# Epigenetics and Periconception Environment

## Proceedings of the EPICONCEPT Workshop 2015 Periconception Environment

**Dubrovnik, Croatia**
**26 - 29 April 2015**


## Editors

**Kevin Sinclair, Alireza Fazeli, Ann Van Soom, Juraj Grizelj**

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# Contents

# About the European Co-operation in Science and Technology

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

- bottom up approach (the initiative of launching a COST Action comes from the European scientists themselves),
- à la carte participation (only countries interested in the Action participate),
- equality of access (participation is also open to the scientific communities of countries which do not belong to the European Union) and
- flexible structure (easy implementation and management of the research initiatives).

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

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

# Welcome from the Chairman

Dear Epiconcept Members

This workshop is again an interesting presentation of the latest developments in epigenetics; how it can influence the world of reproduction and assisted reproduction. We will learn in the lovely medieval city of Dubrovnik how the environment can affect embryonic and gonadal development with a focus on flies, fish and reptiles which are very exotic animals indeed for a veterinarian like me. The next theme deals with tiny changes in environmental conditions. For instance, a change in oxygen concentration can induce huge differences in embryonic development. These alterations can now be unravelled by using the 'omics' approach. Not only the culture conditions can influence embryos and the health of the resulting offsprings but the nutrition of the father and the mother also plays a pivotal role. And finally the general health of the parents is a major factor contributing to the fitness of their offsprings.

So no cigarettes are allowed at the welcome reception, and only one glass of wine. That is my advice to the young students who will attend this workshop, and still plan to reproduce in the near future! This workshop may be an eye-opener for parents to be, but let it not refrain you from reproduction. Fortunately the embryo is so flexible that it can endure most of the harms we impose on it!

I would like to thank the scientific organisers of this meeting, Kevin Sinclair and Anna Navarette-Santos of Working Group 2, for putting together such an interesting programme. Special thanks to my vice-chairman Alireza Fazeli who has been successfully sorting out the finances and many other problems. I am very grateful to Laszlo Tecsi who is making sure that the registration, the refund and the proceedings are in order. Last but not least I want to thank the local organiser Juraj Grizelj and his team for making this meeting possible in this beautiful historic city.

Warm welcome to Dubrovnik!

Ann Van Soom
Chair of Epiconcept

# Welcome from the Organisers

Dear Epiconcept Members

Welcome to Dubrovnik, to the City of Freedom. Our workshop is organized by the Faculty of Veterinary Medicine of the University of Zagreb at the Centre for Advanced Academic Studies.

The uniqueness of the city of Dubrovnik was the reason why we have decided to organise this workshop here. Dubrovnik, the 'Pearl of the Adriatic' is a UNESCO World Heritage Site. This Dalmatian city, which is surrounded with 2000 meters long stone wall, has a rich historic past. Its prosperity was based on maritime activities, and was one of the wealthiest cities in the world at one time. The city and its surrounding area became the independent Republic of Ragusa in 1358. The small state developed a strong defence system coupled with skilled diplomacy, and preserved its freedom until 1808 when it was conquered by Napoleon. Dubrovnik also proud of its title as the 'City of Freedom' because it was the first European state which abolished slavery in 1416, nearly 400 years before the rest of the world. This beautiful historic city was seriously damaged, for the first time in history, by the artillery of Serbia and Montenegro 25 years ago. Our conference venue, the Centre for Advanced Academic Studies was burned to the ground. Many years of tireless work now restored the city and its treasures to their original beauty.

On behalf of the Local Organizing Committee I wish to thank the speakers, authors and co-authors, chairs and delegates. Special thanks to Dr Ann Van Soom and her team for their active support which will guarantee a vibrant scientific forum.

Juraj Grizelj
Local Organiser


*The keys to the success of the Republic of Ragusa was not conquering but trading and sailing under a white flag with the Latin word of Libertas (Freedom). This is still one of the flags of Dubrovnik.*

# Organisers

Alireza Fazeli, United Kingdom
Ann Van Soom, Belgium
Anne Navarrete-Santos, Germany
Juraj Grizelj, Croatia (Chairman)
Kevin Sinclair, United Kingdom
Laszlo Tecsi, United Kingdom

# Programme

## Day 1
## Sunday 26 April 2015

**17:00 – 20:00** **Meeting Registration**

**20:00 – 23:00** **Welcome Dinner**

## Day 2
## Monday 27 April 2015

**07:45 – 09:00** **Breakfast**

**09:00 – 09:15** **Welcome Address**
(15:00)

### Theme 1: Environment

**09:15 – 09:55** **Yael Heifetz, Hebrew University of Jerusalem, Israel**
(40:00) Studying the periconceptional environment in Drosophila: a model organism to bridge unknown gaps

**09:55– 10:35** **Bernd Fischer, Martin Luther University, Germany**
(40:00) Stage-specific effects of environmental contaminants during development

**10:35– 10:50** **Dafni Anastasiadi, Spanish National Research Council (CSIC), Spain**
(15:00) Temperature and farming conditions provoke changes in the epigenome of the European sea bass

**10:50– 11:05** **Aneta Andronowska, Institute of Animal Reproduction and Food Research, Poland**
(15:00) The influence of immune cells on the function of porcine corpus luteum

**11:05 – 11:45** **Coffee and Poster Presentation**

**11:45 – 12:45** **Louis Guillette, Medical University of South Carolina, United States**
(60:00) Environmental factors influence the epigenetics of the developing vertebrate gonad

**12:45 – 14:30** **Lunch**

### Theme 2: Assisted Reproduction

**14:30 – 15:10** **Franchesca Houghton, University of Southampton, United Kingdom**
(40:00) Oxygen tension and epigenetic regulation of developmental genes in embryonic cells

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| **15:10 – 15:50** (40:00) | **Michele Boiani, Max Planck Institute for Molecular Biomedicine, Germany**<br>Phenotypic variation in embryos: contributions of oocyte composition and postfertilization environment under the OMICS lens |
| **15:50 – 16:05** (15:00) | **Andras Dinnyes, Biotalentum, Hungary**<br>Modelling the developing CNS by 3D engineered neural tissue from human iPSC |
| **16:05 – 16:20** (15:00) | **Alfonso Gutierrez-Adan, Spanish National Institute for Agricultural and Food Research and Technology (INIA)**<br>Identification of mRNA splicing factors in bovine sex determination |
| **16:20 – 16:50** (30:00) | **Discussion** |
| **17:00 – 18:00** | **Tea and Poster Presentation** |

## Day 3
## Tuesday 28 April 2015

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|---|---|
| **07:45 – 09:00** | **Breakfast** |
| **09:00 – 09:15** (15:00) | **Laszlo Tecsi, University of Sheffield, United Kingdom**<br>Rules of travel reimbursements |
| | **Theme 3: Parental Nutrition** |
| **09:15 – 09:55** (40:00) | **Noora Kotaja, University of Turku, Finland**<br>RNA regulation during spermatogenesis: importance in male fertility and potential role in epigenetic inheritance |
| **09:55 – 10:35** (40:00) | **Tom Fleming, University of Southampton, United Kingdom**<br>Mechanistic analysis of periconceptional programming |
| **10:35 – 10:50** (15:00) | **Justyna Kolakowska, University of Warmia and Mazury, Poland**<br>The effect of intrauterine environment on proteome profile of the endometrium in pigs (Sus scrofa domestica) |
| **10:50 – 11:05** (15:00) | **Martin Nikolovski, University Saints Cyril and Methodius, Macedonia**<br>Ram spermatozoa motile and metabolic activity – potentially predictable pattern of ejaculate fatty acid composition |
| **11:05 – 11:45** | **Coffee and Poster Presentation** |
| **11:45 – 12:45** (60:00) | **Alireza Fazeli, University of Sheffield, United Kingdom**<br>Tolerance is the key message for potential mothers |
| **12:45 – 14:30** | **Lunch** |

### Theme 4: Parental Health

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|---|---|
| **14:30 – 15:10** (40:00) | **Katie McGhee, University of Cambridge, United Kingdom**<br>Maternal stress, paternal care, and consequences for offspring |
| **15:10 – 15:50** (40:00) | **Martin Sheldon, Swansea University, United Kingdom**<br>Impact of infection of the uterus and the innate immune response on ovarian function |
| **15:50 – 16:05** (15:00) | **Mehmet Kuran, Ondokuz Mayis University, Turkey**<br>Low oocyte maturation temperature successfully support the subsequent bovine embryo development to blastocyst stage in vitro |
| **16:05 – 16:20** (15:00) | **Francisco Otero-Ferrer, Canary Islands Institute of Marine Sciences, Spain**<br>Early nutritional programming windows and seahorse embryo development |
| **16:20 – 16:50** (30:00) | **Discussion** |
| **17:00 – 18:00** | **Tea and Poster Presentation** |
| **20:00 – 23:00** | **Farewell Dinner** |

## Day 4
## Wednesday 29 April 2015

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

# Abstracts of Presentations

**O-1**

*Oral Presentation*


## Anastasiadi, Dafni

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

Anastasiadi D, Piferrer F


# Temperature and farming conditions provoke changes in the epigenome of the European sea bass


The early developmental environment an organism experiences may have a long term impact on the phenotype. Fish represent good animal models for the study of the integration of environmental information via epigenetic mechanisms. Both temperature and farming conditions are shown to be related with lasting changes in gene expression and the phenotype. Here, we used adult European sea bass (Dicentrarchus labrax) subjected to two temperatures (17oC or 21oC) during the thermosensitive period and also, we compared farmed and wild fish. From one hand we explored the DNA methylome using next generation sequencing (NGS) technology and from the other hand we studied specific histone modifications and variants in key genes. In order to measure DNA methylation levels, we prepared and sequenced Reduced Representation Bisulfite Sequencing (RRBS) libraries. Temperature provoked changes in ~3500 cytosines in testis and ~12000 cytosines in muscle, whereas farming conditions affected ~4000 cytosines in testis and ~15000 cytosines in muscle. The great majority of these differentially methylated cytosines arisen both by temperature and farming conditions were physically located in proximity to genes and their regulatory elements including the transcription start sites (TSS). For the purposes of identifying enrichment of two histone modifications and one variant related to regulation of gene expression, we measured the expression of two genes (drmt1 in testis and myog in muscle) and performed ChIP-qPCR experiments using antibodies for both transcriptional activation (H3K4me3 and H2A.Z) and inactivation (H3K9me3) epigenetic markers. In addition, we used RNA-seq to establish possible relationships between epigenetic and gene expression changes. In this study, we have demonstrated that the environmental information received during early development in the sea bass has broad consequences on the epigenome that are revealed later in life.

