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

MEDICAL FACULTY OF CHARLES UNIVERSITY
AND FACULTY HOSPITAL IN PILSEN

GENETICS PILSEN

INTERNATIONAL DAY OF IMMUNOLOGY 2017

XXIIIrd SYMPOSIUM OF IMMUNOLOGY AND BIOLOGY OF REPRODUCTION WITH INTERNATIONAL PARTICIPATION

in memory of Dr. Radslav Kinsky

PROGRAM AND ABSTRACTS

The Castle, Třešť, May 18 – 20, 2017

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XXIIIrd Symposium of Immunology and Biology of Reproduction
with International Participation
The Castle, Třešť, May 18 – 20, 2017

PROGRAM

Thursday, MAY 18, 2017

Arrival and accommodation at the Castle Trest

~19.00 DINNER

Friday, MAY 19, 2017

Breakfast from 7 am

09.00-09.10 OPENING CEREMONY:
Ulcova-Gallova Z., Peknicova J.

Chairpersons: Ulcova-Gallova Z., Jonak J.

09.10-09.30 Chaouat G. (France): An example of evolution concepts regarding a specific pathology.
09.30-09.50 Jankovicova J. (Slovakia): Characterization of tetraspanin protein CD81 on bovine gametes.
09.50-10.10 Antalikova J. (Slovakia): Comparative study of tetraspanins on porcine and bovine oocytes.
10.10-10.20 Frolikova M. (CZ): Key proteins of the egg detected newly on sperm.
10.20-10.40 Michalkova K. (Slovakia): Biochemical characterization of CD-52 like molecule in bull sperm and boar seminal plasma.

10.40-11.10 COFFEE BREAK

Chairpersons: Peknicova J., Madar J.

11.10-11.30 Pavlinkova G. (CZ): Maternal and paternal influences in transmission of diabetes-induced changes.
11.30-11.50 Valaskova E. (CZ): The alterations in protamination and expression of selected testicular genes in diabetic mice in multigenerational study.

11.50-12.10 Margaryan H. (Armenia/CZ): The use of histological methods to detect testicular pathology.
12.10-12.25 Mastikova L. (CZ): Sexual dysfunctions in diabetic women treated with insulin.
12.25-12.40 Stadnikova S. (CZ): The impact of type 1 diabetes on male reproduction.

12.40-14.10 LUNCH

Chairpersons: Hortova K., Antalikova J.

14.10-14.30 Dostalova P. (CZ): How estrogens mediate their effect on sperm cells?
14.30-14.50 Postlerova P. (CZ): Detection of G-protein coupled estrogen receptor (GPER) in the male reproductive tissue and spermatic cells during their development and maturation in pigs.
14.50-15.00 Pohlova A. (CZ): A characterization of sperm surface proteins using monoclonal antibodies.
15.00-15.10 Simonik O. (CZ): Localization of ezrin/radixin/moesin in sperm and their possible association with molecules involved in sperm-egg interaction.
15.10-15.30 Liska F. (CZ): Transgenic centrobin overexpression does not confirm the role of centrobin in spermatid head shaping.

15.30-16.00 COFFEE BREAK

Chairpersons: Jonakova V., Liska F.

16.00-16.10 Kusova M. (CZ): Analysis of normospermiograms in the years 2015-2016.
16.10-16.20 Subrtova N. (CZ): Analysis of female fertility of normosperm partners.
16.20-16.40 Zidkova J. (CZ): Effect of patients‘ treatment with selenium, vitamin E, and zinc on the level of selenoproteins and other antioxidative enzymes in human seminal plasma.
16.40-17.00 Stiavnicka M. (Slovakia): γH2AX as the reflection of DNA damage in human sperm.
17.00-17.20 Jaworek H. (CZ): Prevalence of HPV infection in sperm donors and men treated for infertility: a prospective study.

17.20-17.50 COFFEE BREAK

Chairpersons: Sedmikova M., Zidkova J.

17.50-18.10 Cibulka J. (CZ): Role of thyroid antibodies in spontaneous miscarriages in euthyroid women.
18.10-18.30 Onderova B. (Slovakia): Regulatory T cells and their role in reproductive processes.
18.30-18.50 Sedmikova M. (CZ): Effect of carbon monoxide on aging of porcine oocytes.
18.50-19.10 Dzurillova Z. (Slovakia): Possible role of natural killer T-like cells in immunologically based reproductive disorders.

