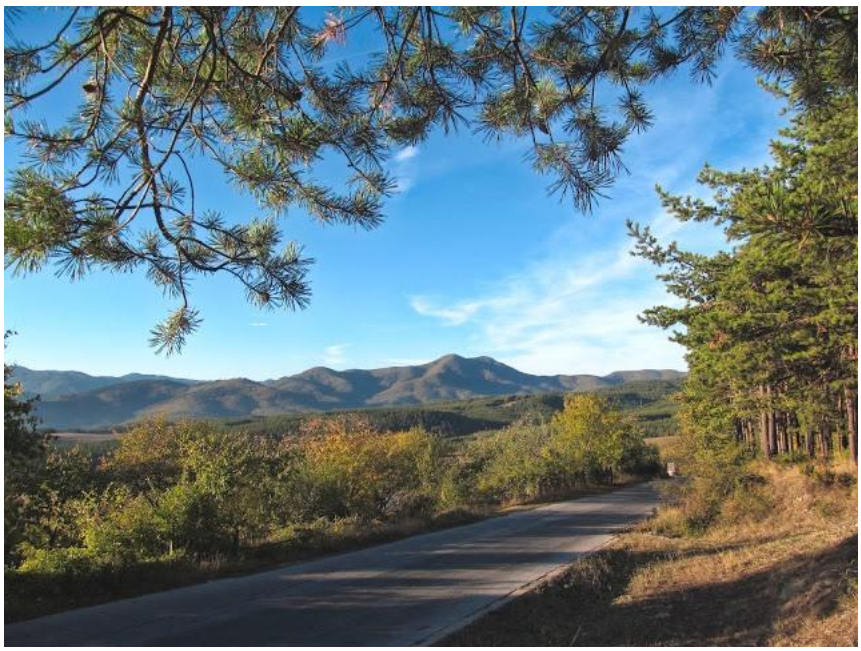
Epigenetics and Periconception Environment



Proceedings of the EPICONCEPT Workshop 2016

Velingrad, Bulgaria
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Editors

Elena Kistanova, Alireza Fazeli, Ann Van Soom, Amos Tandler

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About the European Co-operation in Science and Technology

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

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

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Parental stress before, during and after conception induces epigenetic changes in gametes and embryos. Such epigenetic changes may adversely affect the future health, development, productivity and fertility of the offsprings. Our cooperation in this COST Action focuses on the timeframes and mechanisms of these epigenetic modifications. We plan public engagement activities to inform the general public on the importance of the epigenome and the periconception environment in future food production, health and welfare.

We aim to coordinate various European research activities on epigenetic control of development in order to avoid duplication, set targets and guidance for future research in this field through a large collaborative network.

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Welcome from the Chairman

Dear Epiconcept Members

Welcome in Bulgaria, a country which holds the key land route from Europe to the Middle East and Asia. Here at the crossroads of civilisations we shall examine the crossroads of species under the title ‘Cross-species epigenetics, gametogenesis and embryogenesis’.

I would like to take this opportunity to thank the chairs of Working Group 3, Amos Tandler and Pascale Chavatte-Palmer, who have managed to organise this excellent scientific programme. Our local organiser, Elena Kistanova, has kindly accepted to host the meeting in the beautiful city of Velingrad.

Many thanks to the vice-chair Alireza Fazeli and to Laszlo Tecsi for all his practical arrangements. COST Office is acknowledged for the financial support.
A very warm welcome to all of you in Bulgaria!

Ann Van Soom
Chair of Epiconcept

Alireza Fazeli
Vice-Chair of Epiconcept

Organisers

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Programme

Day 1
Tuesday 17 May 2016

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

19:30 – 21:30 **Dinner**

Day 2
Wednesday 18 May 2016

07:45 – 09:00 **Breakfast**

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

Theme 1: Epigenetics: Disease and Welfare

09:15 – 09:55 **Moshe Szyf, McGill University, Canada**
(40:00) DNA methylation mediating between exposure and phenotype; therapeutic and diagnostic implications

09:55– 10:35 **Vadim Fraifeld, Ben Gurion University, Israel**
(40:00) Aging, longevity and late-onset diseases from the bird's eye view

10:35– 10:50 **Roberta Arena, Polish Academy of Sciences, Poland**
(15:00) Defective placentation in uniparental sheep models

10:50 – 11:30 **Coffee and Poster Presentation**

11:30– 11:45 **Tomer Avidor-Reiss, University of Toledo, United States**
(15:00) Atypical paternal centrioles are essential for progeny embryogenesis

11:45– 12:00 **Milena Georgieva, Institute of Molecular Biology, Bulgaria**
(15:00) Impact of human sperm chromatin organization on sperm quality

12:00 – 12:15 **James Ord, University of Sheffield, United Kingdom**
(15:00) The fright of the father: Paternal fear stress correlates with offspring behavioural alterations in zebrafish (Danio rerio)

12:30 – 14:00 **Lunch**

Theme 2: Epigenetics: Immune System

14:00 – 14:40 **Esteban Ballestar, Bellvitge Biomedical Research Institute, Spain**
(40:00) Epigenetic (de)regulation in the immune system

15:20 – 15:35 **Tiziana Brevini, University of Milan, Italy**
(15:00) Effect of substrate stiffness on 3D cell rearrangement and maintenance of a high plasticity state in epigenetically erased fibroblasts

Theme 4: Epigenetics: Targeted Drug Delivery

14:40 – 15:20 (40:00)	**Paola Arimondo, French National Centre for Scientific Research (CNRS), France** Chemical modulation of epigenetics marks: an example, the targeting of DNA methylation
15:50 – 16:30	**Tea and Poster Presentation**
16:30 – 17:00 (30:00)	**Discussion**
19:30 – 20:30	**Welcome Reception**
20:30 – 21:30	**Dinner**

Day 3
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07:45 – 09:00	**Breakfast**
09:00 – 09:15 (15:00)	**Alireza Fazeli, University of Sheffield, United Kingdom** Rules of travel reimbursements