**O-2**

*Oral Presentation*


## Andronowska, Aneta

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

Andronowska A, Witek KJ, Małysz-Cymborska I


# The influence of immune cells on the function of porcine corpus luteum


The main function of the corpus luteum (CL) is secretion of progesterone (P4) to establish and maintain pregnancy. If pregnancy is not established, the CL undergoes functional (loss of ability to P4 secretion) and structural (disruption of vascular and luteal cells) luteolysis. Moreover, during CL regression, the number of immune cells, especially eosinophils, macrophages and T lymphocytes significantly increases. There is growing evidence that chemokines produced by immune components may be directly or indirectly involved in CL function. We found increased mRNA expression of CXCL10, CXCL9 and CCL8 on day 12 and CXCL2, CXCL8, CCL2, CCL4, CCL5 on day 14 of the estrous cycle. This study was designed to investigate the in vitro effect of: 1)PGF and selected chemokines on luteal progesterone secretion and proliferation and migration of immune cells as, 2)immune cells on luteal progesterone secretion. Purified PBMCs and PMNs were analyzed for migration and activation after PGF and chemokine treatment. Mixed luteal cells (steroidogenic and endothelial) isolated from the CL on days 12-14 of the cycle were used to determine the effect of chemokines, PGF, PMNs and PBMCs on progesterone production. We found that both activated PMNs and PBMCs inhibited P4 production. Significant decrease in P4 synthesis by mixed luteal cells was also observed after PGF and CCL2, CXCL2, CCL4, CCL8 treatment. CXCL8 had the strongest effect on PMNs and PBMCs migration comparing to other chemokines. PGF did not affect either PBMCs proliferation or PBMCs and PMNs migration. We did not observed the significant effect of selected chemokines on PBMCs proliferation after 24 h stimulation. In summary, PGF had no direct effect on immune cells, but both chemokines and immune cells influenced on luteal cell function by inhibiting the progesterone production.



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

*Oral Presentation*

## Boiani, Michele

Department Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Germany

Boiani M, Taher L, Fuellen G, Suzuki Y, Drexler H, Makalowski W, Arauzo-Bravo MJ, Nordhoff V, Schlatt S, Wang B, Pfeiffer MJ, Schwarzer C

# Phenotypic variation in embryos: contributions of oocyte composition and postfertilization environment under the OMICS lens

Observed differences in uniformity between monozygotic twins who were raised apart suggest that, besides the allelic makeups of oocytes, other factors contribute variation to mammalian embryos. Variation is a substrate for selection. The developmental quality of oocytes is determined, in part, by a repertoire of maternally transcribed mRNAs, which accumulate and translate into proteins during oocyte growth and maturation. These maternal products support early development prior to EGA under the provisions of the environment. Oocytes become transcriptionally silent as they approach the time of ovulation, when they contain – in mice – approx. 25 pg mRNA and 17000 pg protein. These figures highlight the necessity of a dual molecular definition of oocyte quality and derivative embryo development (transcriptome + proteome). We use genetic tools, micromanipulation techniques and a combined RNA-seq/LC-MS/MS approach to investigate the origin of quantitative variation in mouse embryos, in relation to the oocyte and to the environment in which it develops. Between inbred mouse strains, oocytes differ in the endowment of several proteins, including maternal-effect factors and epigenetic modifiers, in a way that can hardly be predicted from mRNA abundance. Within strains, developmental potential following fertilization differs according to the preimplantation environment e.g. culture medium, with positive correlation between blastocyst rates and fetal rates. It is possible that embryo culture conditions rescue poor-quality oocytes, or that the definition of oocyte quality is contingent on the environment. Emerging challenges include 1) closing the gap of depth between oocytes' detected proteome and transcriptome; 2) including the information of sub-oocyte localization of mRNAs or proteins when interpreting OMICS data; and 3) raising the sensitivity of OMICS methods to the level of single cell analysis, so as to foster transferability to human reproduction studies.

*Poster Presentation*

## Cebrian-Perez, Jose Alvaro

Department of Biochemistry and Molecular and Cellular Biology, University of Zaragoza, Spain

Gonzalez-Arto M, Hamilton TSR, Serrano E, Aguilar D, Gaspar E, Arruga D, Perez-Pe R, Muino-Blanco T, Casao A, Cebrian-Perez JA

# Identification and immunolocalization of melatonin-synthesis enzymes in the ram genital tract

Melatonin is an ubiquitous molecule presented in a wide range of organisms and involved in multiple functions. Circulating melatonin in vertebrates is secreted by the pineal gland, although it can also be synthetized in various extra-pineal tissues such as the gastrointestinal tract, skin or ovarian follicle. We have recently reported both seasonal and daily variations of melatonin levels in ram seminal plasma. The objective of this study was to determine the presence of the two main enzymes involved in the melatonin synthesis, serotonin-N-acetyltransferase (AANAT) and hydroxyindol-O-methyltransferase (ASMT), in the gonads and accessory sexual glands of rams by RT-PCR, quantitative real time PCR and immunohistochemistry. RT-PCR analysis demonstrated that both AANAT and ASMT mRNAs are present in all studied tissues. The expression of these genes was also positive in the ram pineal gland extracted mRNA, which was used as a positive control for both AANAT and ASMT. Quantitative real time PCR also showed that the AANAT and ASMT mRNA expression is higher in testis than in the other tissues. Moreover, immunohistochemistry revealed that AANAT and ASMT proteins are localised on the testis Leydig cells. Our findings evidenced for the first time that AANAT and ASMT are present in the gonad, epididymis and sexual glands of ram. This evidence also suggests that the ram genital tract has the enzymatic mechanism for the melatonin synthesis, and that melatonin might be a key regulator of sperm functionality.

This work was supported by CICYT-AGL2013-43328 and DGA-A26FSE.

**P-5**

*Poster Presentation*


## Cieslak-Lechniak, Dorota

Department of Genetics and Animal Breeding, Poznan University of Life Sciences, Poland

Pawlak P, Cieslak A, Warzych E, Malyszka N, Madeja Z, Lechniak D


# Follicular fluid and oocytes from prepubertal and cyclic gilts differ in respect to fatty acid content and the number of lipid droplets


Some of the fatty acids present in follicular fluid were shown to exert a positive impact on the developmental competence of oocytes and embryos in vitro whereas some of FAs a negative effect. Therefore the aim of the present project was to analyse the fatty acid profile of porcine FF and to determine the number of lipid droplets in oocytes of prepubertal (P) and cyclic (C) gilts. Additionally we aimed to investigate the effect of puberty of the FF donor (P, C) on the number of lipid droplets in P and C oocytes matured in vitro. Cumulus-oocyte complexes and follicular fluid were collected from P and C ovaries. COCs form prepubertal and cyclic gilts were matured in vitro with FF from P and C ovaries to elucidate a possible effect of FF donor puberty on oocyte quality. FA profile of follicular fluid was analysed by gas chromatography. Number of lipid droplets in the oocytes were analysed by fluorescence confocal microscop. The gilt puberty (P,C) significantly influenced the fatty acid profile in the follicular fluid. FF from P gilts contained more total fatty acids (650 vs 570 ug/ml) as well as more particular FAs (eg oleic, palmitic) with a distinct effect on oocyte competence. On the other hand the FF from C gilts was characterized by higher concentration of the linoleic acid. Lipid droplets were less abundant in pre-IVM oocytes from P gilts than C gilts (247 vs 320). The effect of puberty of the FF donor on oocyte quality is currently being evaluated and will be presented on the conference. This study shows different follicular growth environments of prepubertal and cyclic gilts oocytes. In conclusion the donor puberty significantly affected FA profile in the follicular fluid. We suggest that FF composition may influence the oocyte quality since C oocytes contained more lipid droplets. Moreover higher concentration of palmitic acid in FF of prepubertal gilts may negatively affect oocyte quality what has been previously shown for bovine oocytes.

**P-6**

*Poster Presentation*


## Czelejewska, Wioleta

Faculty of Biology and Biotechnology, Department of Animal Physiology, University of Warmia and Mazury, Poland

Czelejewska W, Dziekonski M, Okrasa S, Zmijewska A


# Transcriptomic profiles of porcine pituitary gland during the estrous cycle and early pregnancy


Major regulatory component of hypothalamic-pituitary-gonadal axis (HPG) is gonadotropin-releasing hormone (GnRH). For a long time, it has been recognized as a sole hypothalamic factor responsible for regulating LH and FSH secretion from the pituitary gland. In the last few years new neuropeptides - gonadotropin-inhibitory hormone (GnIH) and kisspeptins - emerged as a crucial components of the mechanism involved in the regulation of reproductive functions in females. Nevertheless, information about molecular mechanisms controlling changes in gonadotropin secretion from the pituitary of cyclic and pregnant gilts are still lacking. Therefore, the aim of this study was to compare transcriptomic profiles of the pituitaries obtained from pigs on Days 15-16 of the estrous cycle (n=4) and pregnancy (n=4). For this purpose Porcine (V2) Gene Expression Microarray, 4x44K (Agilent Technologies) was used. Microarray data analysis using GeneSpring 13.0 revealed that 1329 genes were differentially expressed in the pituitaries of pregnant vs. cyclic pigs (fold-change ≥ 1.2, $p < 0.05$). We were able to annotate 608 genes; 224 up-regulated and 384 down-regulated. Functional classification analysis, made with PANTHER (Protein Analysis Through Evolutionary Relationships), indicated groups of differentially expressed genes involved among others in GnRH receptor, PPAR and Jak-STAT signalling pathways. Moreover, some transcripts potentially related to the modulation of gonadotropin secretion by opiod peptides also appeared to be differentially expressed. Presented microarray study and preliminary data analysis allow to undertake further investigation of specific genes and molecular pathways putatively engaged in the local modulation of gonadotropin secretion at the pituitary level in cyclic and pregnant pigs.