19.10-19.20 Ripl J.: Presentation of firm VWR
19.20-19.25 Sidova L.: Presentaion of firm I.T.A. Intertact s.r.o.

19.45 DINNER (RAUT)

Saturday, MAY 20, 2017

Breakfast from 7 am

Chairpersons: Postlerova P., Tepla O.

09.00-09.20 Nevoral J. (CZ): SIRT1 improves the quality of matured oocytes due to its epigenetic and non-epigenetic targets.
09.20-09.40 Kochova K. (CZ): Bisfenol S negatively effects the quality of mice ovaries.
09.40-10.00 Ghaibour K. (France): Extremely low doses of bisphenol S affects quality of matured mouse oocytes.
10.00-10.20 Tepla O. (CZ): Cryopreservation of human oocytes – results 2015 – 2016 in ISCARE IVF Prague.
10.20-10.40 Bubenickova F. (CZ): Post thaw distribution of sperm subpopulations in the Old Kladruber stallions is affected by extender type and packaging system.

10.40-11.00 CLOSING OF SYMPOSIUM:
Ulcova-Gallova Z., Peknicova J.

11.30-13.00 LUNCH

13.20-15.30 Trip by bus to the historic city of Telc

XXIIIrd Symposium of Immunology and Biology of Reproduction
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ABSTRACTS

Friday, MAY 19, 2017

AN EXAMPLE OF EVOLUTION OF IMMUNOLOGICAL CONCEPTS REGARDING A SPECIFIC PATHOLOGY. THE PREECLAMPSIA "ISLAND WORKSHOPS"

Chaouat G.

U 976 INSERM, Hopital Saint Louis, Paris, France

There has been now 10 issues of the « island workshops », now the Reunion Workshops. We will recall here the salient points of these as an example of evolution od Immunological concepts regarding a specific pathology.

The 1998 prevailing theory was that preeclampsia was ONLY a vascular syndrome. We will discuss the revolution introduced by the primipaternity concepts, as a replacement of the primiparity one, and why it did imply…. Immunology. Data such as duration of sexual cohabitation will also be remembered. Comparison of Indian and creole populations in Mauritius will be emphasised/

A discussion evolved about whether preeclampsia might somehow be the price to pay for a bigger brain. Linked to that M. Elliott focussed on the relationship between oxidative stress and the evolution of placentation in eutherian mammals. I such a context, the role of CNS-1

As regulator of T cells function will be recalled, as well as discussion of the original Medawar paradigm.

The second important topic was the role sperm exposure and T cell activation. Data of Einarson about usage of barrier methods of contraception and those of D Hall about HIV infected women will be linked with the elegant studies of Sarah Robertson.

The metabolic role of Inositol phospho glycans and their detection as an early marker of PE risk will be reported, as well as the role of VEGF sFlt1 both for its pathologic consequences as well as a re-appreciation of the role of NK cells, and data about maternal KIRS and HLA-C Group 2. This drives to the discussion of the role of Complement and thus eventual implication of statins as a treatment. The integrated model of Redman and Sargent will be presented / discussed in its 2017 version.

CHARACTERIZATION OF TETRASPANIN PROTEIN CD81 ON BOVINE GAMETES

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

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

Sperm-egg interaction and fusion represents a key moment of fertilization and it would not happen in mammals without the interaction of the tetraspanin superfamily members including protein CD81. A detailed immunohistochemical localization of CD81 was monitored on bovine oocytes of different maturation stages, as well as during early embryogenesis. There was also a CD81 characterization on bovine sperm carried out. On bovine eggs, CD81 was detected on the plasma membrane of the germinal vesicle, metaphase I and metaphase II oocytes. During fertilization, an accumulation of CD81 molecules in the perivitelline space of fertilized oocytes, which appeared as vesicles associated to plasma membrane, was observed. In majority of bull ejaculated sperm and caput, corpus and cauda epididymal sperm, CD81 was found on the plasma membrane covering the apical acrosome. Although the process of capacitation did not influence the localization of CD81, it was lost from the surface of the acrosome-reacted spermatozoa in bull. Presented results documented aspects of CD81 expression in bovine gametes and by that also suggest their possible importance in fertilization process of cattle.

Supported by grants VEGA-2/0037/16, APVV-15-0196 and bilateral project SAV-AV ČR 15-05.