Theme 3: Epigenetics: Stress and Nutrition

09:15 – 09:55 (40:00)	**Yoav Soen, Weizmann Institute of Science, Israel** Interactions between the gut microbiota and the host germline
09:55– 10:35 (40:00)	**Wim Vanden-Berghe, University of Antwerp, Belgium** From inflammaging to healthy aging: can nutrition reprogram our epigenetic clock
10:35 – 10:50 (15:00)	**Kamila Zglejc, University of Warmia and Mazury, Poland** The influence of restricted diet on in utero activity of methylation complex in pigs
10:50 – 11:30	**Coffee and Poster Presentation**
11:30 – 11:45 (15:00)	**Alexandra Weyrich, Leibniz Institute for Zoo and Wildlife Research, Germany** Paternal heat exposure causes immediate and inherited epigenetic response in wild guinea pigs
11:45 – 12:00 (15:00)	**Alejandro Valdivieso, Spanish Council for Scientific Research, Spain** Temperature and density masculinize a laboratory strain of zebrafish (Danio rerio)
12:00 – 12:15 (15:00)	**Marilin Ivask, University of Tartu, Estonia** Pregnancy rates and blastocyst yields with different transgenic cell lines in bovine cloning
12:30 – 14:00	**Lunch**

Theme 2: Epigenetics: Immune System

14:00 – 14:15 (15:00)	**Parisa Norouzitallab, Ghent University, Belgium** Probing the phenomenon of trained immunity in invertebrates during a transgenerational study, using brine shrimp Artemia as a model system
14:15 – 14:30 (15:00)	**Yael Heifetz, Hebrew University of Jerusalem, Israel** Perceiving the blastocyst: trophoblast cells affect endometrial epithelial cell expression of microRNAs
14:30 – 14:45 (15:00)	**Eliahu Aflalo, Ben Gurion University, Israel** RNA interference and monosex culture of freshwater prawn Macrobrachium rosenbergii

Theme 4: Epigenetics: Targeted Drug Delivery

14:45 – 15:00 (15:00)	**Jaana Peippo, Natural Resources Institute Finland, Finland** The effect of L-carnitine supplementation during IVM and/or IVC on sex ratio of transferable bovine embryos
15:00 – 15:15 (15:00)	**Kartik Baruah, Ghent University, Belgium** A phenolic compound induces the expression of molecular chaperone hsp70 in Artemia host by modification of the histone proteins
15:15 – 15:30 (15:00)	**Desislava Abadjieva, Institute of Biology and Immunology of Reproduction, Bulgaria** Effect of the bioactive substances supplementation to rabbits during the sexual maturation on the expression of the oocyte-specific genes
15:30 – 15:45 (15:00)	**Jean-Pierre Ozil, French National Institute for Agricultural Research (INRA), France** The Ca^{2+} regime at fertilization is determined by the formulation of the culture media
15:50 – 16:30	**Tea and Poster Presentation**
16:30 – 17:00 (30:00)	**Discussion**
17:00 – 17:15 (15:00)	**Farewell Address**
20:00 – 23:00	**Farewell Dinner**

Day 4
Friday 20 May 2016

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

Abstracts of Presentations

O-1

Oral Presentation

Abadjieva, Desislava

Department of Embryo Biotechnologies, Institute of Biology and Immunology of Reproduction, Bulgaria

Abadjieva D, Chervenkov M, Mladenova V, Kistanova E

Effect of the bioactive substances supplementation to rabbits during the sexual maturation on the expression of the oocyte-specific genes

Importance of properly nutrition for reproductive status of human and animals is undisputed, but the exactly mechanism of its effect on the folliculogenesis and maturation of quality oocytes, resulted in healthy offspring, is not clear. The phytogenic feed additive microalga Spirulina platensis (SP), used in animal and fish feeds, is a valuable protein source and a cocktail of vitamins, minerals, pigments. Little is known about its influence on the animal reproductive system. Which genes, related to the reproductive success, should be affected by bioactive substances? Members of the TGF-beta family, oocytes-specific genes gdf-9 and bmp 15, are essential for critical functions of the ovary, such as the formation of the oocytes and its development. These signaling molecules determine the sensitivity of ovaries to each stimulus in right place and at a particular time. In the present work we estimated the effect of dietary supplementation of SP to female rabbits during the sexual maturation period on the gdf9 and bmp15 expression. The research was conducted with 14 female white New Zealand rabbits, seven of them were individually treated with 350 mg/kg SP from 2 to 6 months old prior to insemination. The RT-PCR analysis of the gdf-9 and bmp15 genes expression in the oocytes and cumulus cells from does' ovaries after delivery of offspring was performed. The SP supplementation to female rabbits during the sexual maturation leaded to an increase of the BMP-15 mRNA level in the oocytes and cumulus cells and the GDF-9 mRNA level in the oocytes. No significant changes in the expression of GDF-9 in the cumulus were established. The results show strong evidence that the including the biological active substance in rabbit feed during the sexual maturation can affect the oocytes-specific genes that have big importance for the reproductive success.

Research was supported by co-financing of COST from NSF of the Ministry of Education and Science, Bulgaria.

Oral Presentation

Aflalo, Eliahu
Department of Life Sciences, Ben Gurion University, Israel

Aflalo ED, Shpak N, Sagi A

RNA interference and monosex culture of freshwater prawn Macrobrachium rosenbergii

Differences between males and females with respect to aquacultural outputs highlight the need for novel technologies and management specifically tailored to monosex crustacean aquaculture. In one of the most economically important freshwater species, the prawn M. rosenbergii, sexual dimorphic patterns are significant, by which fractions of males grow considerably larger than females, presenting an advantage for all-male culture. Alternatively, culture of females is suggested to be more suitable for intensification through high stocking densities and uniform size at harvest. Sexual differentiation in decapod crustaceans is mediated by the insulin-like androgenic hormone-switch (IAG-switch). Presence of IAG dictates maleness, thus it's silencing causes a sexual switch into femaleness. Recently a pioneering RNAi-based technology, harnessing the IAG-switch to generate monosex populations of M. rosenbergii was developed. Two main possible pathways are suggested with respect to the function of injected dsMr-IAG. The first is post transcriptional gene silencing (PTGS), termed siRNA pathway. This suggested pathway involves the Dicer-RISC machinery that employs exogenous dsRNA, homologues to mRNA sequence, and involves proteins such as Dicer-2 and Argonaute-2. The second is transcriptional gene silencing (TGS), termed miRNA pathway. This pathway involves the Dicer-RITS machinery which harnesses proteins such as Dicer-1 and Argonaute-1. Inducing the TGS pathway and RITS complex is assumed to cause heterochromatic modifications that prevent transcription. Revealing the transient mode of action of the above technology is crucial as RNAi might act through one of the above mechanisms resulting either in modulating of mRNA translation through the miRNA pathway or in mRNA degradation through the siRNA pathway. The findings will be discussed in light of the two potential schemes with respect to IAG silencing in the context of monosex prawn aquaculture.