This research was supported by the National Science Centre, Grant No. 12.650.018-300/529-0206-0941

*Oral Presentation*

## Dinnyes, Andras
Biotalentum Research, Biotalentum, Hungary

Dinnyes A, Ochalek A, Chandrasekaran A, Bellak T, Szegedi V, Varga E, Zhou S, Teglasi A, Kovacs E, Szczesna K, Schmidt B, Avci H, Hyttel P, Kobolak J

## Modelling the developing CNS by 3D engineered neural tissue from human iPSC

The early stages of the developing central nervous system (CNS) are usually not accessible for studies in several species, including humans. Several CNS diseases might originate at early development. Three-dimensional (3D) cultures have shown more physiological relevance (improved cell survival, enhanced neuronal differentiation, better cell–cell and cell–matrix interactions) compared to 2D cell cultures. Differentiation of human induced pluripotent stem cells (iPSCs) into 3D engineered neural tissue (ENT) is recapitulating to some extent events during early CNS development, thus provides advantages to study neurodevelopmental disorders, preclinical neural drug candidate tests and neuro-toxicology. We reprogrammed fibroblasts and mononuclear blood cells into iPSCs and differentiated them into compact 3D ENTs using an air-liquid interface based, scaffold-free system without added growth factors (Krause et al., 2009). After 6 weeks of differentiation the ENTs were characterized with multi-electrode array (MEA), calcium imaging and immunocytochemical (IHC) methods. The majority of cells differentiated into neurons with spontaneous firing activity and shown evidence of functional synapses. IHC confirmed the presence of various neuronal and glial markers (beta III Tubulin, MAP2, NF200kD, GFAP, OSP) and different synaptic proteins. In conclusion, these findings demonstrate that 3D ENTs might be suitable for studying different neurological and psychiatric disorders originating during early development.

This work was supported by EU FP7 projects (STEMMAD, PIAPP-GA-2012-324451; EpiHealth, HEALTH-2012-F2-278418; EpiHealthNet, PITN-GA-2012-317146; D-BOARD, FP7-HEALTH-2012-INNOVATION-1-305815).

**O-8**

*Oral Presentation*


## Fazeli, Alireza

Department of Human Metabolism, University of Sheffield, United Kingdom

Fazeli A


# Tolerance is the key message for potential mothers


After the publication of the first report on the alteration of the de novo synthetized oviductal proteins in response to spermatozoa nearly 23 years ago, today we have a much better and clearer view regarding the changes taking place on the arrival of gametes and embryos in the female reproductive tract. Recent pieces of evidence repeatedly point to the proteomic, genomic and epigenomic alterations caused by the presence of gametes and embryos in the maternal tract. The exact purpose of these changes is not known. They are probably involved in mediating the final maturation of the gametes, regulating the early development of embryo(s), taking part in the implantation process and establishing the pregnancy. They may even have inter-generational effect on the epigenomic profile of the offspring. In my presentation I will provide examples from my own and other laboratories in order to explain the role of the maternal communication with gametes and embryos in various reproductive processes. I will highlight the missing link between the innate immune regulation and the success of reproduction. A simple observation of newly synthetized oviductal proteins in response to spermatozoa is leading the way to new theories of immune tolerance and discoveries of novel inter-cellular communication mechanisms today.

**O-9**

*Oral Presentation*


## Fischer, Bernd

Department of Anatomy and Reproductive Biology, Martin Luther University Halle, Germany

Fischer B, Biemann R, Schadlich, K, Schmidt J-S, Schindler M, Pendzialek M, Navarrete Santos A


## Stage-specific effects of environmental contaminants during development

Prenatal development is the most vulnerable ontogenetic stage in life. Alterations of uterine developmental conditions may threaten, harm or misprogram further development. Maternal metabolic diseases and external environmental contaminants (EC) belong to the most serious risk factors. About 800 chemicals are known or suspected to be capable of interfering with metabolic and/or endocrine regulatory mechanisms. Among the implicated disorders obesity currently attracts high attention. Adipogenesis during early development is affected by ubiquitous EC, but also by maternal insulin-dependent diabetes mellitus. Among the many EC present in the environment, plasticisers (phthalate DEHP), industrial chemicals (PCB) and anti-fouling preservatives (tributyltin, TBT) have been studied on their effects on adipogenesis. Female mice were exposed via food (in vivo study) and mouse embryos and stem cells were exposed in vitro, employing single compounds and mixtures in various dosages and at different developmental stages. DEHP-fed mice and their offspring (F1) showed an increase in body weight, food intake and visceral fat. In blastocysts derived from F2 females the expression of adipogenic target genes was altered. In murine mesenchymal stem cells DEHP and TBT affected adipogenesis stage-specifically and by PPAR dependent and PPAR independent mechanisms. It is noteworthy that also a maternal diabetes altered the lipid metabolism early in development (rabbit blastocysts). To conclude, various factors and mechanisms are able to affect the metabolism of embryonic cells. Metabolic plasticity of embryonic cells may be a temporary advantage securing further development but – highly likely - on the expense of an altered metabolism with effects on prenatal development and adult metabolic diseases.


Supported by EU (FP7-REEF No 212885 and EpiHealth N0 278418) and DGKL (Foundation for Pathobiochemistry and Molecular Diagnostic).

**O-10**

*Oral Presentation*


## Fleming, Tom

Centre for Biological Sciences, University of Southampton, United Kingdom

Fleming TP


## Mechanistic analysis of periconceptional programming


The periconceptional (PC) period is sensitive to environmental influences which may change the programme of development and have lasting effects on disease risk into adulthood. We have investigated maternal undernutrition during PC development in mice (low protein diet, LPD). Restricting LPD to just the preimplantation period with normal nutrition thereafter (Emb-LPD) is sufficient to induce cardiovascular, metabolic and behavioural disease risk in adult offspring. Emb-LPD programming initiates through reduction in insulin and branched-chain amino acid (BCAA) concentrations within maternal serum and/or uterine fluid. Blastocysts sense this metabolite change via the mTOR signal pathway. The blastocyst becomes programmed even if transferred to control mothers. Similarly, in vitro culture of embryos in reduced insulin and BCAAs lead to similar adult disease phenotype after transfer. Programmed embryos undergo compensatory responses within the extra-embryonic cell lineages (trophectoderm; primitive endoderm) to promote nutrient retrieval during gestation and support fetal growth. These responses comprise increased proliferation, endocytosis and motility of affected tissues and include epigenetic changes that persist after diet treatment. In contrast, embryonic lineages show evidence of increased apoptosis and reduced survival signalling. Emb-LPD compensatory responses induced within extra-embryonic lineages cause increased perinatal growth. However, perinatal weight in programmed offspring correlates positively with disease risk in later life. A continuum of biological processes therefore exists linking poor maternal PC diet with adult disease risk. Funded through BBSRC, EU FP7 EpiHealth and EpiHealthNet.

**P-11**

*Poster Presentation*


## Gandolfi, Fulvio

Department of Animal Sciences, University of Milan, Italy

Gandolfi F, Pennarossa G, Maffei S, Brevini TAL


# Genome-wide methylation profile related to different plasticity states


Development is regulated by epigenetic mechanisms that gradually decrease cell potency to a phenotype-related expression pattern. This process drives cells along different plasticity states and is still to be fully understood. To further investigate these aspects, we used a protocol where a differentiated adult cell is pushed into a higher plasticity state, though the use of a de-methylating agent, and then converted to a different cell lineage. We characterized the genome-wide methylation profile during the different steps of the conversion protocol. We analyzed untreated human skin fibroblasts (phenotype 1, Phe1), fibroblasts exposed to 5-azacytidine (post 5-aza), and cells re-addressed to the new phenotype (phenotype 2, Phe2). DNA methylation frequency of Phe1 cells was 54.07±2.7%, while 5-aza treated cells showed a significant decrease (31.17±2.4%). De-methylation affected the whole genome, with a decrease of methylation in the promoter (6.8%), inside (18.9%), downstream (1.5%), and unknown (3.3%) regions. This was associated with a clear morphological change. Cells lost fibroblast elongated aspect, showed a round smaller shape, with reduced cytoplasm and larger nuclei. Phe2 cells displayed increased methylation levels (40.54±1.2%), in all regions analyzed: promoter (8.8%), inside (24.9%), downstream (1.9%), and unknown (4.2%). In agreement with this, cells acquired a new morphology, exhibiting an epithelioid pattern. Venn diagram of functional genes indicated that 27/1037 genes remained expressed in all samples, 929/1037 were differentially expressed in Phe1 vs post 5-aza, and 81/1037 when comparing Phe1 vs Phe2. The data obtained demonstrate that different plasticity states display specific methylation patterns and epigenetic roadmaps. These are related to gene expression profile changes and are accompanied by morphological modifications that are likely to cooperate in the control of cell fate transition, commitment and phenotype specification.

**P-12**

*Poster Presentation*


## Gasselin, Maxime

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

Gasselin M, Prezelin A, Boutinaud M, Debournoux P, Neveux A, Mottin A , Kiefer H, Jammes H


# Global DNA methylation levels in bovine peripheral blood mononuclear cells and in milk somatic cells


Epigenetic modifications such as DNA methylation play a role in regulating gene expression and consequently in biological processes like health and diseases. In human, studies provide evidences that the methylome in peripheral blood mononuclear cells (PBMC) can reflect disease susceptibility (Reinius et al, 2012). In dairy cows, the post calving period is characterized by changes associated with an immunosuppression increasing the susceptibility to mastitis as well as post-partum uterine diseases. This study aims to quantify the global DNA methylation levels at two lactation stages (J15 and J60) in PBMC and in milk leukocytes and epithelial cells purified as previously described (Boutinaud et al, 2013). 52 lactating Holstein were housed at two INRA's experimental dairy farms. Parity, milk production and quality, health and diet were notified. Global DNA methylation (MeDNA) was analyzed by a global quantification using a LUminometric Methylation Assay targeting CpG rich regions. 94 samples of PBMC showed MeDNA variation from 72 to 86%. However, no significant difference in MeDNA was found between breeding, parity and lactation stages. In blood, the proportion of different cell types was determined by cytometry and was found stable during the lactation period. DNA methylation amount does not correlate with each cell population count. The analysis of 48 matched samples (milk leukocytes - epithelial cells – PBMC) demonstrated that MeDNA was cell type specific but not modified with the lactation period or the parity. Some cows exhibited diseases like mastitis or uterine post-partum disease. MeDNA in PBMC from these pathological cows was not significantly altered in comparison with MeDNA in PBMC from healthy individuals. This study highlights that MeDNA is cell type specific. However, the change does not be associated with breeding, parity or health in dairy cattle. Studies to identify of specific changes in DNA methylation using genome-wide analysis are in progress.

