to intending mothers can epigenetically modify DNA in their progeny (determined by Restriction Landmark Genome Scanning) and lead to adult offspring that are obese, have altered immune responses to antigenic challenge, are insulin resistant, and have elevated blood pressure. Intriguingly, these epigenetic and phenotypic effects are most pronounced in male offspring. Subsequent studies in the rat revealed common phenotypic effects which are also male specific. More recent (unpublished) studies using MBD-Seq together with qPCR have confirmed sex-biased dietary-mediated epigenetic alterations to DNA methylation and gene expression in sheep offspring; and have identified key pathways involved in insulin signalling and endoplasmic reticulum stress associated with insulin resistance. Attention has now turned to how nutrient-mediated modifications to one-carbon metabolism within the ovarian follicle, egg and pre-implantation embryo lead to epigenetic alterations in DNA methylation. Initial observations indicate that key enzymes within the methionine cycle are not, or are very poorly, expressed and active in these tissues, with subtle differences existing between species. Mathematical models predict consequences for intra-cellular trans-methylation, the magnitude of which may be more pronounced when ovarian somatic cells and zygotes are cultured in vitro in the presence of non-physiological concentrations of one-carbon cycle related metabolites. These predicted effects are currently being tested experimentally and preliminary findings will be discussed.

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Poster Presentation

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Identification of two novel classes of genes inheriting functionally important methylation from the oocytes

A functional role for DNA methylation has been well-established at imprinted loci, which inherit methylation uniparentally, most commonly from the mother via the oocyte. Many CpG islands not associated with imprinting also inherit methylation from the oocyte, though the functional significance of this, and the common features of the genes affected, is unclear. We have identified two major subclasses of genes associated with these gametic differentially methylated regions (gDMR), genes important for brain and testis function, respectively. The gDMR at these genes retain the methylation acquired in the oocyte through preimplantation development, but become fully methylated post-implantation by de novo methylation of the paternal allele. Each gene class displays unique features, with the gDMR located at the promoter of the testis genes, but intragenically for the brain genes. Significantly, demethylation using knockout, knockdown or pharmacological approaches in mouse stem cells and fibroblasts resulted in transcriptional activation of the testis genes, but transcriptional repression for the brain genes. As well as the standard 5-methylcytosine (5mC) type of methylation, the brain genes acquire high levels of 5-hydroxymethylation (5hmC) in adult brain tissue. This is not inherited from the oocyte or early embryo but instead appears to be acquired quite late post-implantation, and is not seen on the testis or imprinted genes. Neither of the other gDMR classes inherited significant 5hmC levels from oocytes either. The gain in 5hmC is not at the expense of 5mC, but instead appears to be in addition to it. The locations of the gDMR, as well as methylation levels and effects on transcription, were also conserved in human cells. Our results suggest: 1) there are two other gene classes inheriting methylation from the oocyte which has functional significance 2)protection of the unmethylated allele against post-implantation methylation is crucial for imprint maintenance 3)5mC can efficiently repress transcription at the testis promoter CpG islands 4)intragenic 5mC weakly promotes transcription at brain genes 5)brain genes, but not testis genes, acquire 5hmC in the brain, where they are highly transcribed. These novel gene classes may be dysregulated by environmental exposures in either mother or offspring which cause demethylation.

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Poster Presentation

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The influence of prolactin on mRNA expression of chemokines and chemokine receptors mRNA in the porcine corpus luteum on day 14 of the estrous cycle and early pregnancy

Prolactin (PRL) is a multifunction protein hormone involved in growth and maturation of ovarian follicles and the regulation of luteal steroidogenesis during early luteal phase. The mechanism of PRL luteotropic or luteolytic action on porcine corpus luteum (CL) is still unknown. Thus, the purpose of this study was to determine the influence of PRL on the mRNA expression of chemokines (CCL2, CCL4, CCL5, CCL11, CXCL2, CXCL8, CXCL9, CXCL12) and their receptors (CCR1, CCR2, CCR5, CXCR2, CXCR3, CXCR4) on day 14 of the estrous cycle and pregnancy.

CLs were collected from mature gilts on 14 day of early pregnancy or estrous cycle (PRL injections on days 12 and 13) and from identical non-treated groups (control). To determine genes expression a Real-Time PCR was used. Data were analyzed by two-way Anova, followed by Fisher's LSD post hoc test.

In the case of pro-inflammatory and potential luteolytic factors: CCL2, CCL11 and CXCL8, a significant increase of mRNA expression was observed in CLs of cycling gilts after PRL treatment compared with the control gilts ($p<0.02$) . Additionally, the PRL injection dropped CXCR2 and CXCL12 ($p<0.02$) mRNA expression in cycling gilts whereas increased CXCL9 and CXCR3 ($p<0.03$) mRNA expression in pregnant gilts. We also noticed a higher expression of CCL11 ($p<0.003$) and CXCL8 ($p<0.002$) in CLs of cycling gilts after PRL treatment compared with pregnant gilts received PRL. Moreover, PRL stimulated expression of CCL4 ($p<0.04$) in pregnant gilts when compared with cycling gilts. No significant differences in mRNA level of any other genes was observed.

Our observations revealed that during late luteal stage PRL may be a one of the important factors stimulating intensive secretion of luteolytic chemokines. Moreover, during early pregnancy PRL stimulate CCL5 and CXCL9 production, which are involved in leukocyte recruitment in corpus luteum and thus indirectly may stimulate its regression and loss of pregnancy.

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