O-3

Oral Presentation

Arena, Roberta

Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Poland

Arena R, Zacchini F, Ptak GE

Defective placentation in uniparental sheep models

Evolutionary theory propose that genomic imprinting plays a role in the mother-offspring interaction in mammals. Indeed, imprinted genes are involved in placental development. Uniparental embryos characterized by only-maternal (parthenogenotes-PAR) or only-paternal (androgenotes-AND) genome represent useful model to study the etiology of imprinting-dependent placental defects. The aim of this study was to evaluate parental (only maternal or only paternal) contribution to early placental development using ovine model. To this aim uniparental (androgenotes-AND, parthenogenotes-PAR) and biparental (CTR) sheep embryos were in vitro produced and transferred to synchronized recipient sheep. Then conceptuses were collected at day 20 of pregnancy and placental tissues were processed for histological evaluation. Our analysis revealed an abnormal structure of trophoectodermal epithelium only in PAR tissues, in particular disorganized epithelial layer and reduced number and improper clusterization of binuclear cells. Moreover, we found impaired vasculogenesis in both uniparental placentae. Vasculogenesis was delayed in PAR and significantly reduced in AND. Our preliminary findings suggest a strictly cooperative role of maternal and paternal genome in early placentogenesis. Further investigation will clarify the molecular mechanisms behind the observed defects.

O-4

Oral Presentation

Arimondo, Paola

Pharmacochemistry and Cancer Epigenetic Regulation Unit, French National Centre for Scientific Research (CNRS), France

Arimondo PB

Design and use of chemical tools to modulate gene expression based on the targeting of DNA methyltransferase

DNA methylation is involved in the regulation of gene expression and plays an important role in normal developmental processes and disease. In particular, the epigenetic landscape is altered in cancers where abnormal hypermethylation leads to silencing of certain genes such as tumor suppressor genes. In mammals, DNA methyltransferases are the enzymes responsible for DNA methylation on the position 5 of cytidine in a CpG context. Few direct enzyme inhibitors are known and those have several drawbacks. In order to identify novel inhibitors, we developed three chemical strategies. First a fluorescent High-Throughput Screening for the inhibition of the murine catalytic Dnmt3a/3L complex on the chemical library of the Muséum Naturelle d'Histoire Naturelle and found twelve hits with low micromolar activities. Two molecules efficiently reactivated YFP gene expression in a stable HEK293 cell line by promoter demethylation. Second, based on molecular modeling studies of quinoline inhibitor SGI1027 in the crystal structure of M.Hha I C5 DNA methyltransferase, suggesting that the quinoline and the aminopyridimine are important for the interaction with the substrates and the protein, we synthesized twenty-five new derivatives. Four compounds induced the reexpression of a reporter gene, controlled by a methylated CMV promoter, in leukemia KG-1 cells. Third, we carried out a modulation study of the non-nucleoside inhibitor N-Phthaloyl-L-tryptophan or RG108. The indole, carboxylate and phthalimide moieties were modified. Two constrained compounds and two NPys derivatives were found at least 10-fold more potent than the reference compound. The cytotoxicity on the tumor DU145 cell line of the most potent inhibitors was correlated to their inhibitory potency. Finally, docking studies were conducted in order to understand their binding mode. Altogether, these studies provide insights for the design of the next-generation of DNMT inhibitors.

O-5

Oral Presentation

Avidor-Reiss, Tomer

Department of Biological Sciences, University of Toledo, United States

Avidor-Reiss T, Khire A, Fishman E, Jo K

Atypical paternal centrioles are essential for progeny embryogenesis

Centrioles are conserved, self-replicating, microtubule-based 9-fold symmetric subcellular organelles that are essential for proper cell division and functions. Most cells have two centrioles and maintaining this number of centrioles is important for animal development. However, how animals gain their first two centrioles during reproduction is only partially understood. It is well established that in most animals, the centrioles are contributed to the zygote by the sperm. However, in humans, insects, and many other animals, the sperm centrioles are modified in their structure and protein composition, or they appear to be missing altogether. In these animals, the origin of the first centrioles is not clear. We have discovered that Drosophila melanogaster sperm has a novel and atypical second centriolar structure that we named the proximal centriole-like structure (PCL). The PCL contains centriolar proteins but lacks microtubules and has a structure distinct from a typical centriole. Nevertheless, the PCL functions analogously to a centriole in the zygote; after fertilization, it recruits PCM, forms astral microtubules and found in one of the spindle pole, and provides a platform for the formation of a new centriole. Therefore, our data suggests that insect sperm provides two centrioles and suggest a universal mechanism of centriole inheritance among animals that include atypical centrioles. We have also discovered that both the typical and atypical centrioles of Drosophila melanogaster sperm centrioles are remodeled during spermiogenesis. The ultrastructure and protein composition of the two centrioles is modified during spermiogenesis, resulting with two atypical centrioles in the spermatozoa. Paternal protein mutants that regulate this remodeling affect the resulting embryo. Altogether, our findings demonstrate that atypical paternal centrioles play a role in pre- and post-fertilization to ensure embryogenesis.

O-6

Oral Presentation

Ballestar, Esteban

Epigenetics and Cancer Biology, Bellvitge Biomedical Research Institute, Spain

Ballestar E

Epigenetic (de)regulation in the immune system

Immune cell differentiation and activation depend on the timely regulation of gene expression changes, which rely on the interplay of lineage-specifying transcription factors and epigenetic mechanisms. These are coupled with upstream signalling pathways and extracellular factors released in the bone marrow, blood and tissue environments. In the myeloid branch of the immune system, differentiation leads to the generation of dendritic cells, macrophages, osteoclasts and myeloid derived suppressor cells, among other cell types. These processes are highly relevant for DNA methylation studies not only because the sets of transcription factors involved in each step are very well characterized, but also because these post-replicative processes appear to involve methylcytosine dioxygenase TET2, a key enzyme in active demethylation. My presentation will focus on aspects related to the targeting and functional relevance of DNA methylation changes, the implication of different enzymes, and their interplay with transcription factors and upstream signaling pathways. Results from our group open up perspectives on the elements that target DNA (de)methylation enzymes and also provide novel targets for potential modulation of the differentiation commitment towards these different related cell types of the innate immune response. Finally, I will also discuss how these elements can be dysregulated in the context of inflammation and cancer.

Oral Presentation

Baruah, Kartik

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

Baruah K, Norouzitallab P, Bossier P

A phenolic compound induces the expression of molecular chaperone hsp70 in Artemia host by modification of the histone proteins

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

O-8

Oral Presentation

Brevini, Tiziana

Laboratory of Biomedical Embryology, University of Milan, Italy

Pennarossa G, Manzoni EFM, Zenobi A, Gandolfi F, Brevini TAL

Effect of substrate stiffness on 3D cell rearrangement and maintenance of a high plasticity state in epigenetically erased fibroblasts

Development is driven by epigenetic mechanisms, that regulate chromatin structure and gene transcription programs. The possibility to interact with the epigenetic signature of differentiated cells, switching the original phenotype into a different one, has been recently demonstrated. Here we drive human fibroblasts into a high plasticity state, using the epigenetic eraser, 5-aza-CR. Cells were plated on plastic dishes (A) or on polyacrilammide gels with stiffness of 5kPa(B) and 1kPa(C), erased with 5-aza-CR and cultured in ESC medium for 17 days. Morphological analysis were carried out. The expression of OCT4 and NANOG was assessed. 5-aza-CR treated cells lost the fibroblast's elongated morphology and became rounded, with larger and granulated nuclei. After 17 days, B cells formed 3D-spherical structures of 100 µm in diameter, while C cells arranged in larger aggregates of 168 µm in diameter. A cells retained a monolayer distribution for the entire length of the experiment. 3D changes were accompanied by significant differences in gene expression levels. After 5-aza-CR, cells actively expressed OCT4 and NANOG. However, while A cells turned them down by day 6, B and C cells steadily transcribed these genes until day 17. Interestingly, we observed a correlation among substrate stiffness, 3D cell behavior and high plasticity-related gene expression levels. C cells, that were kept on a very soft substrate (1kPa) and arranged in larger structures, displayed a higher expression of high plasticity-related genes than B cells. Altogether, the data confirm that epigenetic erasing induces a high plasticity state in terminally differentiated fibroblasts. The use of an adequate substrate induces distinctive cell rearrangement and specific substrate-cell and cell-cell interactions. These responses are likely to be related to the activation of specific mechanotransduction pathways that promote, maintain and support the acquired high plasticity state in a matrix dependent mode

P-9

Poster Presentation

Chadio, Stella

Department of Animal Science and Aquaculture, Agricultural University of Athens, Greece

Symeon GK, Pagonopoulou O, Goliomytis M, Kalogiannis D, Charismiadou M, Bizelis I, Deligeorgis SG, Chadio S

Metabolic effects of maternal overnutrition in rabbit offspring

The aim of the present study was to examine the effects of maternal overnutrition imposed during two periods of gestation on growth and metabolism in rabbit offspring. Twenty-four primiparous non lactating rabbit does were artificially inseminated and randomly divided into three treatment groups: Control (C), fed 100% of the energy maintenance requirements throughout pregnancy and O1 and O2 groups fed 150% of the energy requirements from 6th to 19th and from 20th to 27th day of gestation, respectively. Body weight and feed intake for both mothers and offspring were recorded weekly. Maternal blood samples were collected on days 0, 18, 26 of gestation and on day 40 postpartum and blood samples from offspring were obtained on days 35, 45, 55 and 65 of age for glucose, insulin and leptin levels determination. At 75 days of age all rabbits were euthanized, internal organs were weighted and tissue samples from liver, perirenal fat and L. lumborum muscle were obtained for further analyses. No significant differences were detected for BW or metabolic hormones and glucose concentrations of does during the gestational period. Feed consumption was significantly ($p<0.05$) greater in overfed does (groups O1 and O2) compared to Control, but the average daily intake was less than the 150% of maintenance energy requirements offered. Litter size and weight did not differ among treatments. However, birth weight as well as liver and perirenal fat weight at slaughter were significantly higher in O2 offspring, compared to other two groups. In addition, a tendency for higher blood glucose concentration was detected in the offspring of O2 group, while no differences among groups were observed for insulin and leptin levels.

P-10

Poster Presentation

Czelejewska, Wioleta

Department of Animal Physiology, University of Warmia and Mazury in Olsztyn, Poland

Czelejewska W, Dziekonski M, Gajewska A, Okrasa S, Zmijewska A

Analysis of the porcine pituitary transcriptome during the estrous cycle and early pregnancy

The anterior pituitary secretes hormones involved in the regulation of many physiological processes including those responsible for proper functioning of reproductive system during the estrous cycle and pregnancy. Days 12-13 of pregnancy in the pig are crucial for maternal recognition of pregnancy and in consequence for reproductive success. The aim of this study was to compare transcriptomic profiles of pituitaries harvested from cyclic and pregnant gilts on Days 12-13 to identify alterations in gene expression connected with the appearance of pregnancy. Total RNA isolated from pituitaries obtained from cross-bred pigs (Large White × Polish Landrance) on chosen days of the estrous cycle (n=4) and pregnancy (n=4) was used to perform microarray study (Porcine V2 Gene Expression Microarray, 4x44K, Agilent Technologies). The obtained data analysis was done by means of GeneSpring GX Software and bioinformatic tools, i.e. DAVID and GeneMANIA. The analysis of microarray data revealed significant changes in expression of 520 genes (FC ≥ 1.5, $p < 0,05$); 70 transcripts were up-regulated and 450 down-regulated in pregnant vs. cyclic gilts. Among pathways significantly enriched with differentially expressed genes were those connected with the regulation of actin cytoskeleton, adipocytokine, TGF-beta and calcium signaling. Exemplarily, genes encoding NOS3 (nitric oxide synthase 3), GAD1 (glutamate decarboxylase 1), NPR1 (natriuretic peptide receptor 1) appeared to be down-regulated (FC > 2.0), but genes encoding OAZ3 (ornithine decarboxylase antizyme 3), RGS14 (regulator of G-protein signaling 14) and SSTR5 (somatostatin receptor 5) were up-regulated (FC > 2.0). In conclusion, obtained data indicate that the expression of genes at the pituitary level in the pig is submitted to changes during early pregnancy (Days 12-13) comparing to respective days of the estrous cycle.

This research was supported by the National Science Centre, Grant No. 2012/07/D/NZ4/04177

O-11

Oral Presentation

Fraifeld, Vadim

Shraga Segal Department of Microbiology, Immunology and Genetics, Ben Gurion University, Israel

Fraifeld V

Aging, longevity and late-onset diseases from the bird's eye view

In spite of enormous efforts and accumulated knowledge, our capabilities for tackling aging and age-related (late-onset) diseases (ARDs), and ultimately to promote longevity, are still very modest. What is lacking – essential data on key players, efficient analytic tools, or both? What stands behind a well-established observation that the prevalence of atherosclerosis, cancer, neurodegeneration, diabetes type II increases progressively later in life? The obvious fact that both aging and late-onset diseases are time-dependent processes? Interaction between them, which however retains a substantial degree of independence? Or a common molecular basis, which makes aging and ARDs hardly, if any, distinguishable? Here, I will try to address these principal questions from the systems biology perspective, in the context of interaction networks, gene expression signatures, and comparative analysis of longevity.

O-12

Oral Presentation

Georgieva, Milena

Molecular Genetics Laboratory, Institute of Molecular Biology, Bulgaria

Georgieva M, Stefanova V, Staneva D, Miloshev G

Impact of human sperm chromatin organization on sperm quality

Nowadays infertility, also called childlessness, affects at least one in every four couples. The WHO has recently shown that its incidence among couples steadily increases and has accepted this condition as a disease in order to induce social and scientific interest. Plentiful of reasons for childlessness have been reported, most of them complex, some yet undefined. The last prevents the prospect for appropriate reactions in the battle with infertility and anxieties continue growing. Generally, childlessness could be due to problems with female, male or both sexes' reproductive systems. Actually, female infertility causes are better studied and understood while male infertility seems more obscure and hitherto difficult to be assessed. Male germ cells differentiate from haploid round spermatids to flagella-containing motile sperm, in the process of spermiogenesis. This process is distinct from somatic cell differentiation in that during several cell divisions the majority of the histones are replaced sequentially by protamines, facilitating chromatin hyper-compaction. This process of histone-to-protamine transition is an excellent model for the investigation of transgenerational epigenetics. Here, we present the application of an innovative approach for assessing sperm chromatin organization and its impact on male fertility. We have used the Chromatin Comet Assay (ChCA) combined with Fluorescence Activated Cell Sorting (FACS). The application of both methods allowed complex assessment of sperm chromatin compaction and exact evaluation of sperm DNA damage. Results unambiguously show that chromatin compaction of sperm nucleus together with DNA damage index are parameters with utmost importance for the quality of sperm and therefore have a major impact on male fertility. Additionally, our recent data about remaining histones in chromatin of abnormal sperm cells will be further discussed in the light of transgenerational epigenetic inheritance and male infertility.

O-13

Oral Presentation

Heifetz, Yael

Department of Entomology, Hebrew University of Jerusalem, Israel

Sanchez-Lopez JA, Carmel I, Spiller D, White M, Fazeli A, Heifetz Y

Perceiving the blastocyst: trophoblast cells affect endometrial epithelial cell expression of microRNAs

The window of embryo implantation is a critical period in which the developing blastocyst must communicate with a receptive maternal endometrium and set the environment for its arrival. The cytotrophoblasts of the blastocyst interact directly with the maternal endometrial epithelial cells (EECs) to pave their way for the invasion of the uterine decidua. These trophoblasts have been found to express a variety of miRNAs which can be used as a simple and economical way of prompting the EECs for implantation allowance. To evaluate the capacity of the trophoblast to influence EECs behaviour, we monitored the interactions between trophoblast and EECs using a miR193a-sensor carrying two reporter proteins. This miR has been found expressed during murine embryo implantation and in the culture media of human blastocysts and has been related to the capacity of the embryo to implant. The EECs carrying the miR-sensor were co-incubated with trophoblast spheroids (JAR cell line) and imaged by time-lapse confocal microscopy. We found that the endometrial cells reacted to the presence of the trophoblast delaying significantly the onset time and level of expression of the reporter proteins independently of miRNA expression. The EECs found in close proximity to the trophoblast showed a delay in the onset time of their expression while the cells furthest away from the spheroid expressed the reporters as cells without the influence of the trophoblast. The time lapse analysis demonstrated that the presence of JAR spheroids increased the expression levels of miR-193a in endometrial and human embryonic kidney cells regardless of the distance in comparison to the control sensor. These results suggest that the trophoblasts are able to prompt the EECs locally for implantation through mechanisms dependent and independent of miRNAs.

O-14

Oral Presentation

Ivask, Marilin

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Pregnancy rates and blastocyst yields with different transgenic cell lines in bovine cloning

Somatic cell nuclear transfer (SCNT) has potential applications in agriculture and biomedicine. SCNT is currently the most used technique to produce transgenic cattle. However, the efficiency of bovine cloning remains low in spite of numerous attempts to improve it, in particular, pregnancy and full-term development rates. The aim of this study was to determine the influence of different transgenic cell lines on the blastocyst yield and pregnancy rates using SCN. Two fibroblast cell lines, derived from 2-3 months old female fetuses (L3 and L4), were transfected with the vectors containing human insulin (INS) or follicle stimulating hormone (FSH) cDNA sequences under β-casein promoter. The four transgenic cell lines were then used for SCNT. The embryos were cultured until blastocyst stage and then transferred to heifers. Altogether 385 SCNT blastocysts were produced in 14 experiments. The average blastocyst rate was 13% and pregnancy rate 21.1%. Thirty embryos were transferred in groups of 1 or 2 embryos to 19 heifers. From the transfers only 4 heifers became pregnant. Three of the pregnancies were lost during the first months. One L4-FSH pregnancy developed until 8.5 months, but the calf died in utero two weeks before the planned cesarean section. The possible reason might have been large offspring syndrome common to SCNT as the stillborn calf weighed 53 kg. As the data was limited, no influential difference of cell lines on the blastocyst yield and the in vivo development potential of SCNT embryos could be determined. Although our SCNT embryos developed to blastocyst stage, no full-term development was achieved. The low efficiency of SCNT is probably due to epigenetic errors causing most of the developmental problems of clones. Much more research is needed to learn about the molecular mechanisms to improve transgenic SCNT outcome.

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Poster Presentation

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The interleukin 1β system in oviducts of pig during 2-3 days of the estrous cycle and early pregnancy

Interleukin 1β (IL-1β) can act through specific receptor (IL-R) in the presence of accessory protein (IL-RacP) and endogenous antagonist (IL-Ra). It was previously showed that porcine embryos express IL-1β which could affect communication between them and the uterus during the time of maternal recognition of pregnancy and implantation. It is still not known if IL-1β system is also expressed in porcine oviducts. Embryos of pigs are present in oviducts only during 2-3 days after fertilization. Thus, the aim of this study was to determine mRNAs (non-divided oviduct) and proteins expression of IL-1β, IL-R, IL-Ra and IL-RacP in ampulla and isthmus as well as the concentration of IL-1β in oviductal flushings harvested from pigs on 2-3 days of the estrous cycle (n=5) and early pregnancy (n=5), by using real time PCR, immunofluorescence staining and ELISA, respectively. The mRNAs expression of IL-1β, IL-R, IL-Ra and IL-RacP was higher ($P<0.05$) in oviducts of gravid in comparison to non-gravid pigs. IL-1β protein expression was lower ($P<0.05$) in ampulla and isthmus in pregnant vs. cyclic pigs, however the differences were not detected ($P>0.05$) for IL-R protein expression. IL-Ra protein expression was higher ($P<0.05$) in ampulla of pregnant pigs but did not differ between pregnant vs. cyclic pigs in isthmus. Protein expression of IL-RacP was higher ($P<0.05$) in ampulla and isthmus of pregnant vs. cyclic pigs. Concentration of IL-1β in oviductal flushings was greater ($P<0.05$) in 2-3 days of pregnancy than 2-3 days of the estrous cycle. In conclusion, obtained results showed the presence of all components of IL-1β system in oviducts of pigs during 2-3 days of the estrous cycle and pregnancy. Moreover, this data strongly suggest that IL-1β may affect functions of porcine oviduct. We postulate, that action of IL-1β system may be important for communication between embryos and oviducts during 2-3 days of pregnancy.

O-16

Oral Presentation

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Probing the phenomenon of trained immunity in invertebrates during a transgenerational study, using brine shrimp Artemia as a model system

The invertebrate's innate immune system was reported to show some form of adaptive features, termed trained immunity. However, the memory characteristics of innate immune system and the mechanisms behind such phenomena remain unclear. Using the invertebrate model Artemia, we verified the possibility or impossibility of trained immunity, examining the presence or absence of enduring memory against homologous and heterologous antigens (Vibrio spp.) during a transgenerational study. We also determined the mechanisms behind such phenomenon. Our results showed the occurrence of memory and partial discrimination in Artemia's immune system, as manifested by increased resistance, for three successive generations, of the progenies of Vibrio-exposed ancestors towards a homologous bacterial strain, rather than to a heterologous strain. This increased resistance phenotype was associated with elevated levels of hsp70 and hmgb1 signalling molecules and alteration in the expression of key innate immunity-related genes. Our results also showed stochastic pattern in the acetylation and methylation levels of H4 and H3K4me3 histones, respectively, in the progenies whose ancestors were challenged. Overall results suggest that innate immune responses in invertebrates have the capacity to be trained, and epigenetic reprogramming of (selected) innate immune effectors is likely to have central place in the mechanisms leading to trained immunity.

Oral Presentation

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The fright of the father: Paternal fear stress correlates with offspring behavioural alterations in zebrafish (Danio rerio)

A rapidly growing body of evidence suggests that paternal experience may leave imprints on the biology of the next generation, via non-genetic factors transmitted in the sperm. Echoing previous findings in rodents, we present an experimental framework to study heritable effects of paternal exposures in zebrafish (Danio rerio). Using a well-characterised fear stimulus which simulates exposure to a predator, we show that an acute fear episode either prior to, or during the onset of spermatogenesis in adult male zebrafish is associated with pronounced increases in anxiety-like behaviours in larval offspring. In particular, the incidence of freezing behaviour and thigmotaxis were significantly increased in the offspring of stressed fathers compared to the offspring of non-stressed fathers. The results imply the possible existence of a novel transgenerational adaptive mechanism which, by increasing anxiety, may serve to enhance offspring survival in high-risk environments.

Oral Presentation

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

In mammals, fertilization triggers a series of Ca2+ oscillations the frequency, number and duration of which are highly variable. Several reports demonstrate that the culture media used at fertilization affects the developmental potential and may have postnatal consequences. Given the considerable use of in-vitro fertilization technologies in human, the elucidation of the programming role played by the formulation of the culture media remains a critical challenge. In order to assess the functional impact of the culture media better, we questioned whether the number of Ca2+ oscillation, the time period between oscillations and the total duration of the Ca2+ regime at fertilization in the presence of different commercial culture media makes it possible to establish a quantitative connection between media formulation and the developmental consequences. We measured the Ca2+ signal induced by ICSI in the presence of M16, KSOM, Vitrolife and Cook medium in the mouse. The results show that the parameters of Ca2+ signaling at fertilization are highly determined by the formulation of the culture media. KOM triggers twice as much Ca2+ oscillations than M16 and M16 has twice as much Ca2+ oscillations than Vitrolife and Cook media.

M16, eggs display 9.5 ± 2.6 Ca2+ transients for 3h10min±43min (n=18).
KSOM, eggs display 18.8 ± 7 Ca2+ transients for 3h22min±36min (n=18).
Vitrolife, eggs display 5.8 ± 0.8 for 2h8min±25min (n=12)
Cook, eggs display 5.5 ± 1.4 transients for 2h8min±34min (n=15)

In conclusion, the culture media formulation appears to be a critical determinant in egg functioning. It remains to be learned what the degree of linkage is between the formulation of the culture media at fertilization and the postnatal response, and whether the parameters of the Ca2+ regime can be used to reveal such a linkage.

O-19

Oral Presentation

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The effect of L-carnitine supplementation during IVM and/or IVC on sex ratio of transferable bovine embryos

Mobilization of embryo lipids 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 of oocytes and/or embryo culture on sex ratio of transferable embryos. Oocytes were matured in TCM199 with glutamax-I (Gibco™; Invitrogen Corporation, Paisley, UK) supplemented with 0.25 mM Na-pyruvate, 100 IU/ml penicillin, 100 µg/ml streptomycin, 2 ng/ml FSH (Puregon, Organon, Oss, Netherlands), 1 µg/ml β-estradiol (E-2257) and 10% heat inactivated FBS (Gibco™, New Zealand) at 38.5C in maximal humidity in 5% CO2 in air. Zygotes were cultured in G1/G2 media (Vitrolife, Göteborg, Sweden) supplemented with FAFBSA (4 mg/ml) at 38.5C in maximal humidity in 5% O2, 5% CO2 and 90% N2. The treatment groups were: C/C = control (no L-carnitine), C/LC = 1.5 mM L-carnitine in IVC, LC/C = 2.5 mM L-carnitine in IVM, LC/LC= L-carnitine in IVM (2.5 mM) and IVC (1.5 mM). Transferable embryos (N = 451) were collected on day 8 (IVF=day 0) for diagnosis of sex by PCR. On day 8 the sex ratios (% males) of transferable embryos were 52.3%, 65.8% ($p<0.05$, chi square -test), 61.5% ($p<0.05$, chi square –test) and 58.4% for the C/C, C/LC, LC/C and LC/LC groups, respectively. Taken together, supplementation of IVC or IVM with L-carnitine increases the proportion of male embryos among transferable embryos in the presented conditions.

P-20

Poster Presentation

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The impact of ploidy and incubation temperature on global DNA methylation profiles in Atlantic salmon embryos

Artificially induced triploid Atlantic salmon show potential for commercial production by: reducing genetic impact of farmed escapees on wild populations, protecting intellectual property and reducing adverse effects of sexual maturation. Embryonic incubation temperatures impact long-term developmental factors in both diploids and triploids, for instance prevalence of malformations which triploids are prone to. Epigenetic factors, such as DNA methylation (DNAme), may influence embryonic developmental factors in triploid fish. However, no investigations have been published on DNAme profiles during embryogenesis to date, either in artificially induced polyploid fish or in conventional diploid Atlantic salmon. The present work aimed to study known predictors of Atlantic salmon phenotype: temperature (6, 8 and 11 ˚C) and ploidy (diploid and triploid), throughout embryogenesis and the effect on global DNAme profile. Impact on utilisation of Free Amino Acids was also assessed. Overall, lower incubation temperatures improved embryo survival and reduced hatch malformation prevalence. In both ploidy DNAme profiles continue to increase from blastulation, beyond gastrulation and into somitogenesis. Triploid DNAme levels are maintained at lower levels throughout somitogenesis compared to diploids. Similarities between ploidy in DNAme at eyeing and hatching stages indicate dosage effects. No clear impact of temperature on DNAme was observed. Conversely no effect of ploidy was observed on endogenous FAA concentration, however, a strong impact of temperature and developmental stage was observed. Results indicate that long-term effects of deformity development typically observed in diploids and triploids under high embryonic temperatures, may not be caused by changes in the methylome although verification is required. Importantly, artificially induced triploidy is shown to impact DNA methylation in early embryonic development which has implications for DNA reprogramming.

O-21

Oral Presentation

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Interactions between the gut microbiota and the host germline

Unlike vertically-transmitted endosymbionts which have broad effects on their host's germline, the gut microbiota is transmitted horizontally and is not known to influence the germline. By using the fly, D. melanogaster, as a model organism, we show that extracellular gut bacteria have diverse impacts on their host germline. Removal of the gut bacteria represses oogenesis, expedites maternal-to-zygotic-transition in the offspring embryos and unmasks hidden phenotypic variation in mutants. We further show that the main impact on oogenesis is linked to the lack of gut Acetobacter species, and we identify the host Aldh gene as a significant mediator of repressed oogenesis in Acetobacter-depleted flies. These interactions between the gut microbiota and the germline have clear implications for reproduction, developmental robustness and adaptation.

P-22

Poster Presentation

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Effect of Melissa officinalis L. extract on the methylation state in mouse embryonic fibroblast cells

Melissa officinalis L. (MO) is a medicinal plant with well-known antioxidant, antiviral, antibacterial, antifungal, sedative effects. However, there is a lack of data on the effect of MO on the epigenetic changes in the cells and especially on DNA methylation. Our goal was to evaluate the effect of MO aqueous extract on the DNA methyltransferase 1 (Dnmt1); Dnmt3a, and Dnmt3b mRNA transcript levels in mouse embryonic fibroblast cells. Stock solution of the plant extract was made by water extraction with boiling deionized water in the ratio 1:10 (w/v) microwaved for 5 minutes. Two-fold dilutions (1:2 to 1:128) from the stock solution of the extract were used for evaluation of cytotoxicity on mouse embryonic fibroblasts (MEF) by MTT assay. Subsequently, the cells were treated with the IC50 and the first non-cytotoxic dilutions of the extract. Samples were collected 24 hours later and subjected to qPCR in order to determinate DNA methyltransferases mRNA transcript levels. Our results indicate that Dnmt1 levels in MEF treated with IC50 dilution (1:8) and first non-cytotoxic extract dilution (1:32) are significantly higher ($p<0.05$, Dunnett's test) than in the untreated cells. The level of Dnmt3a and Dnmt3b mRNA transcripts in treated and untreated cells does not have significant difference. The presented preliminary results show that treatment of mouse embryonic fibroblasts with M. officinalis aqueous extract leads to increase in the levels of Dnmt1 mRNA, but has no effect on Dnmt3a, and Dnmt3b.

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O-23

Oral Presentation

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Szyf M

DNA methylation mediating between exposure and phenotype; therapeutic and diagnostic implications

Early life exposures are known to have long-lasting impact on the phenotype later in life. What are the mechanisms that mediate between exposure and long-term effects on physical and mental health? We hypothesize that epigenetic processes such as DNA methylation mediate the impact of exposure on the phenotype later in life. DNA methylation is a mechanism that marks genes during development and provides identical DNA sequences with different identities. We propose that DNA methylation plays a similar role in determining the "experiential" identity of DNA. We will review data from humans and nonhuman primates that is consistent with the idea that early experiences result in system wide changes in DNA methylation that are detectable later in life. We will also present evidence that common exposures later in life such as drugs and injury are mediated by alterations in DNA methylation in both the brain as well as systemically. We will present data suggesting that DNA methylation variations can define quantitative traits such as size distribution and that these are reversible by epigenetic manipulations. The idea that DNA methylation is mediating the effects of early exposures on later phenotypes and thus defining quantitative traits has important implications for human health and agriculture alike in both the diagnostics, prevention and therapeutic fields. DNA methylation biomarkers could be used to screen for past exposures, to predict high risk for developing pathology later in life as well as response to therapeutic interventions. Epigenetic approaches and biomarkers could guide animal farming and agriculture strategies. Epigenetic marks are potentially reversible and therefore epigenetically mediated phenotypes could potentially be reprogrammed by epigenetic therapeutics. Examples of reversing experience triggered phenotypes such as cocaine addiction and chronic pain through an epigenetic approach will be discussed.

O-24

Oral Presentation

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Temperature and density masculinize a laboratory strain of zebrafish (Danio rerio)

The zebrafish (Danio rerio) laboratory strain is slightly different from the wild strain due to the many crossings carried out during many generations. This caused the loss of the telomeric region of chromosome 4, which harbored the putative sex gene. Hence the sex determination system in laboratory strains of zebrafish behaves as a polygenic system. This provides a unique system where to test and understand the effect of environmental factors on sex determination and differentiation in fish. It is well known that environmental factors can be stressors that alter the homeostatic state of the fish, and even if these stressors are applied during the earlier stages of development of zebrafish they can modulate the final sex. In this study, zebrafish of the AB strain was exposed to either elevated temperature or rearing at high density during their differentiation period, to determine how these two factors alter population sex ratios. In addition, we were interested to determine the possible existence of transgenerational effects temperature. Here we exposed one zebrafish generation either to normal (control; 28°C) or elevated (34–36°C) temperature and report the effect on sex ratios in the subsequent unexposed (F1 and F2) generations, searching for transgenerational epigenetic inheritance, and trying to establish whether the effect is paternally or maternally transmitted. We also report the effects of density on sex ratios, establishing the threshold in terms of rearing density in which the effects on sex ratio become apparent.

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O-25

Oral Presentation

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Vanden-Berghe, W

From inflammaging to healthy aging: can nutrition reprogram our epigenetic aging clock?

The progressively aging population in Europe is reflected in an increasing number of people suffering from age-related chronic diseases such as metabolic syndrome, diabetes, cardiovascular disease, cancer, osteoporosis, arthritis, and dementia. The heterogeneity in biological aging, chronological age, and aging-associated disorders in humans has been ascribed to different genetic and environmental factors (i.e., diet, pollution, stress) that are closely linked to socio-economic factors. Today, human diet is believed to have a major influence on both the development and prevention of age-related diseases. Recently, the field of “epigenetics” has added a new bridge between nutrition and healthy ageing: besides genetic instructions encoded in DNA which allow correct synthesis of functional protein/RNA molecules, epigenetic instructions which modify the DNA structure further determine the relative amounts of each protein/RNA molecule to be synthesized by the cell. Moreover, whereas genetic instructions are stable after birth, epigenetic instructions dynamically anticipate to environmental (dietary) factors from conception throughout life. Altogether, our health or disease state strongly relies on a delicate balance of genetic (“nature”) and epigenetic (“nurture”) instructions. Increasing evidence reveals that nutrition adds reversible cumulative epigenetic chemical modifications (acetylation, methylation, phosphorylation, glycosylation, etc.) to histones, DNA and RNA. Upon reaching specific tresholds, diet specific epigenetic changes are translated into gene expression changes which contribute in (un)healthy ageing. In this lecture, the epigenetic impact of nutrition and medicinal phytochemicals will be discussed related to chronic inflammation disorders, aging disease prevention and healthy epigenetic aging.

O-26

Oral Presentation

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Paternal heat exposure causes immediate and inherited epigenetic response in wild guinea pigs

Epigenetic modifications, of which DNA methylation is the best studied one, are a mechanism to convey environmental information through generations via parental germ lines. The majority of studies have focused on the maternal transmission of epigenetic information to the offspring, whereas the paternal role in transgenerational transmission has received little attention. Here we show that exposure to a temporally increase in ambient temperature led to changes in DNA methylation patterns in exposed males and were transmitted to their male offspring. Five F0 adult male guinea pigs, a phenotypically and genetically heterogeneous mammal species, were exposed to an increase in ambient temperature for two months. Reduced representation bisulfite sequencing revealed differentially methylated regions (DMRs) in liver samples of F0 fathers before and after heat treatment, as well as in liver and testes of F1 sons sired before and after heat treatment. Since testicular methylation changes imply transmission to the F2 generation, exposure of fathers to increased temperature resulted in rapid and heritable epigenetic modifications that were transmitted paternally. In the context of climate change this mechanism is increasingly relevant for the survival of exposed populations with rising global temperatures.

O-27

Oral Presentation

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The influence of restricted diet on in utero activity of methylation complex in pigs

DNA methylation is maintained by the methylation complex – tripartite motif containing 28 (TRIM28) and zinc finger protein 57 (ZFP57). TRIM28 and ZFP57 are expressed in embryos, oocytes and adult ovaries but, its presence and activity in porcine uterine tissues were not determined. We suppose that restricted diet applied during periconceptional period may affect epigenetic programming especially during the periimplantation period. Therefore, the aim of the study was to determine the expression of TRIM28 and ZFP57 in uterine tissues collected from gilts fed during periconceptional period restricted diet (n = 5) or normal diet (n = 5). The expression and abundance of TRIM28 and ZFP57 was estimated using Real-time PCR and immunofluorescence, respectively. In utero presence and activity of methylation complex was studied during the periimplantation period. Our results showed the expression of TRIM28 and ZFP57 mRNAs in uterine tissues of studied gilts. Endometrial expression of TRIM28 and ZFP57 was significantly decreased in restricted-diet-fed gilts in comparison to normal-diet-fed gilts ($P < 0.01$). However, the myometrial expression of TRIM28 and ZFP57 did not differ ($P > 0.05$) in studies gilts. The immunofluorescence showed that total endometrial abundance of TRIM28 and ZFP57 was significantly increased in restricted-diet-fed gilts in comparison to normal-diet-fed gilts ($P < 0.05$). Total myometrial quantity of TRIM28 and ZFP57 did not differ in normal- and restricted-diet-fed gilts ($P > 0.05$). Therefore, the presence of TRIM28 and ZFP57 in uterine tissues may indicate that methylation complex plays an important role to protect from global DNA demethylation in the uterus during early pregnancy. Female undernutrition during periconceptional period affects in utero activity of TRIM28 and ZFP57 during periimplantation period.

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