**P-13**

*Poster Presentation*


## Gonzalez-Bulnes, Antonio

Comparative Physiology Laboratory, Spanish National Institute for Agricultural and Food Research and Technology (INIA), Spain

Gonzalez-Bulnes A, Torres-Rovira L, Astiz S, Chavatte-Palmer P, Lopez-Bote CJ, Ovilo C


## Sex matters even in prenatal life

Currently, it is well established that consequences of prenatal nutritional programming are influenced both by the timing, degree and duration of the challenge and by the adaptive response of the mother and the conceptus. However, the most recent studies suggest that the adaptive responses of the foetuses are also strongly modulated by their sex. Hence, females, which are more critical for the survival of the species, would have better survival and developmental traits than males. Most of the data are based in studies performed in rodents, but evidences in large mammals and humans are elusive. Pigs are a reliable large animal model for translational research in intrauterine growth retardation (IUGR) and subsequent prenatal programming. We are working with a swine model, the Iberian pig, which is characterized by the development of a thrifty genotype with effects on food intake, body weight and fat deposition; Iberian pigs become obese in case of food excess. The IUGR offspring have compromised health status and reduced growth potential in both Iberian and lean commercial breeds. These effects are similar in males and females from lean breeds, but the Iberian pigs have a clear sexual dimorphism. The Iberian males affected by nutritional restriction have reduced growth potential like the offspring from lean swine breeds; IUGR in Iberian males cannot be compensated during postnatal growth. Conversely, Iberian females show an early-postnatal catch-up growth, as early as during lactation, and weight and size are compensated at weaning. Such sex-related differences in early-postnatal developmental patterns may be caused by sex-related prenatal differences in developmental and metabolic traits between male and female Iberian foetuses exposed to prenatal programming. These differences are not found in foetuses from lean breeds, confirming a genotype-related effect which would favour the development of female Iberian offspring in case of environmental challenges.

**P-14**

*Poster Presentation*


## Gould, Joanna

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Gould JM, Smith PJ, Airey CJ, Warricker F, Egar J, Marfy SJ, Fleming TP, Willaime-Morawek S


# Maternal protein restriction around conception reduces the neural stem cell pool during mouse fetal brain development and alters neuronal differentiation


Maternal protein malnutrition during pregnancy and lactation compromises brain development in late gestation and after birth, affecting structural, biochemical and pathway dynamics with lasting consequences for motor and cognitive function. However, the importance of nutrition during embryogenesis for early brain development is unknown. We have previously shown maternal low protein diet confined to the preimplantation period (Emb-LPD) in mice is sufficient to induce cardiometabolic and locomotory behavioural abnormalities in adult offspring. Here, we report, using in vivo and in vitro techniques, that Emb-LPD and sustained LPD reduce neural stem cell (NSC) and progenitor cell numbers through suppressed proliferation rates in both ganglionic eminences and cortex of the early (E14.5) fetal brain. Moreover, Emb-LPD causes remaining NSCs to upregulate the neuronal differentiation rate in compensation beyond control levels. These data demonstrate poor maternal nutrition around conception, already associated with adult behavioural deficit, adversely affects early brain development.

**P-15**

*Poster Presentation*


## Grybel, Katarzyna

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Grybel K, Canon E, Pendzialek M, Schindler M, Gurke J, Blachere T, Godet M, Fischer B, Navarrete-Santos A, Duranthon V


# Investigation of DNA methylation role in periconceptional programming in early stage embryos from rabbit diabetic pregnancy model


DNA methylation is a conservative epigenetic marker. A correct DNA methylation pattern is essential for embryonic development. Mammalian embryos gain a complete de novo DNA methylation design around implantation. DNA methylation is a potential mechanism of periconceptional programming, suggesting that the DNA methylation pattern of developing embryo might be affected in distinct ways, depending on nutritional and hormonal signals. To expand this idea we have investigated the DNA methylation pattern of the promoter region of the POU5F1 (Oct4) pluripotency gene in preimplantation rabbit embryos at day 6 p.c (early gastrulation stage). The blastocysts were collected from healthy and diabetic rabbits. The POU5F1 promoter contains four conservative regions, which are embracing important elements for its transcription: proximal and distal enhancers (PE-1A, PE-1B, DE-1A), a SOX2/Oct4 binding site and hormone responding element (HRE). We have characterized the CpG islands methylation at all mentioned regions in two embryonic tissues: embryoblast and trophoblast, using bisulfite treatment, cloning and sequencing. The analysis has been successfully performed for the trophoblast tissue yet. A higher methylation of the CpG islands for HRE and at the beginning of first exon has been noticed in the trophoblast of diabetic embryos. A hypomethylation in trophoblast from diabetic embryos comparing to trophoblasts from healthy pregnancies was visible for proximal enhancer 1A (PE-1A) in frame of conservative region 3 (CR3). In this context, DNA methylation can be considered as a form of embryo developmental plasticity, which can promote metabolic disorders in adult life.

*Oral Presentation*

## Guillette, Louis

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Guillette LJ

## From sentinel species to human health: the epigenetics of environmental health

Wildlife, domesticated and laboratory animals have been used to predict detrimental human health effects from environmental variables for decades. There is growing concern, however, that exposure to low levels of 'endocrine-active' contaminants early in embryonic development coupled with altered climate can lead to altered phenotypes, and disease. Although each species is unique, molecular, cellular and physiological systems are conserved allowing insight into the process of human health from 'sentinel species' studies. A large and growing literature has now demonstrated that 1) classical gene mutations likely account for less than 20% of known disease (in many cases as low at 8 – 10 %), 2) linear dose response curves poorly predict adverse responses to low levels of environmental contamination and exposure to complex mixtures, and 3) altered gene expression, via epigenetic mechanisms, can be induced by varying diets, stress and low level exposure to various environmental contaminants, including metals and organics. These epigenetic modifications are being readily linked to predisposition for disease. This talk reviews, in part, the work done by my laboratory on wildlife species, such as the American alligator, examining the effects of various environmental contaminants on the development and functioning of the endocrine and reproductive systems from the epigenetic to organismal level. I will relate this work to implications for modern human health care as well as environmental management and conservation.

**O-17**

*Oral Presentation*


## Gutierrez-Adan, Alfonso

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Ramos-Ibeas P, Pericuesta E, Laguna-Barraza R, Forde N, Maillo V, O'Gaora P, Bermejo P, Rizos D, Lonergan P, Gutierrez-Adan A


# Identification of mRNA splicing factors in bovine sex determination


We have reported transcriptional differences between male and female bovine embryos occurring during preimplantation before gonadal sex determination. Regulation at the transcription level is believed to be the essential mechanism governing the mammalian sex determination cascade to produce sex differences in the gonads; however, the mechanisms controlling sex differences prior to sexual differentiation of the gonads and outside of the gonads are unknown. We propose that alternative splicing could play a critical role in these sex-determining pathways in mammals. Using the GeneChip Bovine Array Affymetrix®, we analyzed the differential gene expression of mRNA splicing factors between male and female bovine embryos/conceptuses on Day 7 and 19 of pregnancy. Sex of the embryo or conceptus affected the expression of 47 (Day 7) and 111 (Day19) splicing factors, of which the expression of 17 (Day 7) and 72 (Day 19) were increased in males. Six splicing factors are located on the X-Chr (RMBX2, CSTF2, GEMIN8, USP9X, NONO, and RBM3) and one on the Y-Chr (ZRSR2Y). The ontology analysis of all differentially expressed genes between male and female bovine/conceptus indicated that the sex of the embryo/conceptus significantly affected 102 biological processes. However, this would be expected if alternative splicing is the cause of the dimorphism, as it usually affects all aspects of cell biology. We have also cloned a new gene on the bovine Y-Chr, ZRSR2Y, which has three paralogues encode for mRNA splicing factors: U2AF1, U2AF1L4, and ZRSR2 (located on the X-Chr of all mammals studied thus far). ZRSR2Y was found to be uniquely expressed in all of the bovine male tissues analyzed except the spleen, and in male bovine and porcine embryos. Preliminary analysis in bovine embryos indicates that ZRSR2Y is also expressed in the genital ring at Day 39, before testis cords are distinguishable (Day 42), and it is upregulated ahead of DMRT1, a critical gene in male sex determination.

**O-18**

*Oral Presentation*


## Heifetz, Yael

Department of Entomology, Hebrew University of Jerusalem, Israel

Yael H


# Studying the periconception environment in Drosophila: a model organism to bridge unknown gaps


For animals with internal fertilization, the female reproductive tract is more than just a locale for gametes to convene; it facilitates the passage of gametes, gamete activation, gamete fusion and embryo development. In insects and mammals, complex processes of differentiation and tissue morphogenesis at specific development stages prepare the female reproductive tract for attaining this functional goal. It is now accepted that this environment - the milieu of the female reproductive tract at and around the time of mating (the periconception environment) - dynamically changes in a way that is essential for successful fertilization and that impacts the progeny's future phenotype. However, little is known about how the periconception environment and its composition are regulated. Moreover, it is not clear how the periconception environment responds to / is affected by the outside environment. To address these issues, we are using the fruit fly Drosophila melanogaster to investigate how the reproductive tract environment responds to external stimuli. In Drosophila, secretory glands and secretory epithelial cells along the female reproductive tract are the sources for molecules that create this environment. Though insects and mammals are distantly related, their genome and physiology indicate that many aspects of their reproductive biology are conserved. In my talk I will present the power of Drosophila as a wonderful model to make inroads into the mechanisms that set up the reproductive tract periconception environment. I will further discuss how Drosophila can provide a foundation for future work on the female reproductive tract in diverse animal lineages, including humans, to better understand how the reproductive tract environment prompts successful fertilization.

**O-19**

*Oral Presentation*


## Houghton, Franchesca

Centre for Human Development, Stem Cells & Regeneration, University of Southampton, United Kingdom

Houghton FD


## Oxygen tension and epigenetic regulation of developmental genes in embryonic cells

Human embryonic stem cells (hESCs) derived from the inner cell mass of the blastocyst, proliferate by self-renewal and can give rise to all cells of the body. Thus, they have great potential for regenerative medicine and the treatment of degenerative disorders such as Parkinson's disease and type 1 insulin-dependent diabetes. hESCs are notoriously difficult to maintain in culture as the cells have a propensity to spontaneously differentiate. Fundamental research is therefore required to understand how pluripotency is regulated, not only to improve hESC maintenance, but also advance the efficient and uniform differentiation of cells down specific lineage pathways. In vivo, the blastocyst resides in a low oxygen tension and in vitro, culture at reduced oxygen levels have been shown to be beneficial for embryo development. Thus, the atmospheric oxygen conditions routinely used to maintain hESCs may not be optimal. Research in my lab has found that culture at a reduced oxygen tension is beneficial for the maintenance of a highly proliferative, pluripotent population of cells compared to atmospheric oxygen. These effects are mediated by a family of hypoxia inducible factors (HIFs), with HIF-2α regulating the long term response to hypoxia in hESCs. Culture under hypoxic conditions induce epigenetic changes in the proximal promoters of pluripotency genes which are associated with transcription, while a heterochromatic state exists in hESCs maintained at atmospheric oxygen conditions. Together, our data highlights environmental oxygen tension as being a critical regulator of hESCs.


Work funded by the Gerald Kerkut Trust, MRC UK, and the University of Southampton.

**P-20**

*Poster Presentation*


## Hulinska, Pavlina

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Hornak M, Kubicek D, Broz P, Hulinska P, Hanzalova K, Machatkova M, Rubes J


# Universal approach for aneuploidy detection in embryos of farm animals using Next-Generation sequencing-based protocol


Aneuploidy presented in embryos is a significant cause of embryo developmental arrest, abortions, stillbirths or a birth of affected individual. In farm animals, the data on frequency of aneuploidy and its nature is lacking. During in vitro production (IVP) of farm animals, it is suggested that in vitro processes, such as oocyte maturation, IVF and embryo cultivation, adversely influence chromosome segregation, which results in an increased aneuploidy, and in turn in lower IVP efficiency. Thus, our goal was to develop universal technology capable of detecting aneuploidy of all chromosomes across all significant farm animal species applicable to a single cell. Whole genome amplification by PicoPlex WGA kit (Rubicon Genomics, Inc.) was performed to amplify DNA from blastomeres derived from porcine and bovine embryos. Amplified DNA was quantified and DNA library containing sequencing adaptors and unique barcodes was prepared for each sample using Nextera XT DNA Sample Prep Kit (Illumina, Inc). Up to 48 individual samples were barcoded, pooled and sequenced on MiSeq sequencing system (Illumina, Inc.). Further, raw sequencing data were de-barcoded and FASTQ files produced for each sample. Then, FASTQ files were aligned to reference genome (susScr3 or BosTau8) with Burrows-Wheeler Aligner (BWA) algorithm. Unmapped and multi-mapped reads were filtered out. Finally, the number of reads was counted for each chromosome and its 10Mb region (bin). Bin read counts were normalized to the total read count for each sample and were compared to the bin read counts in an amplified male euploid reference sample. Sample reference bin read counts ratio >1.3 and <0.7 was indicative of chromosomal region gain and loss, respectively.



Supported by grant COST CZ - LD14104 of the Ministry of Education of the Czech Republic.

**P-21**

*Poster Presentation*


## Ivask, Marilin

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Ivask M, Plaas M, Jaakma U, Koks S


# Production of human growth hormone in the milk of transgenic mice


The production of biopharmaceutical proteins in the mammary glands of transgenic dairy animals promises to provide high-quality therapeutic medicine for humans at an acceptable cost. The aim of this study was to generate human growth hormone (hGH) transgenic mice to test functionality of the expression vector for the potential scale-up of hGH production in the milk of transgenic dairy animals. Two hGH transgenic embryonic stem cell lines were used for injecting into blastocysts to generate chimeras. Altogether 11 chimeric pups were born which were assessed by coat colour and PCR. The chimeras were mated with wild type mice and milk was collected from F1 female animals. Different tissues were collected from F1 females for qRT-PCR analysis. The F1 females were separated from pups and injected with 2 UI of oxytocin. The collected milk was defatted by centrifugation and stored at -20°C. The qRT-PCR results from tissues of non-lactating F1 females showed no expression of hGH. Analysis of hGH transgenic milk with Western blot showed that hGH can be detected from mouse milk. The estimated concentration from Western blot analysis was in range of 0.2-0.6 mg/mL. The presence of hGH in the transgenic milk was also verified by LC-MS/MS using human pituitary growth hormone as a standard. In conclusion, our study provides supporting evidence to explore the production of human growth hormone in the milk of dairy animals.



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**P-22**

*Poster Presentation*


## Jammes, Helene

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

Guillomot M, Prezelin A, Kiefer H, Beaujean N, Aguirre-Lanvin T, Salvaing J, Jammes H


# Analysis of methylated DNA contents and distribution in bovine placental and foetal tissues after somatic nuclear reprogramming


DNA methylation (methylated (5-mC) and hydroxymethylated (5-hmC) DNA), is highly dynamic during mammalian embryogenesis. After fertilization, a loss of 5-mC of maternal (passive process) and paternal (active process) genomes takes place; 5-hmC accumulates in the paternal pronucleus and decreases progressively with the embryonic cleavage. In blastocyst, the first cell differentiation is associated with higher levels of 5-mC in the inner cell mass compared with the trophectoderm. During foeto-placental development, the patterns of 5-mC and 5-hmC are established according to cell specification and tissue differentiation. In some bovine blastocysts produced by somatic cell nuclear transfer (SCNT), a global DNA hypermethylation has been observed in the trophectoderm. Defaults of placental and foetal development are frequent after SCNT in mammals. Whether these pregnancy pathologies are associated with alterations of global DNA methylation remains to be analysed. In this study, the patterns of DNA methylation were determined in extra-embryonic and foetal tissues during pregnancies obtained by artificial insemination (AI) versus SCNT i) by a global quantification using a LUminometric Methylation Assay and ii) by immunohistochemistry with specific antibodies. Great variations in methylation levels between extra-embryonic and fœtal tissues at D60 of gestation were observed from 30% to 90% (chorionic villi > chorion > liver > heart > brain > amnion > allantois). Chorionic methylation content increased significantly from D18 until term of gestation. Effects of SCNT on trophoblast methylation level in blastocyst were conserved in trophoblast at D18 and D40 and in cotyledons at G60 only. However, by immunocytochemistry, only the mesenchymal part of chorionic villi was stained using 5-mC antibody and an unexpected 5-hmC staining was found in various placental and extra-embryonic cell types, suggesting a role of this epigenetic mark in all lineages in bovine.

**P-23**

*Poster Presentation*


## Kaczmarek, Monika Marzena

Molecular Biology Laboratory, Institute of Animal Reproduction and Food Research, Poland

Kaczmarek MM, Mendoza T, Kozak LP


# Molecular reprogramming of the hypothalamic neural circuits controlling reproductive functions observed in the second generation, not exposed to malnutrition during early postnatal life


Although early life nutrition appears to be an important determinant for optimal mammalian reproduction, the precise neurobiological mechanisms that contribute to this phenomenon are not understood. Thus in the present study we hypothesized that transient malnutrition experienced during early development affects the maturation of the HPG-axis and subsequent reproductive performance of the second generations, not exposed to malnutrition. We used a well-established mouse model of undernutrition, causing severe leptin and insulin deficiency, to show that progeny (F2) of neonates exposed to malnutrition during lactation acquire gender-specific molecular reprogramming of the hypothalamus and delayed puberty. In our mouse model, F1 mice were breed according to their nutritional protocol (undernourished, LUN=L; control, CON=C) to generate F2 progeny originating from control (CxC), mixed (CxL, LxC), and undernourished (LxL) parents. We observed that both female ($P < 0.05$) and male ($P < 0.001$) F2 progeny showed delayed puberty when only mother (LxC) or both parents (LxL) were undernourished. qRT-PCR analysis performed on the hypothalamus of 21-day old F2 progeny showed altered expression of several genes, including kisspeptin-signaling related genes. Although Kiss1 mRNA levels were downregulated in LxC and LxL F2 progeny irrespective of gender ($P<0.05$), Leprv1 expression was upregulated only in LxC and LxL F2 males (vs. CxC, $P<0.001$). Moreover, gender specific patterns of Hcrt expression was observed in F2 progeny, with decreased levels in CxL and LxL females (vs. CxC, $P<0.01$ and $P<0.0001$, respectively) and increased in CxL, LxC, and LxL males (vs. CxC, $P<0.05$, $P<0.01$ and $P<0.0001$, respectively). It seems likely that the molecular and physiological effects of post-natal malnutrition can lead to gender specific changes of hypothalamic neural circuits controlling reproductive functions in the second generation, not exposed to malnutrition during early postnatal life.

**O-24**

*Oral Presentation*


## Kolakowska, Justyna

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Kolakowska J, Franczak A, Souchelnytskyi S


# The effect of intrauterine environment on proteome profile of the endometrium in pigs (Sus scrofa domestica)


Intrauterine environment differs between days 12-13 and 15-16 of pregnancy and corresponding days of the estrous cycle, with progesterone (P4) and estradiol 17β (E2) having potentially strong regulatory role. The aim of our study was to determine the proteome profiles of porcine endometrium harvested during maternal recognition of pregnancy (days 12-13) and the time of peri-implantation period (days 15-16), and to compare these profiles with proteome profiles of the endometrium from the respective days of the estrous cycle. Total proteins were extracted from pregnant endometrial tissue (n = 4 from each period) and cyclic (n = 4 from each period). Tissue extracts were used for two-dimensional gel electrophoresis. Then, MALDI TOF mass spectrometry and ProFound search engine were used to identify proteins which changed expression. Systemic analysis of the identified proteins was performed using GoMiner and Cytoscape tools. We detected in average 500 proteins in proteome maps of the tested samples, and identified 21 proteins differentially expressed between the samples. The identified proteins are classified in 24 biological processes, mostly regulatory and cellular processes and signaling. We focused on exploration of 2 proteins which occurred specifically in pregnant pigs. i.e. DNA mismatch repair protein Msh3 (d 12-13) and kinesin-like protein KIF6 (d 15-16). We propose that these proteins might be important to create proper intrauterine environment for developing embryos. Therefore, we report a number of potential novel regulators of pregnancy in pigs, and perform a validation study of two selected proteins.



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

**O-25**

*Oral Presentation*


## Kotaja, Noora

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Kotaja N


# RNA regulation during spermatogenesis: importance in male fertility and potential role in epigenetic inheritance

Spermatogenesis is a complex differentiation program that includes specialized processes and dramatic epigenetic transitions, and that has to be strictly controlled to prevent the transmission of harmful information to next generations. An alarming decline in the semen quality and reproductive health in Western countries has evoked concern about the adverse influence of environment on spermatogenesis. Male germ cell differentiation is orchestrated by cell type-specific gene expression patterns. In fact, male germ cells have an exceptionally diverse transcriptome that in addition to mRNAs and ncRNAs, also includes a wide range of non-annotated intergenic transcripts. The complex transcriptome creates a demand for efficient RNA regulation. We have demonstrated an importance of the miRNA-mediated post-transcriptional regulation in spermatogenesis by analyzing a conditional Dicer knockout mice. We have also been interested in elucidating the mechanisms that coordinates the male germ cell's transcriptome, especially focusing on cytoplasmic male germ cell-specific RNP granules, such as the chromatoid body (CB) in haploid germ cells. By identifying the molecular composition of the CB, we showed that it accumulates a multitude of different RNA-binding proteins as well as a diverse set of RNAs, the piRNA pathway representing the predominant functional pathway in the CB. The reason for the pervasive transcription of the male germ cell genome is still unclear. It is possible that it is a non-specific consequence of the dramatic chromatin modifications taking place during meiosis and post-meiotic differentiation. However, it also provides an intriguing opportunity to utilize RNA as a tool for epigenetic inheritance. Given the constant flow of cellular RNAs into the CB and its molecular composition, it appears that the CB has a central role in RNA surveillance during the critical time when the haploid male germ cell is preparing its genome and epigenome for fertilization.

**O-26**

*Oral Presentation*


## Kuran, Mehmet

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Sen U, Kuran M


# Low oocyte maturation temperature successfully support the subsequent bovine embryo development to blastocyst stage in vitro


It is known that the temperature of the maturing oocyte's follicular environment in the ovary is 2–3 °C lower than the core body temperature in the cow. However, conventional IVM culture of bovine oocytes is performed at a higher (38.5 °C) than for those oocytes matured within the ovarian follicular environment in vivo. Therefore the aim of the present study was to investigate the effect of 36.5 °C and 38.5 °C incubation temperatures in maturation on nuclear and cytoplasmic maturation of bovine oocytes in vitro. A total of 1245 cumulus oocyte complexes classified as good quality obtained from bovine ovaries were subjected to IVM for 22 hours in a humidified atmosphere of 5% CO2 in air at either 36.5 or 38.5 °C. After in vitro fertilization (IVF), presumptive zygotes were cultured at 38.5°C for 8 days. There were no significant differences between oocytes matured in either at 36.5 or 38.5 °C incubation temperatures in terms of nuclear maturation parameters. 81.7 vs 75.0% of oocytes reached to MII stage in maturation temperatures of 36.5 vs 38.5 °C. Oocytes matured at 36.5 °C resulted in a similar blastocyst yield to those matured at 38.5 °C following (32.6 vs 29.8%) and the quality of blastocysts were also similar. The number of cells in inner cell mass and trophectoderm and total number of cells were similar in blastocysts from oocytes matured either at 36.5 or 38.5 °C maturation temperature. The results of this study indicate that bovine oocytes matured at 36.5°C temperature yield a similar development rate to the blastocyst stage compared to those matured at a conventional maturation temperature. It can be concluded that culture of bovine oocytes during in vitro maturation at 36.5 °C incubation temperature which represent in vivo conditions may provide a suitable thermal environment for the completion of nuclear maturation and subsequent embryo development.

**P-27**

*Poster Presentation*


## Lombo, Marta

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


# Transgenerational paternal inheritance of the toxic effects of bisphenol A


Bisphenol A (BPA) is an endocrine disruptor widespread used in manufacturing of plastics and epoxy resins for food and sanitary items, resulting in a long-term exposure in humans. Relevant concentrations of BPA have been reported in human urine and epidemiologists have established correlations with different pathologies. It is well known that direct exposure to BPA or maternal exposure during pregnancy, promote metabolic alterations, reproductive disorders or cardiovascular diseases. Moreover some reports indicate epigenetoxic effects, including changes in the expression of DNMTs, global DNA methylation or methylation of specific promoters. The potential modification of the germ line might lead to transgenerational inheritance of these toxic effects, but the paternal factor has not been so far studied. In our study adult zebrafish males were exposed to BPA (100 and 2000 ug/L) during spermatogenesis to assess potential effects on F1 and F2 development after mating with non-exposed females. Global DNA methylation and methylation of specific promoters (vasa, pou5f1 and sox2) was analyzed in sperm from F0 and F1, as well as in 48hpf embryos. Malformations were characterized in F1 and F2 larvae 7dph and the expression of 7 genes related to cardiac development (myh6, cmlc2, tbx5, atp2a2b, sox2, insra and insrb) was evaluated in the F1. Results showed a significant increase of the rate of cardiac edema and heart failures in the unexposed progeny up to the F2, as well as downregulation of 5 from the 7 analyzed genes. F1 embryos also showed an increase in global DNA methylation and demethylation of sox2 promoter, whereas no similar effects were noticed in sperm. Our results clearly demonstrate a paternal transgenerational inheritance of the toxic effects of BPA, affecting gene expression and organogenesis in the progeny. The increased rate of malformations in the F2, clearly support changes in the paternal germ line that require a deeper study.

**P-28**

*Poster Presentation*


## Martyniak, Marcin

Faculty of Biology and Biotechnology, Department of Animal Physiology, University of Warmia and Mazury, Poland

Martyniak M, Zlotkowska A, Zglejc K, Franczak A, Kotwica G


# Steroidogenic activity of pig oviduct during early pregnancy


Steroid hormones – estradiol-17β (E2) and progesterone (P4) derived from ovaries are regulators of reproductive tract functions, including oviduct. The appropriate microenvironment in oviduct is necessary for final maturation of gametes and their transport as well as for fertilization and early embryonic development. We have determined that steroids are also produced by endometrium and myometrium of cyclic and pregnant pigs and may affect uterine tissues through local manner. We hypothesizes that tubal cells of early pregnant pigs may be a source of E2 and P4. Steroids produced by oviducts can complement action of those of ovarian origin in order to optimize tubal milieu. Steroid synthesis requires activity of specific enzymes: 3β-hydroxysteroid dehydrogenase/Δ-5-4 isomeraseenzyme (3βHSD) and aromatase P450 (P450arom) - product of CYP19 gene. 3βHSD converts pregnenolone into P4 or androstendion (A4). P450arom converts A4 and testosterone (T) into E2. Thus, in this study, the expression of 3βHSD and CYP19 mRNAs in ampulla and isthmus of oviducts harvested on Days 10-11 (n=4), 12-13 (n=4) and 15-16 (n=4) of early pregnant pigs were determined. The expression of 3βHSD mRNA in both ampulla and isthmus was low on Days 10-11 and 12-13 and increased ($p \leq 0.05$) during 15-16 Days of pregnancy. On Days 12-13 this expression was lower ($p \leq 0.05$) in ampulla vs. isthmus. The expression of CYP19 mRNA in ampulla was similar on Days 10-11 and 15-16 and was lower on Days 12-13 vs. Days 10-11 of pregnancy. CYP19 mRNA expression in isthmus did not differ ($p > 0.05$) between studied days. In conclusion: 1) 3βHSD and CYP19 mRNAs are present in ampulla and isthmus of oviducts in early pregnant pigs, 2) this expression is dependent on days of early pregnancy, 3) the results demonstrate the ability of tubal cells in early pregnant pigs for steroid enzymes production after the time when embryos leave the oviduct.

**O-29**

*Oral Presentation*


## McGhee, Katie

Department of Zoology, University of Cambridge, United Kingdom

McGhee KE, Bell AM


## Maternal stress, paternal care, and consequences for offspring

In many species, the environmental conditions experienced by mothers while pregnant or yolking eggs can affect the prenatal environment that offspring experience during development. For example, maternal stress can expose offspring to elevated levels of maternal stress hormones and result in life-long consequences for offspring behavior and morphology. Additionally, the care that parents provide to offspring after birth or hatching can affect the postnatal environment that offspring experience early in life and being deprived of this parental care has deleterious consequences for offspring of many species. Unlike mammals where mothers provide both the prenatal and postnatal experiences for offspring, many fishes have father-only care. Thus maternal effects due to egg provisioning and paternal effects due to offspring care are separated from one another allowing us to tease apart the importance of prenatal and postnatal experiences on offspring phenotype. Using the threespined stickleback we have found that an ecologically-relevant maternal stress (predator-exposure) has negative consequences for offspring behavior and survival. Furthermore, we have found that stickleback families differ in the sensitivity of their offspring to the care provided by fathers and that the type of care provided by fathers is linked to gene expression differences in offspring brains. Finally, father care is able to compensate for some, but not all, of the effects of maternal stress.

**P-30**

*Poster Presentation*


## Muino-Blanco, Teresa

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# Involvement of JNK and p38 MAPK pathways in ram sperm capacitation and apoptosis


Unlike most other species, ram spermatozoa are difficult to capacitate in vitro, and the involvement of Epidermal Growth Factor (EGF) signal-regulated MAP Kinase ERK1/2 in sperm capacitation has been described in sheep. The purpose of this study was to determine the role of the other two EGF receptor-induced MAP kinases, JNK and p38, not only in ram sperm capacitation but also in the triggering of the apoptotic pathway and the relationship between these processes. We have evidenced for the first time the presence of these kinases, JNK and p38, in ram spermatozoa by indirect immunofluorescence and western-blot analysis, and their activation during in vitro capacitation with EGF. To verify the involvement of the JNK and the p38 pathways in ram sperm physiology, as two signal mechanisms that contribute to the modulation of capacitation and apoptosis, we tested the effect of adding specific JNK (SP-600125) and p38 (SB-203580) inhibitors in a dose-dependent manner during incubation in EGF-capacitating conditions (3 h). The obtained results showed an inhibition effect on capacitation at a concentration of 100 µM, with a decrease in both the percentage of capacitated-sperm pattern assessed by the chlortetracycline (CTC) fluorescence assay and protein tyrosine phosphorylation associated with capacitation. Likewise, we found reduced levels of apoptotic markers (phosphatidylserine translocation, activation of caspases and DNA fragmentation) in the presence of these inhibitors. These findings will contribute to our understanding of the biochemical pathways involved in mammalian sperm functionality, and the relationship between capacitation and apoptosis. This opens new perspectives for the study of regulatory mechanisms in spermatozoa that could help in the improvement of ram semen preservation protocols.



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**O-31**

*Oral Presentation*


## Nikolovski, Martin

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# Ram spermatozoa motile and metabolic activity – potentially predictable pattern of ejaculate fatty acid composition


Spermatozoa contain considerably high proportion of polyunsaturated fatty acids (PUFA) thus being prone to peroxidation (PO). According to the “membrane pacemaker theory”, regarding spermatozoa, we hypothesized that fatty acid (FA) composition in the ejaculates could dictate spermatozoa metabolic profile whilst retaining vitality and resilience to PO. Samples were collected from four high performing rams (Pramenka) by artificial vagina. Standardized cell concentrations (~300x106 in TRIS-PBS) were evaluated on CASA for compliance with velocity (VSL-μ/s) and linearity index (STR) cut-off nominal values (VSL ≥60, STR ≥0.8). Total of 91 ejaculates have been categorized in three groups: A (not complying with either nominal), B (complying with one nominal), and C (complying with both nominal), each containing 18, 36 and 37 samples, respectively. FA values have been acquired on gas chromatography with flame ionization detector (GC-FID), expressed as percentile units (mean ±SEM %). PUFA and docosahexaenoic acid (C22:6n3) had detectable peaks in 100% of the cases, while monounsaturated FA (MUFA) in 90%. Individual bias effect was excluded following comparison of ranks between subjects. PUFA and C22:6n3 pointed significantly highest values in A and lowest in C (P=0.0001), being highly correlated (r=0.95, P=0.0001). MUFA resulted with highest concentration in C and lowest in A (P=0.0004). These results indicate that ejaculate motility patterns could have predictable FA composition, possibly deducing the inter-species limits of the theory to in-species level. Higher presence of MUFA in ejaculates characterized by high velocity and linearity could be explained due to possible existence of cellular altering mechanisms that promote this lipid construction, more resilient to PO. The opposite presumption would apply for PUFA. For justification of our findings further experimentations and tests are needed to exclude possible biases and small sample heterogeneity.

**P-32**

*Poster Presentation*


## Ord, James

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# Exploring environmentally-induced epigenetic effects in the live-bearing fish, Poecilia reticulata


Exposure to environmental stress at early developmental stages may induce changes to gene expression, via epigenetic mechanisms. Such changes, which affect the phenotype of an individual, may persist into adulthood and even manifest as disease. P. reticulata, being a viviparous (live-bearing) fish, offers a unique model system for studying the effects of stress in the maternal environment during gestation. This project will assay three major maternal stress factors for effects on the offspring phenotype: dietary restriction, predation signals, and immune stimulation. Offspring will be screened for possible phenotypic changes, which may manifest in development, morphology, and behaviour. Once a phenotypic change linked with maternal stress is identified, possible underlying epigenetic mechanisms will be explored. The role of communication between the embryo and the maternal tract via RNA interference is of particular interest.

**O-33**

*Oral Presentation*


## Otero-Ferrer, Francisco

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# Early nutritional programming windows and seahorse embryo development


The seahorse embryo undergoes an interaction with the male's pouch that resembles mammalian implantation. Based on these statements, the aim of this work was to determine the effects of pre- and peri-conception diet on embryonic growth in seahorse offspring. To understand when the males exert the dietary effects on the offspring, the experimental design involved wild-sourced or commercially available seahorse diets, establishing dietary windows before, during and after the time of conception. Seahorse pairs were separated, and the male's feeding regime was changed from wild to commercial diet during the various nutritional windows. The female's diet remained constant (wild-caught) all through the experiment. Physical dimensions of 1-day old offspring from each pair's first brood were measured and the biochemical composition of both, diets and newborns also analysed. Males fed with suboptimal diet (commercial) before conception, produced offspring whose body size was abnormally low compared to wild fed males. Moreover when suboptimal diet was provided around the time of conception, it had also negatively impacted the offspring, producing poor embryonic growth rates and asynchronous body development. Males entirely fed during pregnancy with commercial diets showed smaller newborns but there morphological development was normally synchronized compared to control (Male Wild). Regarding biochemical analysis, males fed with suboptimal diets reduced the docosahexanoic/eicosapentanoic fatty acid ratio in their progeny, showing the contrary trend for eicosapentanoic/arachidonic ratio compared to wild fed males. The preliminary analysis conducted confirmed our hypothesis regarding male sensitive nutritional periods affecting embryo development in terms of biometry, morphology and biochemical composition. Moreover our results suggested that signals produced by diet could induce chemical variations in the male pouch that affect early embryo developmental programme.

**P-34**

*Poster Presentation*


## Ozil, Jean-Pierre

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## The regime of Ca2+ oscillations at fertilization is determined by the culture media formulation


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 quantitative 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.

**P-35**

*Poster Presentation*


## Pawlak, Piotr

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# Follicular fluid and oocytes from prepubertal and cyclic gilts differ in respect to fatty acid content and the number of lipid droplets


Some of the fatty acids present in follicular fluid were shown to exert a positive impact on the developmental competence of oocytes and embryos in vitro whereas some of FAs a negative effect. Therefore the aim of the present project was to analyse the fatty acid profile of porcine FF and to determine the number of lipid droplets in oocytes of prepubertal (P) and cyclic (C) gilts. Additionally we aimed to investigate the effect of puberty of the FF donor (P, C) on the number of lipid droplets in P and C oocytes matured in vitro. Cumulus-oocyte compelxes and follicular fluid were collected from P and C ovaries. COCs form prepubertal and cyclic gilts were matured in vitro with FF from P and C ovaries to elucidate a possible effect of FF donor puberty on oocyte quality. FA profile of follicular fluid was analysed by gas chromatography. Number of lipid droplets in the oocytes were analysed by fluorescence confocal microscopy. The gilt puberty (P,C) significantly influenced the fatty acid profile in the follicular fluid. FF from P gilts contained more total fatty acids (650 vs 570 ug/ml) as well as more particular FAs (eg oleic, palmitic) with a distinct effect on oocyte competence. On the other hand the FF from C gilts was characterized by higher concentration of the linoleic acid. Lipid droplets were less abundant in pre-IVM oocytes from P gilts than C gilts (247 vs 320). The effect of puberty of the FF donor on oocyte quality is currently being evaluated and will be presented on the conference. This study shows different follicular growth environments of prepubertal and cyclic gilts oocytes. In conclusion the donor puberty significantly affected FA profile in the follicular fluid. We suggest that FF composition may influence the oocyte quality since C oocytes contained more lipid droplets. Moreover higher concentration of palmitic acid in FF of prepubertal gilts may negatively affect oocyte quality what has been previously shown for bovine oocytes.

**P-36**

*Poster Presentation*


## Peippo, Jaana

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# Mobilization of intracellular lipids by supplementation of IVM and IVC media with L-carnitine improves bovine embryo quality


Mobilization of embryo lipid by supplementing culture media with metabolic activator is one of the promising tools to improve bovine embryo quality. The present study investigated the effect of L-carnitine supplementation during in vitro maturation (2.5 mM) and embryo culture (1.5 mM) on embryo developmental rates, quality and gene expression profiles. Treatment groups were: T1=IVM+LC, T2=IVC+LC, T3=(IVM and IVC)+LC and control. In vivo produced embryos were included in all analyses. Total cell counts and number of apoptotic cells were evaluated using Tunnel-Hoechst assay. The activity of mitochondria and intensity of lipid were measured using fluorescent probes. Expression of selected candidate genes was profiled using real-time PCR. Although there was an increase in blastocyst rate in T2 (44.4%) and T3 (42.1%) groups compared to T1 (39.2%) and control (38.2%), it was not statistically significant. Embryos cultured with L-carnitine and in vivo had greater total cell numbers than the controls. Noteworthy, the percentage of apoptotic cells from the total number of cells was greater in the controls than in L-carnitine treated and in vivo derived blastocysts. Cytoplasmic lipid content was reduced by 1.8, 2.7, 2.4 and 5.1 times in T1, T2, T3 and in vivo produced blastocysts compared to their control counterparts. Whereas, intracellular mitochondria density was increased by 2.0, 4.8, 4.5 and 6.3 folds in embryos cultured with L-carnitine and in vivo. Genes regulating lipid oxidation (CPT2 and CPT1B), fatty acid transport (SLC27A1) and mitochondria transcription (TFAM) were up-regulated while a lipid storage marker transcript (PLIN2) was down-regulated in embryos cultured in presence of L-carnitine and in vivo compared to controls. Taken together, the lipolytic effect of L-carnitine was linked with increased mitochondrial activity and improved quality of produced embryos which will most likely enhance their survival after cryopreservation and transfer to recipients.

**P-37**

*Poster Presentation*


## Perrier, Jean Philippe

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# Epigenetics of bovine semen: tools to DNA methylation analysis


Spermatogenesis is a long process requiring several epigenetic modifications to obtain a stably packed chromatin. Recent studies suggest that stochastic and environmental disturbances in this genome-wide epigenetic reprogramming may lead to epigenetic aberrations of semen quality, fertility and embryo development (Boissonnas et al., 2013). Establishing reference values and profiles of spermatozoa DNA methylation provides the opportunity to identify biological disturbances in epigenetic marks, as well as protective measures. Here, we present tools to monitor sperm DNA methylation at different scales and to identify individual and temporal variations in the methylation contents and patterns. The global quantification of DNA methylation amount (MeDNA) estimated by LUminometric Methylation Assay, demonstrated a species specificity (bovine, porcine, ovine, human and murine semen). In bovine, the MeDNA wasn't higher in semen than in somatic tissues as well as between fresh and cryopreserved semen. However, breed-dependent variations of semen MeDNA were found. To identify methylated regions in bovine semen, we used immunoprecipitation of methylated DNA followed by hybridization on a bovine promoter microarray (MeDIP-chip). The microarray targets the upstream region (-2000/+1360bp) of 21416 genes (UMD3.1 assembly). After normalization of the data, enriched probes were identified using ChIPmix (Martin-Magniette et al., 2008). Genomic DNA from sperm (4 fertile bulls), liver (19 females) and fibroblasts (3 females) were analyzed. Differentially Methylated Regions between tissues (T-DMRs) were identified using Spatstat R package. T-DMRs in sperm were enriched for genes involved in the spliceosome (DAVID analysis). Most of these T-DMRs were demethylated in sperm. We developed successful and relevant tools to analyze the methylation statue of bovine semen. Now we could analyze the environmental impact on the sperm epigenetic profile according to the spermogram and fertility.

**P-38**

*Poster Presentation*


## Reliszko, Zaneta

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# Analysis of pathways and functions regulated by miRNA transferred by maternal blood during early pregnancy in pig


Early pregnancy in all mammalian species is a complex process, controlled by several genes involved in e.g. remodeling of endometrium, conceptus elongation, and implantation. These genes can be regulated by tissue specific, short (17-25 nt), non-coding RNAs called microRNAs (miRNAs). There are at least two ways that miRNAs can be packaged and transported using extracellular vesicles or pathways involving RNA-binding proteins, and released into maternal circulation. In our previous studies we detected three miRNAs (miR-23b, miR-26a, and miR-125b) in the maternal serum, showing elevated levels during early pregnancy in pigs. In the present study we hypothesized that these miRNAs present in the maternal circulation can be local as well as peripheral regulators of gene expression, involved in multilevel early embryo-maternal crosstalk. TargetScan analysis tool allowed us to predict 1127, 885, 848 target genes for miR-23b, miR-26a and miR-125b, respectively. Ingenuity Pathway Analysis showed that sets of genes targeted by these miRNAs can be involved in local functions, such as development of embryonic cells, elongation of conceptus, vascularization and development of extraembryonic tissues, as well as proliferation, development and differentiation of epithelial cells. Among canonical pathways these participating in local (e.g. Wnt/β-catenin, TGFβ, FGF, and IGF1) as well as peripheral (e.g. GnRH or prolactin) signaling were identified. Our in silico analyses confirmed potential and important regulatory role of miR-23b, miR-26a, and miR-125b during early pregnancy in the pig. It seems likely that miRNAs released in to maternal circulation are not only the sign of ongoing local embryo-maternal crosstalk but also can be involved in more distal gene expression regulation. However, further studies are needed to prove our hypothesis.



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**P-39**

*Poster Presentation*


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# Methylation levels at the GNAS locus is not associated with field fertility in Holstein bulls


Prediction of male fertility remains a major goal to promote animal insemination (AI). The combination of several markers of sperm quality provides promising results but remain still unsatisfactory. The crucial role of epigenetics in sperm functions and fertilization efficiency is now recognized. A better understanding of epigenetic mechanisms such as DNA methylation may highlight new key criteria that could improve prediction models. Recently, an increased methylation level at the imprinted Gnas locus has been observed in infertile boars (Congras et al., 2014). Our study evaluated the relationship between CpG methylation status at this locus and the field fertility (SF) score of Holstein bulls. Firstly, 56 CpG positions of GNAS locus were analyzed by bisulfite conversion and pyrosequencing using gDNA from 25 Holstein bulls. A negative correlation was observed between SF and methylation status. The two most informative CpG were selected for further investigation using an extended cohort (113 bulls; SF mean=-1.6±7.0 [-30 to +7.5]). Low methylation levels were observed (CpG#1: 26.7±3.4%; CpG#2: 14.4±2.4%). High variability between bulls (min/max %: 18.3/34.4 and 9.7/20.8) and bull families was noted (e.g. mean CpG#1 methylation percentage inner bull families ranging from 21.3±0.2% to 32.3±1.9%), suggesting that the genetic background may affect the methylation status. However, the correlation between SF and the methylation level was lossed. More studies will probably be required to explore the link between the GNAS methylation variability and fertility efficiency or embryo developmen. In conclusion, the methylation status at the Gnas locus does not provide accurate information to improve fertility prediction models. However, our results pave the way to study the relationship between genetics and methylation status at specific or genome-wide levels (ANR SeQuaMol).

**O-40**

*Oral Presentation*


## Sheldon, Martin

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Sheldon IM


# Impact of infection of the uterus and the innate immune response on ovarian function


Microbial infections impair ovarian function. Using postpartum dairy cattle as an in vivo model, there is evidence that bacterial infections of the uterus perturb ovarian follicle and corpus luteum function. In addition, conception rates are reduced compared with unaffected animals, even after successful resolution of the clinical disease. Furthermore, pathogen-associated molecules are detected in ovarian follicles of diseased animals in vivo. In vitro, challenging granulosa cells or cumulus-oocyte complexes with pathogen-associated molecules stimulates inflammatory responses. Granulosa cells express receptors involved with innate immunity, including the Toll-like receptors, and the innate immune responses by ovarian cells are conserved across species. Binding of pathogen-associated molecular patterns to Toll-like receptors activates cellular MAPK signalling pathways, and stimulates the secretion of cytokines and chemokines. In addition, pathogen-associated molecules perturb cumulus-oocyte function and disrupt oocyte development. However, the impact of pathogens and their molecules on the embryo warrant further investigation.

**P-41**

*Poster Presentation*


## Soen, Yoav

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## Impact of the gut microbiome on the germ-line and reproductive system of the fly


The impact of changing the extracellular gut microbiome is typically thought to be limited to one generation of the host. In this talk, I will present new findings showing that the gut microbiome in flies influence the offspring generation by modifying the germline and the reproductive features of the parent female. Specifically, I will show that lack of these gut bacteria disrupts oogenesis, expedites maternal-to-zygotic-transition in the offspring and unmasks hidden phenotypic variation in mutants. I will also show that the main impact on oogenesis is caused by Acetobacter species and that their impact on the ovary is mediated by Aldh which we identified as a novel effector of oogenesis. These findings uncover a new dimension of host-microbe interactions with significant implications for reproduction, developmental robustness and adaptation.

**P-42**

*Poster Presentation*


## Turkmen, Serhat

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Turkmen S, Izquierdo M


# Testing long effects of parental nutritional programming and a 'remainder' juvenile programming on lipid metabolism and growth in Sparus aurata


Nutritional programming is widely studied in vertebrates and it has been shown that nutritional stimuli during developmental stages may trigger short or/and longterm effects on several physiological functions of the organism. Studies about nutritional programming on different fish species also showed that functioning of certain metabolic pathways involved in lipid and carbohydrate metabolism of juveniles may be influenced by an early diet. Our research on sea bream revealed that nutritional programming through broodstock nutrition is very effective and improves the ability of 4 month juveniles to use vegetable oils (VO) and vegetable meals (VM). However, it is still unknown longer term effects of this type of programming. The present study examined the longterm influences of programming through broodstock nutrition in 16 month juveniles. Therefore, sea bream broodstock were fed 4 different replacement levels of fish oil (FO) by VO. FO replacement by VO affected growth of 45 day and 4 month-old juveniles, as well as Δ6 desaturase gene expression. Besides, when 4 month-old juveniles were fed with a VO-VM based diet, fish from broodstock fed VOs utilized more effectively this diet and showed a higher growth. Afterwards, all fish were fed with a standard fishmeal/FO based commercial diet for 16 months. Then, fish were challenged with a VM/VO based diets for 2 months. The results showed that the influence of parental feeding had disappeared on 16 month-old fish. However, those fish that were challenged at 4th month with a VM-VO based diet significantly showed the effect of parental feeding, suggesting that the nutritional challenge at 4 months may acted as a “remainder” effect added of the parental programming. Furthermore, long effects of nutritional programing and a remainder diet on fatty acids and gene expression involved in lipid metabolism were studied.



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**P-43**

*Poster Presentation*


## Ulbrich, Susanne

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# Maternal low-dose exposure to estradiol-17β reduces sex-specific differences in the mRNA expression profile of porcine preimplantation embryos


Maternal exposure to estrogens is known to induce long term epigenetic effects in the offspring. We recently demonstrated changes in body composition after oral low-dose estradiol-17β (E2) treatment of different doses (0, 0.05, 10 and 1000 µg E2/kg body weight daily) during pregancy. To analyze the possible direct impact of E2 during the preimplantation period, we repeated the experiment and fed sows E2 from insemination until slaughter at day 10 of pregnancy. Maternal tissues and blastocysts were analyzed 1 h after the last E2 application. In maternal plasma, bile, skeletal muscle, heart muscle and endometrium, a significantly higher concentration of total estrogens (estrone, E2, estradiol-17α), conjugated E2 and conjugated total estrogens was determined in the high dose group. Notably, conjugated estrogens were as well significantly increased in the 10 µg E2/kg body weight group. Thus, a direct effect on the endometrium may be assumed. A transcriptome analysis by RNA Seq done after sexing of the maternally exposed day 10 blastocysts revealed a dose dependent increase of 14, 17 and 28 differentially expressed genes (DEG) in the treatment groups, respectively. Validation of a subset of genes was performed using qPCR. Transcriptome changes in the embryos depicted a higher number of up-regulated genes in female blastocysts, especially in the high dose group (238 vs. 13 in males). This contributed to female embryo transcriptomes being more similar to male embryo transcriptomes of the high dose group. The comparison of the same treatment groups between the sexes demonstrated a dose dependent decrease in DEG, with more genes higher expressed in male control blastocysts (63 vs. 5 in females). Overall, low-dose estrogen effects were demonstrated in maternally exposed developing blastcysts, potentially pointing towards the possibility of lasting epigenetic effects originating from early preimplantation estrogen exposure.

**P-44**

*Poster Presentation*


## Velazquez, Miguel

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# Advanced maternal age in mice causes adverse programming of blastocysts leading to overgrowth, glucose intolerance and abnormal organ allometry in postnatal female offspring


The use of assisted reproductive technologies (ART) in women of advanced maternal age (AMA) is increasing. However, long-term effects on offspring derived from ART-AMA models are relatively unexplored. Blastocysts from young (8 weeks, Young-ET offspring) and old (8 months, Old-ET offspring) female mice (C57BL/6 mated with CBA males) were flushed and transferred to young recipients (MF1). All animals were fed with standard chow. At postnatal week 30 a glucose tolerance test was performed and animals were culled for organ allometry. Data was analysed with a multilevel random effects regression model. Postnatal body weight did not differ significantly in males. However, an increase in body weight from week 13 onwards was observed in Old-ET females. Old-ET females also showed a greater peak glucose concentration at 30 min during the glucose tolerance test. Spleen weight and several organ:body weight ratios (i.e. heart, lungs, liver and spleen) were decreased in Old-ET females. Blood pressure data are currently being analysed. Nevertheless, our data show that offspring from aged mouse embryos can develop altered phenotypes during postnatal development even under a normalised maternal in vivo environment, especially in female offspring. Furthermore, these altered phenotypes are programmed by the blastocyst stage and are independent of gestational litter size.



Funded by EU FP7 EpiHealth.

**P-45**

*Poster Presentation*


## Zglejc, Kamila

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# The expression of zinc finger protein 57 (ZFP57) in the endometrium of earlypregnant and cyclic pigs – the potential ability for DNA methylation


Zinc finger protein 57 (ZFP57) is a member of KRAB zinc finger proteins and the element of methylation complex involved in genomic imprinting. Inhibition of ZFP57 causes loss of DNA methylation marks at multiple imprinted gene regions. There are many examples that environmental and physiological factors affect on the female organism during critical periods of pregnancy, such as early implantation and maintenance of pregnancy. We hypothesize that, the intrauterine environment specifically during periimplantation period which is determined by the activity of uterine tissues may not only affect embryonic development but also the health of the offsprings. There are reasons for which we suppose that physiological status such as pregnancy can affect epigenetic programming especially during the periimplantation period. Therefore, the aim of this study was to determine the expression of ZFP57 in endometrial tissue collected from cyclic gilts (days 15 to 16; n=5) and pregnant gilts (days 15 to 16; n=5). The endometrial expression and present of ZFP57 was estimated using Real-time PCR and immunofluorescence. Our results showed the presence of ZFP57 mRNA expression in the endometrium of studied gilts. The expression of ZFP57 was significantly decreased in pregnant in comparison to cyclic pigs. However, the immunofluorescence showed, that the total endometrial quantity and content of ZFP57 in the endometrium did not differ in pregnant and cyclic pigs. In conclusions, the results have shown, that the presence of embryos in the uterus can affect endometrial expression of ZFP57 on the gene level. During early pregnancy the potential to DNA methylation can be significantly decreased. These preliminary study indicates the potential role of ZFP57 in the regulation of epigenetic programming of the uterus during early pregnancy.

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