COMPARATIVE STUDY OF TETRASPANINS ON PORCINE AND BOVINE OOCYTES

Antalikova J.[1], Jankovicova J.[1], Simon M.[1], Secova P.[1], Michalkova K.[1], Horovska L.[1], Chmelikova E.[2], Postlerova P.[3]

[1]Laboratory of Reproductive Physiology, Centre of Biosciences Slovak Academy of Sciences, Institute of Animal Biochemistry and Genetics, Bratislava, Slovak Republic
[2]Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Czech Republic
[3]Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., Biocev Vestec, Czech Republic

Tetraspanins are multifunctional molecules located in specific microdomains on the plasma membrane. Thanks to their ability to enter in molecular partnerships with other members of tetraspanin family or other proteins they can form tetraspanin web and thereby affect many cellular functions. Well described involvement of tetraspanins in somatic cell immune response, cell migration, viral infections, metastasis formation etc., definitely led to suggestion of their participation in the similar processes occurring during gametes interaction. Up to date, although an extensive study proved the fact that tetraspanins CD9 and CD81 are really directly involved in gamete interaction of mammals, their precise role in the fertilization process is not clear yet. In the present study, we compared the localisation of these two tetraspanins on bovine and porcine oocytes in different stage of development. We examined the study of the possible role of CD9 and CD81 molecules in the fertilization process of cattle using polyclonal antibodies to CD9 and CD81 in *in vitro* fertilization assay. In our experiment, no significant reduction in a number of fertilized eggs and cleavage of zygotes after pre-treatment of oocytes with CD9 and CD81 antibodies were observed. The obtained results contribute to data in the scope of bovine and porcine species comparison in the field of fertilization process in mammals.

Supported by grants VEGA-2/0037/16, APVV-15-0196, BIOCEV project CZ.1.05/1.1.00/02.0109 from the ERDF, project of Ministry of Agriculture of the Czech Republic (NAZV) No. QJ1510138, and bilateral project SAV-AV ČR 15-05.

KEY PROTEINS OF THE EGG DETECTED NEWLY ON SPERM

Frolikova M.[1], Sebkova N.[1], Dvorakova-Hortova K.[1,2]

[1]Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., Biocev Vestec, Czech Republic
[2]Biocev Group, Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

Fertilization is a key physiological event that include sperm-egg binding and sperm-egg fusion. Many different proteins participate in these processes. Up to now two crucial molecules were found, Izumo1 on sperm and Juno on egg. The presence of these two molecules is essential during fertilization but there are probably also other proteins required when some can substitute others, if defected or missing. Proteins localized on 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, CD81 and integrins. On the egg β1 integrins associate with CD81, CD9 and other tetraspanins and mediate connection between them and actin cytoskeleton. These proteins were described to play several specific roles on the egg but most importantly they are binding partner of sperm surface proteins.
Interestingly, current studies show that same molecules are also present on sperm. We detected CD81 protein in the acrosomal cap of mouse intact sperm head and over the equatorial segment after completed acrosome reaction and in parallel, we also detected β1 integrins in the same structures. Moreover, we confirmed results of Ito et al. 2010 and we also detected protein CD9 on mouse sperm head. These findings suggest that the same structure like tetraspanin web could be present on sperm and therefore we hypothesis that same molecules participate during sperm-egg binding forming homologous horizontal protein-protein interactive network. However, further clarification is needed.

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. P502-14-05547S, and by the Institutional support of the Institute of Biotechnology RVO: 86652036. We acknowledge the Light Microscopy Core Facility, IMG CAS, Prague, Czech Republic (supported by grants Czech-Bioimaging – LM2015062, "Centre of Model Organisms" OPPK (CZ.2.16/3.1.00/21547) and "Biomodels for health" (LO1419)) for their support with obtaining STED and SIM super- resolution microscopy data presented in this presentation.

BIOCHEMICAL CHARACTERIZATION OF CD52-LIKE MOLECULE IN BULL SPERM AND BOAR SEMINAL PLASMA

Michalkova K.[1], Simon M.[1], Antalikova J.[1], Jankovicova J.[1], Secova P.[1], Horovska L.[1], Postlerova P.[2]

[1]Laboratory of Reproductive Physiology, Centre of Biosciences, Slovak Academy of Sciences, Institute of Animal Biochemistry and Genetics, Bratislava, Slovak Republic
[2]Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., Biocev Vestec, Czech Republic

The aim of this study was to analyse the biochemical properties of the CD52-like antigen in bull sperm and boar seminal plasma using monoclonal antibody (mAb) IVA-543 prepared in the laboratory at CBs SAS. Human CD52 is a GPI-anchored protein with molecular weight in the range from 25 to 29 kDa, which is expressed on all lymphocytes and in the male genital tract. Except human, CD52 has been described in mice, rats, chimps, dogs and pigs. In all species, CD52 is characterised by several common features, such as a post-testicular production in the male genital tract, similar expression in epididymal epithelium and sperm membrane distribution. CD52 molecule is also considered as a major sperm maturation antigen. Western blot analysis showed that the molecular weight of bull CD52-like antigen in sperm extract is 18-22 kDa whereas in seminal plasma 16-20 kDa. Two-dimensional electrophoresis of bull sperm extract and boar seminal plasma showed pI of CD52-like protein in the range from 3.1 to 3.6 and 4.5 to 5.5, respectively. Molecular mass of boar seminal plasma CD52-like appeared as 20-28 kDa. Neuraminidase treatment of bull sperm extract caused 2 kDa reduction of CD52-like molecular weight, which suggests sialic acid modification. However, presumably glycosylation of the antigen was not confirmed after glycopeptidase F and O-glycosidase treatments, since molecular weight of the protein was unchanged. Treatment of bull sperm with phosphatidyl-inositol specific phospholipase C (PI-PLC) suggested that antigen is GPI-anchored, because the ratio of mAb IVA-543 reactive sperm decreased by 70%. This findings was also supported by immunoprecipitation of bull antigen remained in supernatant after the PI-PLC treatment of sperm. Molecular weight of immunoprecipitated protein was 20 kDa. Nevertheless, the protein in bull sperm and boar seminal plasma recognized by our mAb IVA-543 is required for the CD52 molecule confirmation by mass spectrometry.

Supported by grants VEGA-2/0037/16, APVV-15-0196, BIOCEV project CZ.1.05/1.1.00/02.0109 from the ERDF and bilateral project SAV-AVCR 15-05.

MATERNAL AND PATERNAL INFLUENCES IN THE TRANSMISSION OF DIABETES – INDUCED CHANGES

Pavlinkova G.[1], Valaskova E.[2], Zatecka E.[2], Bohuslavova R.[1], Elzeinova F.[2], Margaryan H.[2], Peknicova J.[2]

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

Clinical and experimental studies demonstrate that the exposure to diabetic environment, can increase susceptibility for diabetes in the offspring and that both maternal and paternal factors contribute to offspring phenotypes.
Diabetic pregnancy has been associated with higher risk of adverse outcomes, such as perinatal mortality and congenital birth defects, compared to non-diabetic pregnancy. In addition to direct teratogenicity of maternal diabetes, the intrauterine and early postnatal environment can influence cardiovascular and metabolic health of offspring later in life. This phenomenon is also termed fetal or developmental programming. The offspring of diabetic pregnancy show differences in metabolic, cardiovascular and inflammatory variables compared to the offspring of non-diabetic mothers.
Additionally, a number of recent studies show transgenerational transmission of metabolic phenotypes from males to their offspring. We focused our studies on the effects of diabetes on the male reproductive system in type 1 diabetes model. We showed that direct exposure to the diabetic environment negatively affects the testis morphology, sperm viability, sperm concentration, and sperm characteristics. Additionally, paternal diabetes induces unfavourable changes in the subsequent F1 and F2 offspring generations and thus, an increased risk for infertility disorders in the offspring. Our data are in line with other studies calling for new strategies to improve metabolic health not only in women of reproductive age but also in potential fathers in order to reduce susceptibility to diabetes and related pathologies in subsequent generations.

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.

THE ALTERATIONS IN PROTAMINATION AND EXPRESSION OF SELECTED TESTICULAR GENES IN DIABETIC MICE IN MULTIGENERATIONAL STUDY

Valaskova E.[1], Zatecka E.[1], Doros A.[1], Pavlinkova G.[2], Bohuslavova R.[2], Elzeinova F.[1], Margaryan H.[1], 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 Molecular Pathogenetics, Institute of Biotechnology CAS, v.v.i., Biocev Vestec, Czech Republic*

According to the World Health Organization (WHO), 15% of couples in reproductive age suffer from infertility problems, and up to 60% cases are due to male infertility. Causes of this condition could be genetic background, environmental factors and various diseases, including diabetes mellitus (DM). The aim of this multigenerational study was to investigate the effects of DM on sperm parameters and expression of selected testicular genes using FVB inbred mouse strain, and reveal possible paternal transmission to subsequent male generations.

In the parental generation, DM type 1 was artificially induced at 6 weeks of age by a chemical substance streptozotocin, which causes destruction of pancreatic β cells. One week after an injection, blood glucose levels were measured and mice with level higher than 13.9 mmol/L were considered as diabetic and analysed. Subsequent F1 and F2 generations were not exposed to the streptozotocin.

The critical and unique step during spermatogenesis, especially in the elongation of spermatids, is a replacement of the most histones by protamines (PRMs). This process involves transition proteins (TNPs), which help to bind protamines to form a highly compact nucleoprotamine complex. We assessed the qualitative and quantitative changes of these essential sperm nuclear proteins, which condense chromatin and may play role in epigenetic regulations. On the protein level, protamine 1 to protamine 2 ratio (P1/P2), a marker of male fertility, was changed in the sperm not only in the parental diabetic generation, but also in subsequent F1 and F2 offspring generations. Moreover, we focused on the mRNA level of these proteins. In diabetic animals, we found alterations in the gene expression of *Prm1* and *Prm2* compared to controls in parental generation. The upregulation of *Prm1* was observed in parental generation and it was inherited to F1 and F2 diabetic offspring. We also analysed the expression of other important genes involved in spermatogenesis (*Tnp1, Tnp2, Sycp1, Sycp3, Wt1, Sox8, Cldn11, Gja1*). The alterations

in expression of *Tnp1* and *Tnp2* were detected in parental diabetic mice, and the upregulated trend of Tnp1 was transmitted to F1 diabetic offspring. Compared to the control, relative expression of genes enconding markers for spermatocytes *Sycp1* and *Sycp3* were downregulated in the testes of parental diabetic mice, which can lead to impaired individual stages of spermatogenesis and consequently affect developing sperm. Our results have shown that diabetic condition caused changes in protamination in sperm, and in the gene expression of *Prm1* and *Prm2* as well. We have also observed alterations in genes important during a meiotic phase of spermatogenesis (*Sycp1* and *Sycp3*) and genes important during spermiogenesis (*Tnp1, Tnp1*). We have observed transgenerational effect through the protamine ratio and changes in the gene expression of *Prm1*. These transmitted alterations indicate increased susceptibility to the infertility of the next generation via male germ cells.

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.

THE USE OF HISTOLOGICAL METHODS TO DETECT TESTICULAR PATHOLOGY

Margaryan H.[1], Pavlinkova G.[2], Bohuslavova R.[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 Molecular Pathogenetics, Institute of Biotechnology CAS, v.v.i., Biocev Vestec, Czech Republic*

Histological analysis of the testes may help to determine the scope of tissue damage under the influence of various substances (endocrine disruptors) as well as metabolic diseases (diabetes mellitus). Using hematoxylin & eosin stained sections, the morphometric evaluations of seminiferous tubules can be performed, particularly the tubule diameter and thickness of the seminiferous epithelium.
In our case, we used a panel of antibodies and mouse model to evaluate changes in the testicular tissue affected by metabolic disease - diabetes mellitus.
Selected antibodies against different proteins were used for detection of germ cells during spermatogenesis, mitotic and meiotic division, tissue architecture and other parameters:

Antibody	Proteins / Marker
VEGF-A	Vascular endothelial growth factor A is the molecular marker for type B spermatogonia to evaluate mitosis and the production of type B spermatogonia. Marker for primary spermatocytes and spermatids.
Wt1	Wilms tumor protein. *Wt1* gene encodes transcription factor that plays an important role in cellular development and cell survival. Marker of Sertoli cells.
Connexin 43 (Cx43)	Gap junction proteins levels is response for different function for cooperation between cells, synchronization physiological activities, growth control, regulation of development. Marker of cell-cell communication.

N Cadherin	Cadherins are calcium dependent cell adhesion proteins may contribute to the sorting of heterogeneous cell types. They play important roles in cell adhesion, forming adherens junctions to bind cells within tissues together. Marker of cell-cell signal transfers.
Ki-67	Antigen KI-67 is a nuclear protein, which is expressed in proliferating cells, and it is necessary for cellular proliferation. Marker of mitosis.
PhH3	Phospho-histone H3, Histones play a central role in transcription regulation, DNA repair, DNA replication, and chromosomal stability. Marker of mitosis.
SCP3	Synaptonemal complex protein 3 is the component of the transverse filaments SCP formed during meiotic prophase. Marker of meiosis.

The histology of control testes showed smooth rounded seminiferous tubules flanked by embryonic epithelium at various stages of spermatogenesis. In contrast, in the testes of diabetic mice, thinner disrupted germinal epithelium was noticeable, although all the distinct developmental stages of spermatogenesis, including spermatozoa in the lumen of the seminiferous tubules were identified. We observed high individual variations in testicular histology within the diabetic group. Some seminiferous tubules showed the early spermatocytes and spermatids, which did not form regular "columns". Premature spermatocytes or some seminiferous tubules were anthropic without any cells in the tubule lumen.

The panel of antibodies can be used to test differences in healthy and damaged organs in other mammals, including human.

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

SEXUAL DYSFUNCTIONS IN DIABETIC WOMEN TREATED WITH INSULIN

Mastikova L.[1,3], Stechova K.[1], Stadnikova S.[1,3], Pavlinkova G.[3], Pastor Z.[2], Andrashko V.[1], Kvapil M.[1]

[1]Department of Internal Medicine, 2nd Faculty of Medicine, Charles University in Prague and Faculty hospital Motol, Prague, Czech Republic
[2]Department of Obstetrics and Gynaecology, 2nd Faculty of Medicine, Charles University in Prague and Faculty hospital Motol, Prague, Czech Republic
[3]Laboratory of Molecular Pathogenetics, Institute of Biotechnology CAS, v.v.i., Biocev Vestec, Czech Republic

Diabetic women suffer more often from irregular menstruation, anovulation cycles and fertility issues, but there are much less studies focusing on their sexual life. Female sexual dysfunctions (FSD) are defined by WHO as changes when people cannot participate in their sexual life as they would like. Prevalence of FSD is 20%.
We analysed sexual functions of 39 sexually active insulin-dependent diabetic women with the standardized questionnaires FSFI (Female Sexual Function Index) and FSDS (Female Sexual Distress Scale). Depression rate was evaluated by BDI (Beck's Depression Inventory). We also determined parametres as HbA1c, total cholesterol, LDL, HDL, TG, urea, creatinine, TSH, fT4 as well as the presence of chronic diabetic complications.
72% of women had sexual intercourse at least once a month. According to FSFI 56% of women fulfilled the criteria of FSD, 44% by FSDS and 15% suffered of depresion. 23 women were treated by CSII and only 9% of them never disconnected the pump during sex. Recurring genital infections appeared within 44% and 21% suffered from recurring urinary tract infections.
In the future we would like to focus on pathogenetic principle of this issue (including study on animal model) as well as on the other means how diabetes influences female sexuality.

Supported by: Project no. 15-30880A of Czech health research council.

THE IMPACT OF TYPE 1 DIABETES ON MALE REPRODUCTION

Stadnikova S.[1], Kubatova A.[2], Macek M.[3], Paulasova P.[3], Stechova K.[1], Pavlinkova G.[4], Peknicova J.[2]

[1]Department of Internal Medicine, Motol University Hospital, Prague, Czech Republic
[2]Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., Biocev Vestec, Czech Republic
[3]Department of Biology and Medical Genetics, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic
[4]Laboratory of Molecular Pathogenetics, Institute of Biotechnology CAS, v.v.i., Biocev Vestec, Czech Republic

DM as a risk factor of male dysfertility has been recognized only recently. The aim of our study is to determine direct effects of type 1 diabetes (T1DM) on male reproductive function. 33 male patients with T1DM were approached so far and 27 agreed to participate in our study. Participants aged 23 - 47 years (median 33 years) and suffering from diabetes for 0 - 27 years (median 15 years) were assessed for presence of erectile dysfunction, depression and sexual quality of life via various questionaries (i.e. SQOL, IEEF-5, Beck depression inventory). They were also screened for diabetic complications and comorbidities - diabetic retinopathy, neuropathy, nephropathy, autoimmune thyroiditis, cardiovascular disease, hypertension and hyperlipidaemia. Serum levels of the hormones (estradiol, prolactin, testosteron, SHBG, LH, FSH and PSA) were obtained. Samples of semen were collected from the patients who were able to ejaculate (24/27). Routine analysis (semen volume, pH, viscosity, sperm count, motility and morphology) according to the WHO criteria was conducted immediately after collection. Samples were further processed for DNA quality analyses such as TUNEL assay, comet assay, Annexin V binding, ubiquitination and sperm quality testing by using monoclonal antibodies against acrosome proteins. We intent to connect detailed clinical data with these sensitive molecular techniques to determine influence of diabetic environment on spermatogenesis.

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.

HOW ESTROGENS MEDIATE THEIR EFFECT ON SPERM CELLS?

Dostalova P., Zatecka E., Ded L., Dvorakova-Hortova K., Peknicova J.

Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., Biocev Vestec, Czech Republic

Estrogens are steroid hormones that regulate many events during sperm cell development including spermatogenesis, epididymal maturation and sperm maturation which takes place in the female reproductive tract. The underlying mechanism of this process is not entirely clear and there is still much more to be clarified. The estrogen response is mediated through the classical estrogen receptors (ERs), namely estrogen receptor 1/alpha (ESR1), estrogen receptor 2/beta (ESR2) and a membrane G protein-coupled estrogen receptor 1 (GPER). Since the sperm nucleus is densely packed and transcriptionally inactive, the genomic estrogen signaling, where the activation of ERs leads to binding of ERs to DNA and results in changes in gene expression of target genes, cannot be involved in the estrogenic effect on sperm. On the other hand, the later described non-genomic pathway, that starts at the membrane and occurs within the seconds to minutes, is consider to be involved in estrogenic effect on sperm. We detected both classical ERs in mouse and boar sperm. The immunolocalization of ERs was in the acrosomal region of sperm head and for mouse also in the tail. More interestingly, we found two population of boar sperm based on the positivity or negativity to ESR1. Further, we showed that estrogens regulate boar sperm capacitation in a time and dose dependent manner and that only some boar individuals responded to the estrogen environment. Finally, we used agonists and antagonists of classical ERs and GPER to shed light on the involvement of estrogen receptors in mouse and boar sperm capacitation. In both model organisms, ESR1 appears to be the key receptor responsible for estrogen mediated events, while ESR2 and GPER probably play minor or no role during capacitation.
Our results indicate that estrogens activate ESR1 which leads to procapacitation effects and that the level of ESR1 expression may reflect the sensitivity of the cell to estrogen stimuli. This is of particular interest, as it is known that many endocrine disruptors change the expression of ERs and thus this may represent one of the possible mechanism how endocrine disruptors contribute to increasing fertility issues.

This work was supported by BIOCEV project CZ.1.05/1.1.00/02.0109 from the ERDF, by the Grant Agency of the Czech Republic No. GA-14-05547S, 539 and by the Institutional support of the Institute of Biotechnology RVO: 86652036.

DETECTION OF G-PROTEIN COUPLED ESTROGEN RECEPTOR (GPER) IN THE MALE REPRODUCTIVE TISSUE AND SPERMATIC CELLS DURING THEIR DEVELOPMENT AND MATURATION IN PIGS

Krejcirova R.[1], Sommerova V.[1], Rajmon R.[1], Postlerova P.[1,2]

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

Effect of estrogenic substances on the male reproductive tissue and development of gametes is an essential for the reproductive success. Estrogens affect the target cells via estrogen receptors (ERs) by both genomic and non-genomic pathways. The G-protein coupled estrogen receptor (GPER) is a transmembrane receptor mediating rapid non-genomic responses of estrogens. ERs have been found in the male reproductive tract in many mammalian species including humans. GPER has not been detected in reproductive tissues of pigs yet. Its presence has been only described in boar ejaculated spermatozoa (*Rago et al., J Anat 2014;224(6):732-6*). We detected GPER in testicular and epididymal tissues and in epididymal spermatozoa using specific antibody G-19 by immunofluorescent microscopy. In the testis GPER was found in Leydig and spermatic cells. In epididymis, we immunolocalized GPER in plasma membrane of the secretory epithelium and in spermatozoa at the lumen of cauda epididymis tubule. In spermatozoa isolated from the epididymis GPER was localized in the acrosome and flagellum. Our results confirmed the presence GPER in boar epididymal sperm for the first time. The localization of GPER in the plasma membrane of epididymal secretory tissue and spermatozoa suggests the possible involvement of estrogens not only in the sperm development, but in the sperm post-testicular maturation.

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A CHARACTERIZATION OF SPERM SURFACE PROTEINS USING MONOCLONAL ANTIBODIES

Pohlova A.[1,2], Zigo M.[1,3], Doros A.[1], Jonakova V.[1], Dvorakova-Hortova K.[1], 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 Science, Charles University, Prague, Czech Republic*

[3]*Division of Animal Sciences, University of Missouri, Columbia, MO, USA*

Reproduction is a crucial biological process, which leads to a production of new individuals by combining genetic material of two parental organisms. Proteins are a substantial equipment of the spermatic cell and their characterization can help us to find potential diagnostic markers or identify species-specific sperm-*zona pellucida* (ZP) receptors. Thus, a unique panel (17 clones) of monoclonal antibodies against boar sperm surface proteins was prepared by immunization of mice. Firstly, an indirect immunofluorescent technique was used to screen a protein localization on a pig spermatozoa (epididymal, ejaculated and *in vitro* capacitated). Secondly, proteins isolated from the sperm surface of epididymal, ejaculated and *in vitro* capacitated sperm were separated by SDS-electrophoresis and used for antibody detection (Western blot) and a binding study with lectin-labeled ZP glycoproteins (Far Western blot). In the pig model, three proteins, which were recognized by monoclonal antibodies and interacted with porcine ZP glycoproteins, were immunoprecipitated and identified as acrosin precursor, RAB-2A protein, and lactadherin P47 (*Zigo et al., Cell Tissue Res. 2015;359(3):895-908*). Additionally, we tested the whole panel of 17 monoclonal antibodies with the sperm of other mammalian species, such as mouse, bull and human. In these mammalian species we found by using an immunofluorescent technique that a few antibodies recognized proteins localized in the sperm acrosomal region. Antibodies named 1E3, 2E1 and 3B10 recognized proteins on the mouse epididymal sperm and 4C7 and 1E3 antibodies bound to proteins on the bull sperm. Further, we observed a cross-reactivity of these monoclonal antibodies in protein extracts of bull, mouse and human sperm. Especially, antibodies 4C7, 5C5, 1E3 and 2E1 detected proteins in extracts from bull sperm. Preselected antibodies could be used for detection of the sperm-ZP binding receptors in patients with normal and pathological spermiograms and serve as a tool for searching diagnostic fertility markers in the human.

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LOCALIZATION OF EZRIN/RADIXIN/MOESIN IN SPERM AND THEIR POSSIBLE ASSOCIATION WITH MOLECULES INVOLVED IN SPERM-EGG INTERACTION

Simonik O.[1,2], Doros A.[1], Frolikova M.[1], Ded L.[1], Dvorakova-Hortova K.[1,3]

[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*

Although there has been recently made a great progress in knowledge of mammalian fertilization, the mammalian gametes' interaction during their fusion has not been fully comprehended yet. The aim of our study is to localize Ezrin/Radixin/Moesin (ERM) proteins in sperm of different species and to perform a co-immunoprecipitation assays to determine a predicted interaction with proteins involved in sperm-egg interaction. Prior to fertilization, sperm have to undergo physical and biochemical changes during a process of capacitation and acrosome reaction, among which actin polymerization/depolymerization plays an important role. ERM protein family acts as cross-linkers of actin and the plasma membrane. In case of human sperm capacitation ezrin plays the role via interaction with Rho Guanin Nucleotide Dissociation Inhibitor. In correlation to this, a depletion of ezrin negatively affected human sperm capacitation and moreover the inhibition of actin polymerization blocked fertilization *in vitro*. Our results of immuno-localization and Western blot demonstrate a presence of ezrin and also other members of ERM family (radixin and moesin) in mouse, boar and bull sperm. First selected molecule for co-immunoprecipitation assay was CD46 which is suspected to interact with ERM during sperm-egg fusion due to its described role in acrosome integrity transduction pathways and processes before the fusion of gametes. Moreover, moesin probably interacts with CD46 during virus-cell fusion. