INSTITUTE OF BIOTECHNOLOGY
THE CZECH ACADEMY OF SCIENCES, v. v. i., PRAGUE

GENETICS - PLZEN

XXIVth SYMPOSIUM OF IMMUNOLOGY AND BIOLOGY OF REPRODUCTION WITH INTERNATIONAL PARTICIPATION

in memory of Dr. Radslav Kinsky

PROGRAM AND ABSTRACTS

The Castle, Třešť, May 17 – 19, 2018

Published by: Institute of Biotechnology
The Czech Academy of Sciences, v. v. i, Prague
Aranged by: Kubatova A.
Number of pages: 47
Year of issue: 2018

XXIVth Symposium of Immunology and Biology of Reproduction
with International Participation
The Castle, Třešť, May 17 – 19, 2018

PROGRAM

Thursday, MAY 17, 2018

Arrival and accommodation at the Castle Trest

~19.00 DINNER

Friday, MAY 18, 2018

Breakfast from 7 am

9.00 - 9.10 OPENING CEREMONY:
Ulcova-Gallova Z., Peknicova J.

Chairpersons: Ulcova-Gallova Z., Jonak J.

9.10-9.45 **Steger K. (Germany):** Sperm chromatin: Lessons from mouse and man.
9.45-10.05 **Shtapenko O. (Ukraine)**: Effect of zinc glutamate on embryogenesis of female rats.
10.05-10.25 **Antalikova J. (Slovakia)**: Colocalisation of CD9 and CD81 tetraspanins on bull epididymal sperm.
10.25-10.45 **Jankovicova J. (Slovakia)**: What happens with tetraspanins CD9 and CD81 on the egg after fertilization?

10.45-11.10 COFFEE BREAK

Chairpersons: Hortova K., Antalikova J.

11.10-11.30 **Frolikova M. (CZ):** CD9 and CD81 interaction in human and mouse sperm prior to fertilization.
11.30-11.50 **Sanchez N. (Spain):** The role of FcRL3 protein during human sperm-egg interaction.
11.50-12.10 **Secova P. (Slovakia):** Glycoprotein distribution changes after capacitation of bull sperm.
12.10-12.30 **Ded L. (CZ):** Estrogen alters histone-to-protamine transition process and epigenetic profiles in testis and sperm.

12.40-14.30 LUNCH

Chairpersons: Peknicova J., Postlerova P.

14.30-14.50 **Dostalova P. (CZ):** Gestational and pubertal exposure to DEHP results in changes of testicular gene expression in adult male mice.

14.50-15.10 **Valaskova E. (CZ):** The alterations in expression of selected testicular genes and protein level in mice model of diabetes mellitus 2.

15.10-15.30 **Postlerova P. (CZ):** Sperm surface proteins with zona pellucida-binding activity.

15.30-15.50 **Palenikova V. (Slovakia):** Selected human sperm proteins as possible immunogens contributing to idiopathic infertility in women.

15.50-16.10 **Simonik O. (CZ):** Cryopreservation of bull spermatozoa – major obstacles and potential alternatives how to increase cryoprotective efficiency.

17.00-19.00 **Musicians from Moravia**

19.00 DINNER (RAUT)

Saturday, MAY 19, 2018

Breakfast from 7 am

Chairpersons: Nagyova E., Lestan M.

9.00-9.20 **Nagyova E. (CZ):** Some oocyte factors affect differentiation of granulosa cells and cumulus- extracellular matrix organization.
9.20-9.40 **Onderova B. (Slovakia):** Obesity, inflammation and reproduction.
9.40-10.00 **Honzikova M. (CZ):** Could systemic enzyme therapy influence vaginal microbiota?

10.00-10.30 COFFEE BREAK

Chairpersons: Jonakova V., Liska F.

10.30-10.50 **Liska F. (CZ):** Recurrent microdeletions at Xq27.3-Xq28 do not associate with male infertility inthe Czech population.
10.50-11.10 **Lestan M. (Slovakia):** Management of couples with fertility disorders in the context of the MFTHR gene.

11.10-11.20 CLOSING OF SYMPOSIUM
Peknicova J., Ulcova-Gallova Z.

12.00-14.00 LUNCH

XXIVth Symposium of Immunology and Biology of Reproduction
with International Participation
The Castle, Třešť, May 17 – 19, 2018

ABSTRACTS

Friday, MAY 18, 2018

SPERM CHROMATIN : LESSONS FROM MOUSE AND MAN

Steger K.

Department of Urology, Pediatric Urology and Andrology, Section Molecular Andrology
Justus-Liebig University, Giessen, Germany

Histone to protamine exchange in spermatids causes chromatin hypercondensation, while retained histones carry epigenetic information which, at fertilization, is transferred to the oocyte and affects gene expression in the early embryo. Protamine-2 (P2) deficient mice demonstrated the importance of P2 for the production of functional sperm, as P2-deficient sperm are completely immotile and exhibit high levels of DNA fragmentation and severely impaired acrosome formation. However, loss of one P2-allele is tolerated, as heterozygous males are fertile. In human sperm, chromatin immunoprecipitation (ChIP) with an anti-H4K12ac antibody in combination with promoter array analysis demonstrated a significant correlation between H4K12ac-associated gene promoters and the expression level of their stored transcripts. ChIP with an anti-H4K12ac antibody in combination with pyrosequencing revealed 5-10% hypomethylation within CpG islands of selected gene promoters in sperm of fertile donors and it was not significantly altered in subfertile men. Therefore, aberrant histone acetylation within developmentally important gene promoters rather than the DNA methylation status reflects insufficient sperm chromatin integrity in subfertile men.

EFFECT OF ZINC GLUTAMATE ON EMBRYOGENESIS OF FEMALE RATS

Shtapenko O., Hevkan I., Dzen Ye., Slyvchuk Yu., Syrvatka V.

Laboratory of Reproductive Biotechnology, Institute of Animal Biology of NAAS, Lviv, Ukraine

Pregnancy is associated with increased trace elements needs due to the physiologic changes of the female and the metabolic demands of the embryo. The use of chelating compounds with high biological activity, will increase the fertility of female rats by stimulation metabolism and functional activity of the reproductive system. The aim of the research was to determine the effect of Zn glutamate in liposomal form on reproductive function and embryogenesis of female rats. Experimental studies were conducted on female rats Wistar aged 2,5-3 months with body weight of 180-200 g. Rats with a stable rhythm estrous cycle stages of proestrus and estrus were divided into 3 groups: two experimental groups, which were subcutaneously injected with 3mg/kg Zn glutamate in liposomal form one week before fertilization and during fertilization, and a control group, animals of which received distilled water. Possible negative effects of Zn glutamate on gametotoxic and embryo development speculated on the ability to raise the number of live fetuses, number of implantations and resorptions were recorded. Implantation index, preimplantation and postimplantation losses were also evaluated.

Experimental results showed that the administration of Zn glutamate after fertilization significantly increased the number of corpora lutea of pregnancy ($p<0,01$), number of implantation sites ($p<0,05$) and total live fetuses ($p<0,01$) compared with the control group. Analysis of the overall performance of embryonic development in the group that received the Zn glutamate one week before fertilization showed improvement of the embryonic development compared with control group manifested significant increase in the number of live embryos per female to 9,6±0,16 vs 8,7±0,15 that leaded to the decrease of the general gestational losses by reducing preimplantational mortality at 7,29% and, particularly, the postimplantational mortality at 5,39%. The results of the study shown that the subcutaneous injection of organic forms of Zn improves female rats' reproductive performance and is efficacious in enhancing implantation rates. Comparison the period of the administration of Zn glutamate have been not showed significant differences in functional and morphometric indices of pregnancy except lower number of resorption sites, postimplantational and total gestational losses in the group, administered with zinc before fertilization.

COLOCALISATION OF CD9 AND CD81 TETRASPANINS ON BULL EPIDIDYMAL SPERM

Antalikova J., Secova P., Horovska L'., Michalkova K., Simon M., Jankovicova J.

Laboratory of Reproductive Physiology, Centre of Biosciences Slovak Academy of Sciences, Institute of Animal Biochemistry and Genetics, Bratislava, Slovakia

Mammalian spermatozoa released from testes are not competent to fertilize the egg. They have to undergo the maturation process in epididymis including modifications of protein composition by the addition of new proteins secreted by epididymal epithelium. The transfer of CD9-positive microvesicles to bull epididymal sperm was described; the presence of CD9 and CD81 on ejaculated bull sperm was confirmed; moreover CD81 molecule on bull epididymal sperm was detected. The aim of this study was to inspect the presence of CD9 on bull epididymal sperm and to compare the reaction pattern with CD81 molecule. The immunofluorescence analysis revealed the presence of both tetraspanins on all sperm with intact acrosome, but the reaction pattern of antibodies anti-CD81 (Q14) and anti-CD9 (MRP1) was markedly different. Whilst the localisation of CD81 on the apical part of the sperm head remained unchanged, the position of CD9 molecule was changed distally and expanding from equatorial segment of sperm from caput to pattern spreading to whole acrosomal area of caudal sperm. It seems that CD81 molecule is present on sperm plasma membrane already in testes without any changes during the epididymal transport, whilst the CD9 is probably progressively transported to sperm via epididymosomes.

This work was funded by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA-2/0037/16), by the Slovak Research and Development Agency (APVV-15-0196), bilateral projects SAS-CAS (15-05) and SAS-CAS (18-17).

WHAT HAPPENS WITH TETRASPANINS CD9 AND CD81 ON THE EGG AFTER FERTILIZATION?

Jankovicova J., Secova P., Horovska L'. and Antalikova J.

Laboratory of Reproductive Physiology, Centre of Biosciences Slovak Academy of Sciences, Institute of Animal Biochemistry and Genetics, Bratislava, Slovakia

CD81 is the molecule with the highest level of homology with CD9 and both proteins are belonging to the tetraspanin superfamily. In mammalian organism, tetraspanin proteins are widely expressed in many cell types and their activity is mostly concentrated to specific areas of membrane surface; tetraspanin enriched microdomains, where they participate in different cellular functions. The analysis of mouse knockouts with deleted CD9 and CD81 genes showed breakthrough findings about involvement of these proteins in fertilization process in mammals. Whereas our previous experiments revealed the clustered pattern of CD9 and CD81 molecules on plasma membrane of unfertilized metaphase II bovine eggs, this study was addressed to CD9 and CD81 tetraspanins in bovine eggs fertilized by ejaculated sperm in comparison with partenogenetically activated eggs. Immunofluorescent analysis showed the releasement of CD9 and CD81 clusters associated with egg plasma membrane into the perivitelline space of zygotes and embryos independently on the way of egg activation.

This work was funded by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA-2/0037/16), by the Slovak Research and Development Agency (APVV-15-0196), bilateral project SAS-CS (15-05) and SAS-CAS (18-17).Supported by grants VEGA-2/0037/16, APVV-15-0196 and bilateral project SAV-AV ČR 18-17.

CD9 AND CD81 INTERACTIONS IN HUMAN AND MOUSE SPERM PRIOR TO FERTILIZATION

Frolikova M.[1], Postlerova P.[1,2], Cerny J.[3], Jankovicova J.[4], Simonik O.[1,2], Pohlova A.[1,5], Secova A.[4], Antalikova A.[4] and Dvorakova-Hortova K.[1,6]

[1] Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., BIOCEV, Vestec, Czech Republic
[2] Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources, CULS Prague, Czech Republic
[3] Laboratory of Structural Bioinformatics of Proteins, Institute of Biotechnology CAS, v.v.i., BIOCEV, Vestec, Czech Republic
[4] Laboratory of Reproductive Physiology, IABG, Centre of Biosciences, SAS, Bratislava, Slovakia
[5] Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic
[6] Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

Up to now, many molecules were identified to be involved in sperm-egg binding and sperm-egg fusion. However, their precise role and mutual relationship are in most cases still unclear. Proteins localized on a surface of the egg form an extensive network called tetraspanin web in which proteins associate and cooperate. Very important members of the egg tetraspanin web are CD9 and CD81. These proteins were also detected in mammalian sperm, where they are suspected to form active protein network similar like tetraspanin web in egg and probably play a role in sperm-egg membrane fusion. The importance of these two proteins during early stages of fertilization is supported by complete sterility of CD9/CD81 double null female mice. The association of CD9 and CD81 with integrins suggested their role as cofactors participating in integrin-mediated signalling. In our study, we investigate behaviour of CD9 and CD81 in sperm prior fertilization and their mutual relationship within sperm tetraspanin web. The species-specific traits in CD9 and CD81 distribution were compared between mouse and human. A mutual position of CD9/CD81 is shown in human spermatozoa in the acrosomal cap, however in mice, CD9 and CD81 occupy a distinct area. During the acrosome reaction in human sperm, only CD9 is relocated, compared to the relocation of both proteins in mice. The structural modelling of CD9 and CD81 homologous and possibly heterologous network formation was used to propose their lateral Cis as well as Trans interactions within sperm membrane and during sperm-egg membrane fusion.

This work was supported by the project BIOCEV (CZ.1.05/1.1.00/02.0109), from the European Regional Development Fund (www.biocev.eu), by the GACR No. GA-18-11275S, by the Charles University No. SVV260440 , by the Institutional support of the IBT RVO: 86652036, by Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA-2/0037/16), by the Slovak Research and Development Agency (APVV-15-0196), bilateral projects SAS-CAS (15-05) and SAS-CAS (18-17). We acknowledge the Imaging Methods Core Facility at BIOCEV, institution supported by the Czech-BioImaging large RI projects (LM2015062 and CZ.02.1.01/0.0/0.0/16_013/0001775, funded by MEYS CR) and Operational Program Prague Competitiveness (CZ.2.16/3.1.00/21515) funded by European Regional Development Fund for their support with obtaining imaging data presented in this paper. Access to computing and storage facilities owned by parties and projects contributing to the National Grid Infrastructure MetaCentrum provided under the programme "Projects of Large Research, Development and Innovations Infrastructures" (CESNET LM2015042), is greatly appreciated.

THE ROLE OF FCRL3 PROTEIN DURING HUMAN SPERM-EGG MEMBRANE INTERACTION

Sanchez N.[1], Frolikova M.[1], Simonik O.[1], Cerny J.[2], Gregory S.[3], Aflatoonian B.[3], Pacey A.[4], Erickson K.[5], Lam K.S.[6], Liu R.[6], Moore H.[3] and Dvorakova-Hortova K.[1,7]

[1] Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., BIOCEV, Vestec, Czech Republic
[2] Laboratory of Structural Bioinformatics of Proteins, Institute of Biotechnology CAS, v.v.i., BIOCEV, Vestec, Czech Republic
[3] Centre for Stem Cell Biology, University of Sheffield, Sheffield, U.K.
[4] Department of Obstetrics and Gynaecology, University of Sheffield, Medical School, Jessop Hospital for Women, Sheffield, U.K.
[5] Department of Cell Biology and Human Anatomy, University of California, Davis, School of Medicine, California, U.S.A.
[6] Department of Biochemistry and Molecular Medicine, University of California, Davis, School of Medicine, California, U.S.A.
[7] Department of Zoology, Faculty of Science, Charles University, Czech Republic

In the last years, an enormous progress has been made in deciphering changes that gametes undergo during fertilization. However, despite the pivotal importance of understanding gamete interaction in controlling human reproduction, the physiological events and the molecular basis underlying sperm-egg interaction are not still completely understood. Furthermore, our knowledge of human gamete fusion remains even more unknown due to the scarcity of human gametes available for studies and the ethical limitations of their use.

So far, among the molecular actors of sperm-egg interaction, only three genes that encode cell surface receptors have been identified as essential; Juno and CD9, which are expressed on the egg membrane, and Izumo1, located on the surface of sperm. Izumo1-Juno contact, highly conserved in mammals, is the unique and essential receptor-ligand interaction described for the moment. The rapid loss of Juno after its contact with Izumo1 and the fact that Izumo1-Juno union is not sufficient to mediate fusion but enough to promote adhesion between gametes, suggests that there should be alternative receptors other than Juno.

We have recently used a random one-bead-one compound (OBOC) combinatorial peptide library as synthetic egg mimics to screen for novel gamete ligands. The assay led to identify the human immunoglobulin super receptor Fc receptor-like 3 (FCRL3) as a candidate

involve on fertilization. Using human gametes and human cell lines, we are carrying out a comprehensive study of FcRL3 involvement in the gametes adhesion/fusion protein network.

Our preliminary data show that FcRL3 is distributed throughout the ooplasm of human eggs in a dotted pattern but also highly concentrated in the egg membrane which is consistent with a potential role in the adhesion and fusion processes. Moreover, we have observed that Fcrl5, FcRL3 homologous in mouse, co-localizes with the site of sperm attachment where it interacts with Izumo1. Consistently, data from OBOC assay shows a blockage of sperm attachment to the beads upon antibody against IZUMO1 addition. Together, our work suggests a role of FcRL3 as a novel egg fusion ligand for the sperm receptor IZUMO1.

This work was supported by the project BIOCEV (CZ.1.05/1.1.00/02.0109), from the European Regional Development Fund (www.biocev.eu), by the GACR No. GA-18-11275S, by the Charles University No. SVV260440, by the Institutional support of the IBT RVO: 86652036.

We acknowledge the Imaging Methods Core Facility at BIOCEV, Czech-BioImaging large RI projects (LM2015062 and CZ.02.1.01/0.0/0.0/16_013/0001775, funded by MEYS CR), Operational Program Prague Competitiveness (CZ.2.16/3.1.00/21515) and "Projects of Large Research, Development and Innovations Infrastructures" (CESNET LM2015042).

GLYCOPROTEIN DISTRIBUTION CHANGES AFTER CAPACITATION OF BULL SPERM.

***Secova P.*, Jankovicova J. Michalkova K., Horovska L'., Simona M. Antalikova J.**

Laboratory of Reproductive Physiology, Centre of Biosciences Slovak Academy of Sciences, Institute of Animal Biochemistry and Genetics, Bratislava, Slovakia

Sperm capacitation is a complex process that undergoes mature sperm to acquire the fertilization ability. The capacitation-like changes in bull spermatozoa are induced also by cryopreservation procedures probably via the plasma membrane destabilization sufficient to trigger the signalling cascade associated with physiological capacitation. The aim of our study was to monitor the possible differences in glycoprotein distribution on sperm plasma membrane of freshly ejaculated spermatozoa capacitated in commercially supplied medium in comparison with "capacitation" provoked by cryopreservation using the fluorescently labelled lectins: *Pisum sativum* agglutinin (PSA), *Lens culinaris* agglutinin (LCA), Peanut agglutinin (PNA), Wheat germ agglutinin (WGA) in sperm suspension. The similar changes in PSA and LCA binding pattern after capacitation of freshly ejaculated and thawed bull sperm were observed. In the case of PNA binding we observed moderate increase in the number of reactive sperm after thawing caused probably by cryoinjury. Different reaction pattern was recorded after WGA treatment. The changing reaction pattern of freshly ejaculated sperm after capacitation was accompanied by loss of fluorescence contrary to frozen-thawed sperm with changed pattern but preserved WGA signal.

This work was funded by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA-2/0037/16), by the Slovak Research and Development Agency (APVV-15-0196), bilateral project SAS-CS (15-05) and SAS-CAS (18-17).

ESTROGEN ALTERS HISTONE-TO-PROTAMINE TRANSITION PROCESS AND EPIGENETIC PROFILES IN TESTIS AND SPERM

Ded L.[1], Zatecka E.[1], Valaskova E.[1], Frolikova M.[1], Dorosh A.[1], Margaryan H.[1], Elzeinova F.[1,4], Kubatova A.[1], Peknicova J.[1], Paradowska-Dogan A.[3], Steger K.[3] and Dvorakova-Hortova K.[1,2]

[1] Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v. v. i., BIOCEV, Vestec, Czech Republic
[2] Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic
[3] Department of Urology, Pediatric Urology and Andrology, Section Molecular Andrology, Biomedical Research Center of the Justus-Liebig University, Giessen, Germany
[4]Department of Genetic Toxicology and Nanotoxicology, Institute of Experimental Medicine CAS, v.v.i., Prague, Czech Republic

Estrogens are a group of steroid compounds, named for their importance in the estrous cycle. They have been for a long time considered mainly as female hormones, but they also play an important role in regulating male reproductive functions. In our previous studies we described the significant effect of several types of natural and synthetic estrogens on various aspects of sperm and testicular physiology. Despite the large body of evidence about the effect of estrogens on male reproductive functions, the epigenetic effect of estrogens on the process of spermatogenesis and its physiological consequences remains largely unexplored. We carried out transgenerational *in vivo* study on mice to study the effect of synthetic estrogen 17α-Ethynylestradiol (EE2) in two doses (environmental - 2.5 ng/L and anticonception - 2.5 μg/L) on the process of histone-to-protamine transition in testicular tissue, epigenetic profile in testicular cells/sperm in males of P generation and to evaluate the effect of EE2 on subsequent F1, F2 generations (with and without continuous exposure). We observed significantly higher retention of histones in mature sperm nuclei in the group of P males exposed to higher dose of EE2 (55.4 ± 9.7 RFU vs 69.1 ± 11.2 RFU). The effect was also propagated to F1 generation, but not observable in F2 generation. The changes in histones abundancies were also accompanied by the changes in their post-translational modifications (H4K12Ac, H3K27me3, H3K36me2) with the prominent effect on H4K12Ac. Our ongoing work is now focused on the evaluation of the testicular tissue sections and individual testicular cell types to determine the exact points of the effect of EE2 on epigenetic profiles during spermatogenesis. Our further work

on the presented project should bring new important insights into the process of the hormonal regulation of epigenetic functions in males.

This work was supported by the project "BIOCEV" – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University" (CZ.1.05/1.1.00/02.0109), from the European Regional Development Fund (www.biocev.eu), by the Grant Agency of the Czech Republic No. GA-18-11275S, by the Charles University in Prague No. SVV260440, by the Institutional support of the Institute of Biotechnology RVO: 86652036

GESTATIONAL AND PUBERTAL EXPOSURE TO DEHP RESULTS IN CHANGES OF TESTICULAR GENE EXPRESSION IN ADULT MALE MICE

Dostalova P.[1], Zatecka E.[1], Ded L.[1], Dorosh A.[1], Kubatova A.[1], Elzeinova F.[1,3], Valaskova E.[1], Korenkova V.[2], Langerova L.[2], Dvorakova-Hortova K.[1], Peknicova J.[1]

[1] Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., Biocev Vestec, Czech Republic
[2] Laboratory of Gene Expression, Institute of Biotechnology CAS, v.v.i., BIOCEV, Vestec, Czech Republic
[3]Department of Genetic Toxicology and Nanotoxicology, Institute of Experimental Medicine CAS, v.v.i., Prague, Czech Republic

Di(2-ethylhexyl) phthalate (DEHP) is a compound used as a plasticizer in consumer, industrial and medical products. Since DEHP is not covalently bound to the polymer, it can leach from plastics and be available for biological exposure. Several studies have shown adverse effect of DEHP exposure on male reproductive parameters. The aim of this study was to analyse whether DEHP exposure during two critical developmental periods, fetal development and puberty, can cause long-lasting changes observable month/s after the last exposure. Unlike previous studies, we used concentration relevant to human daily exposure - 3 µg/kg b.w./day and concentration 1000 times higher that corresponds to the exposure of patients undergoing medical procedures. CD1 outbred mice were treated with these concentrations either *in utero* (by exposing dams) during gestational days GD 10 – GD 20 or postnatally during puberty (PD 21 – PD 45). In general, mice exposed during their fetal development were more affected by DEHP exposure than mice exposed during puberty. Higher dose of DEHP had higher effect on mice exposed during gestation, on the other hand mice exposed to DEHP during puberty were more affected by the lower dose. Our analysis showed a lower sperm count, slightly decreased sperm quality and changes in anogenital distance and androgen dependent organ after DEHP treatment either during fetal development or during puberty. We did not observed reproductive tract malformations in any treated group. Further, we found changes in expression of several testicular genes among which the most interesting were genes involved in testosterone synthesis or action and genes coding junctional proteins and its regulators. Improper adhesion and communication between Sertoli cell (SC)-SC and SC-germ cells can result in decreased spermatogenesis output and ultimately lead to reduce fertility. As some phtalates may have the same course of action, we hypothesize that the synergic action of several phtalates

and other pollutants during sensitive developmental periods can act on the junctional molecules, thus disrupt the integrity of blood-testis barrier, ectoplasmic specializations and tubulobulbar complexes and consequently contribute to the male infertility.

This work was supported by project BIOCEV (CZ.1.05/1.1.00/02.0109) from the ERDF, from the European 538 Regional Development Fund (www.biocev.eu), by GACR No. GA-18-11275S and by the Institutional support of the Institute of Biotechnology RVO: 86652036.

THE ALTERATIONS IN EXPRESSION OF SELECTED TESTICULAR GENES AND PROTEIN LEVEL IN MICE MODEL OF DIABETES MELLITUS 2

Valaskova E.[1], Zatecka E.[1], Margaryan H.[1], Bohuslavova R.[2], Dvorakova-Hortova K.[1], Pavlinkova G.[2] & Peknicova J.[1]

[1] Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., BIOCEV, Vestec, Czech Republic
[2] Laboratory of Molecular Pathogenetics, Institute of Biotechnology CAS, v.v.i., BIOCEV, Vestec, Czech Republic

Male infertility disorders are a reason for over a half of all cases of infertile couples and they are caused by many factors, such as genetic background, environmental factors, and diseases. One of the suspected factor associated to male infertility is diabetes mellitus (DM). We focused on linkage these two issues as a major goal of this presented study. We performed diabetes mellitus type 2 (DM2) mice model induced by high fat diet to clarify effect of metabolic disease on male reproductive system through the examination of genes expression and proteins quantity in the testis. We assessed the quantitative changes of the testicular genes, which are responsible for proper stages of spermatogenesis and spermiogenesis as well. Furthermore, to reveal molecular changes in testicular tissue we quantified relevant biomarkers of blood-testis barrier (BTB) and genes encoding enzymes reducing reactive oxygen species. In diabetic animals, we found alteration in the gene expression of protamine 1 (*Prm1),* the unique and essential sperm nuclear protein, which condense chromatin and may play role in epigenetic regulations. Compared to the control, relative expression of gene enconding spermatocytes synaptonemal complex 1 (*Sycp1)* was downregulated in the testes of diabetic mice, which could lead to impaired specific step of spermatogenesis, principally meiotic phase. Moreover, to determine effect of high fat diet exposure on blood-testis barrier, we investigated tight junction–associated gene markers in a mouse testis. We detected variations in mRNA level of N-Cadherin (*Cdh2*) and Claudin 11 (*Cldn11*) in DM2 mice. Based on western blot assay, we analyzed a set of the proteins with various cellular functions in male reproduction. We detected decreased level of valosin–containing protein (Vcp, also TER ATpase), which plays a role in ubiquitinylation and it is known as a biomarker for reduced fertility. According to our findings, DM2 mice model revealed changes in spermatic genes expression (*Prm1* and *Sycp1*) and high fat diet exposure in male mice had an impact on Vcp protein level.

This work was supported by the AZV Grant 15-30880A and by BIOCEV project CZ.1.05/1.1.00/02.0109 from the ERDF.

SPERM SURFACE PROTEINS WITH ZONA PELLUCIDA-BINDING ACTIVITY

Postlerova P.[1,2], Pohlova A.[1,3], Zigo M.[1,4], Jonakova V.[1], Jankovicova J.[5], Antalikova J.[5], Dvorakova-Hortova K.[1,6]

[1] Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., BIOCEV, Vestec, Czech Republic
[2] Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Czech Republic
[3] Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic
[4] Division of Animal Sciences, Gynaecology and Women's Health, University of Missouri, Columbia, MO, USA.
[5] Laboratory of Reproductive Physiology, Institute of Animal Biochemistry and Genetics, Centre of Biosciences, SAS, Bratislava, Slovak Republic
[6] Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

Sperm surface proteins play an essential role in the reproductive process. Recognition and binding of spermatozoa to the zona pellucida (ZP) is one of the key steps in mammalian fertilization. Only capacitated spermatozoa are able to undergo the acrosome reaction, to bind to ZP and to fuse with the egg. Throughout the last few decades, great progress has been made towards identification of the sperm proteins that are able to recognize and bind ZP receptors; however, in various mammalian species, their precise determination remains unresolved. We investigated proteins associated with the plasma membrane of spermatozoa and monitored their localization in sperm during their post-testicular maturation and ZP-binding activity. We isolated proteins from the surface of boar ejaculated spermatozoa and prepared a panel (17 clones) of monoclonal antibodies. This panel was used for detection of potential ZP-binding receptors in the sperm acrosomal cap region. Far Western blot binding assay revealed several potential boar and bull sperm proteins with the ZP-binding activity. Three proteins, which were recognized by monoclonal antibodies and interacted with porcine ZP glycoproteins, were identified as proacrosin/acrosin, lactadherin/P47 and Rab-2A protein. We monitored the changes in localization of ZP-binding proteins during their post-testicular maturation using immunofluorescent microscopy and studied sperm protein origin in reproductive fluids and tissues. Some boar sperm surface proteins with the ZP-binding activity were found to origin in the testis or epididymal fluid. Additionally, we tested some monoclonal antibodies from

our panel against boar sperm proteins with spermatozoa of other mammalian species and proved a cross-reactivity of several monoclonal antibodies to proteins of bull, mouse and human sperm in different post-testicular developmental stages. Our panel of monoclonal antibodies can help us to identify species-specific sperm-zona pellucida receptors and may serve as a tool for searching of the diagnostic fertility markers in human and farm animal practice.

This work was supported by CAS (RVO: 86652036), project BIOCEV (CZ.1.05/1.1.00/02.0109) from the ERDF, projects GA-18-11275S, VEGA-2/0037/16, APVV-15-0196 and bilateral project CAS-SAS (18-17).

SELECTED HUMAN SPERM PROTEINS AS POSSIBLE IMMUNOGENS CONTRIBUTING TO IDIOPATHIC INFERTILITY IN WOMEN

Palenikova V.[1,2], Kratka Z.[3], Luxova S.[3], Malickova K.[3,4], Hortova K.[1,5], Postlerova P.[1]

[1] Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i, BIOCEV, Vestec, Czech Republic
[2] Department of Biochemistry, Faculty of Sciences, Charles University, Prague, Czech Republic
[3] Gennet Archa Palace, Prague, Czech Republic
[4] Institute of Medical Biochemistry and Laboratory Diagnostics of General University Hospital and 1st Faculty of Medicine, Charles University, Prague, Czech Republic
[5] Department of Zoology, Faculty of Sciences, Charles University, Prague, Czech Republic

Immunological infertility is a problem for many couples. The immune system may jeopardize fertilization in many aspects, including production of anti-sperm antibodies (ASA). These antibodies bind to the different antigens of spermatozoa and affect the sperm motility, sperm-egg interaction or fusion. The large number of ASA-antigens decreases the quality of diagnostic methods and consequently the treatment. Therefore, our aim was to identify immunogens in ejaculate samples based on their response to the serum of women suffering with idiopathic infertility. The goal was to characterize several common ASA antigens that could lead to a better understanding of principles and mechanisms of these antibodies in order to open new diagnostic and therapeutic possibilities. Methodologically, we isolated proteins from seminal plasma and spermatozoa from ejaculates of patients with different spermiograms. These proteins were separated by SDS-electrophoresis and subsequently subjected to the Western blot analysis. Membranes were incubated with sera of women and detected via secondary antibody against different classes of human immunoglobulins. The obtained results were compared with data from spermiograms, immunoglobulins and women sera. Every sperm samples contained great amount antibody-reactive proteins, and we found several repeated antigens with molecular masses of approximately 75 kDa, 37 kDa and 15 kDa. In addition, serum of an each patient contained ASA that gave unique response to an each male sample. Individually based interaction between women sera and sperm samples is reason why it is so important to establish a common ASA-binding protein for efficient diagnostic tests and subsequently improve therapeutic approaches.

This work was supported by CAS (RVO: 86652036) and project BIOCEV (CZ.1.05/1.1.00/02.0109) from the ERDF and GACR No. GA-18-11275S.

CRYOPRESERVATION OF BULL SPERMATOZOA – MAJOR OBSTACLES AND POTENTIAL ALTERNATIVES HOW TO INCREASE CRYOPROTECTIVE EFFICIENCY

Simonik O.[1,2], Sichtar J.[2], Postlerova P.[1,2], Dvorakova-Hortova K.[1,3], Rajmon R.[2]

[1] Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., BIOCEV, Vestec, Czech Republic
[2] Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic
[3] Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

Cryopreservation enables a long term conservation of spermatozoa. Moreover, together with the artificial insemination they represent the most effective method for dissemination of genetic merit of top bulls. However, several negative non-physiological events such as low temperature, oxidative stress, huge osmotic changes and formation of ice crystals become a challenge for sperm during cryopreservation. Thus, substantial portion of spermatozoa might be irreversibly damaged along with significant decrease of their fertilizing ability. A usage of cryopreservation media such as semen extenders is essential for protection of spermatozoa during whole process of cryopreservation. Although there has recently been made a great progress in an improvement of bull sperm cryopreservation protocols, up to 50% spermatozoa still remains an irreversibly damaged during this process. Except an adjustment of freezing curves, a modification in semen extender composition is main way how to increase cryoprotective efficiency.
The aim of our study was to assess effect of addition of Low Density Lipoprotein (LDL) in 6% concentration (v/v) to selected bull semen extenders Andromed and Bioxcell on functional parameters of bull spermatozoa after thawing. In total, a semen from 7 bulls was collected in five replicates (in standard way) at an insemination centre. The effect of the extender modification was assessed on the basis of sperm motility, mitochondrial and acrosome intactness. Based on kinematic parameters derived from Computer Assisted Sperm Analysis, k-mean cluster analysis was used for classify individual spermatozoon into specific subpopulations (fast, medium, slow). A subpopulation of fast spermatozoa was increased in the presence of LDL in both extenders ($P<0.05$). Moreover, the positive effect of LDL on sperm motility was confirmed by decreasing the percentage of spermatozoa ($P<0.05$). The percentage of spermatozoa with intact acrosome was

increased when LDL was added to Bioxcell ($P<0.05$). On the other hand addition of LDL to Andromed improved mitochondrial integrity after thawing ($P<0.05$).
Our results showed that adding LDL to selected semen extenders considerably ameliorated the functional parameters of bull spermatozoa after thawing, however in the extender dependent manner. Our study unveiled new possibility for improving the results of bull spermatozoa cryopreservation. Nevertheless, for completion of LDL effect on improvement of cryopreservation protocol, following *in vitro* fertilization and *in vivo* studies are necessary.

Supported by CAS (RVO: 86652036) and project BIOCEV (CZ.1.05/1.1.00/02.0109) 307 from the ERDF and National Agency for Agricultural Research grant NAZV 305 QJ1210109

Saturday, MAY 19, 2018

SOME OOCYTE FACTORS AFFECT DIFFERENTIATION OF GRANULOSA CELLS AND CUMULUS- EXTRACELLULAR MATRIX ORGANIZATION

Nagyova E.[1], Scsukova S.[2], Bujnakova - Mlynarcikova A.[2]

[1] *Institute of Animal Physiology and Genetics CAS, Libechov, Czech Republic*
[2] *Institute of Experimental Endocrinology, BRC SAS, Bratislava, Slovakia*

It is known that bidirectional communication between cumulus cells and the oocyte is necessary for oocyte maturation and subsequent development competent oocyte. The first oocyte factor that we investigated was growth differentiation factor 9 (GDF9; Prochazka et al. 2004). We found the expression of *GDF9 mRNAs* in porcine oocytes, cumulus cells (CC) and mural granulosa cells (GC). Recently, we have shown that bone morphogenetic factor 15 (BMP15), another oocyte produced factor, affected differentiation of GC and organization of cumulus extracellular matrix (ECM) in porcine ovarian follicle. Moreover, we detected a significant increase in the expression of *AREG* and *TNFAIP6* (both at 16 h) and *CYP11A1* (at 24 h) in FSH/LH-stimulated oocyte-cumulus complexes (OCC) due to the action of BMP15 compared to complexes cultured only with FSH/LH (Nagyova et al. 2017). The third investigated oocyte-produced factor was fibroblast growth factor 10 (FGF10), since it has been shown that FGF receptors utilized by FGF10 are expressed in bovine CC and oocytes, and that FGF10 affects bovine oocyte maturation *in vitro*. We stimulated porcine GC or OCC *in vitro* with gonadotropins to evaluate principal maturation processes such as resumption of meiosis, cumulus expansion, hyaluronan (HA) synthesis and steroidogenesis in absence /presence of FGF10. Cumulus expansion associated with significant increase in HA synthesis and its retention within expanded cumulus ECM was observed only when medium was supplemented with FSH/LH and serum, without any effect of FGF10. Both, basal and gonadotropin-stimulated progesterone (P4) measured after culture of GC as primary monolayer in the presence of FGF10 was not changed in comparison to control. In contrast, FGF10 increased P4 production by OCC cultured in serum-free conditions.

OBESITY, INFLAMMATION AND REPRODUCTION

Onderova B.[1], Bergendiova K.[2]

[1] *Medirex, Bratislava, Slovakia*
[2] *ImunoVital centrum, Bratislava, Slovakia*

Obesity is a chronic metabolic disorder with an increased fat content in the body composition and a simultaneous weight gain above normal values. It arises from various reasons, such as endocrinological, nutritional, psychological and others. It is a risk factor contributing also to reproductive disorders.

The adipose tissue cells release hormones and immunological factors that can cause a state of hormonal imbalance. Obesity causes a chronic inflammatory state of an organism with increasing CRP values and pro-inflammatory cytokines that are able to increase the readiness of the inflammatory response. Many studies confirm the correlation between higher adipose tissue levels and the production of pro-inflammatory cytokines.

In women with miscarriages of immunological causes, endometrial biopses have shown an increase in the number of leukocytes (mainly of macrophages and NK cells) and their significant activation. Furthermore, there is a higher frequency of cytotoxic T cells responsive to paternal antigens. An abnormally functioning immune system can result in a detrimental response directed against the fetus.

A weight reduction of 10% results in reduced production of pro-inflammatory cytokines, and thus in increased probability of spontaneous pregnancy.

COULD SYSTEMIC ENZYME THERAPY INFLUENCE VAGINAL MICROBIOTA?

Honzikova M.

Mucos Pharma CZ, Pruhonice, Czech Republic

Systemic enzyme therapy (SET) is the treatment method which uses oral application of exogenous proteolytic enzymes of animal origin (trypsin, chymotrypsin) and plant origin (bromelain, papain) in the form of acido-resistant tablets for treating inflammatory conditions of a wide variety of origins. SET medicinal preparations are known for their anti-inflammatory, anti-edematous and immunomodulation effects. Very good results of SET application in the treatment of recurrent respiratory infections in children were a stimulus for exploration of SET efficiency in recurrent vulvovaginal candidiases (RVVC).

10 weeks of SET (Wobenzym) administration in women with RVVC significantly reduced recurrence of this disease not only for a whole one year, but also for the next 3 years (without SET taking) as retrospective analysis showed.

An explanation of the basis for this effect needs further research. We can consider the possible effect of SET on the relationship between a host and yeast – for example preventing of adhesion of yeast to vaginal epithelium by decrease of adhesion receptors expression. Maybe SET can also influence gastrointestinal and vaginal microbiota. Verification using PCR method could be helpful and interesting. We welcome everyone who is interested in participating in such a project.

RECURRENT MICRODELETIONS AT Xq27.3-Xq28 DO NOT ASSOCIATE WITH MALE INFERTILITY IN THE CZECH POPULATION

Chylikova B.[1], Hrdlicka I.[1], Vesela K.[1], Rezabek K.[2], Liska F.[1]

[1] Institute of Biology and Medical Genetics, 1st Faculty of Medicine, Charles University in Prague and General University Hospital, Praha, Czech Republic
[2] Center for Assisted Reproduction, Clinic of Gynecology and Obstetrics, 1st Faculty of Medicine, Charles University in Prague and General University Hospital, Praha, Czech Republic

Genetic causes of male infertility range from point mutations to complex chromosome rearrangements. Recently, 3 recurrent subtelomeric Xq microdeletions were reported to be associated with male infertility in Spanish and Italian males. We tested these microdeletions in men from the Czech Republic – 107 males with pathological sperm evaluation resulting in nonobstructive infertility and 131 males with normal semen. X-chromosome microdeletions were assessed by +/- PCR with three primer pairs for each region Xcnv64 (Xq27.3), Xcnv67 (Xq28) and Xcnv69 (Xq28). Xcnv69 was further characterized by amplification across the deleted region.
We detected presence of Xcnv64 deletion in 3 patients and 14 controls, and Xcnv69 in 3 patients and 6 controls. There was one control and one patient with combined Xcnv64 and Xcnv69 deletions. Xcnv64 was marginally “protective” (adjusted Fisher´s exact test P=0.043), Xcnv69 was not associated (P=0.452). There was no Xcnv67 deletion in our cohort.
The two previously reported X-linked microdeletions (Xcnv64 and Xcnv69) do not seem to confer a significant risk for impaired spermatogenesis in the Czech population. The potential clinical role of the previously reported patient-specific Xcnv67 remains to be determined in a larger study population.

MANAGEMENT OF COUPLES WITH FERTILITY DISORDERS IN THE CONTEXT OF POLYMORPHISM OF THE MTHFR GENE

Lestan M.[1], Belvoncikova S.[1], Kazimirova A.[2], Palenikova J.[1]

[1] *Alergoimmunological Centre, Prešov, Slovakia*
[2] *Centre of Haemostasis and Thrombosis, Prešov, Slovakia*

Repeated pregnancy losses are defined as two or more consecutive abortions, with an incidence of 1-2% of all pregnancies. Over 50% of the causes of these abortions remain unknown. In recent years, research has been also focused on the relationship of MTHFR polymorphism to these pregnancy losses. At present, there are about 40 different mutations of the MTHFR genes, two of which are clinically significant: MTHFR C677T and MTHFR A1298C. If the gene has a heterozygous form MTHFR C677T, a 40% loss of MTHFR enzyme function occurs, with a homozygous form of mutation this loss of functionality is up to 70%. The frequency of the occurrence of polymorphisms is dependent on race (lower occurrence in blacks), geographic factors - TT genotype (the lowest enzyme functionality) occurrence increases in Europe from north to south (4-7% Finland up to 26% southern Italy). Slovakia is one of the countries with an increased incidence of MTHFR polymorphisms, with the risk of reducing the functionality of the MTHFR enzyme and the subsequent possible methylation processes disorders that can lead to fertility disorders at different levels.

There is a general consensus of opinions of haematologists and reproductive immunologists about the management of MTHFR polymorphisms in patients with elevated levels of homocysteine and an anamnesis of repeated pregnancy losses. Different situation is in relation to the association of repeated pregnancy losses with MTHFR polymorphism without elevated level of homocysteine. In the presentation we will present several studies confirming the relationship between MTHFR polymorphism and pregnancy losses even at normal levels of homocysteine. We highlight the issue of MTHFR polymorphisms also in males within a pair with repeated pregnancy losses, with the possibility of pre-implementation genetics.

A separate topic of the presentation is a view of the supplementation of folic acid rsp. methyl folate in couples during preconception and during pregnancy. So far, there is little or no clear evidence to change the general recommendations leading to the replacement of folic acid by other folates, although several studies prefer to use methylated forms of folates and other vitamins in patients with MTHFR polymorphism. There is a large number of studies that clearly confirm the benefit of the preventive use of folic acid, even in patients

with MTHFR polymorphism. On the other hand, the chemical stability of methyl folate is problematic, as well as the absence of a significant number of clinical studies with methyl folate, although some studies are promising. It is still true that the folic acid is a significant success of medicine with a beneficial preventive effect on the reduction of congenital malformations, on the reduction of anaemia of pregnant women and other complications. In the presentation, we address possible risks of folic acid supplementation (masking of vitamin B12 deficiency, potential risk of the action of unmethylated form of folic acid).

AUTHOR INDEX

AUTHOR INDEX

SPONSORS

Strategie AV21

Asco-med

Dynex

I.T.A.

Laboserv

Mucos

Olympus

Schoeller Pharma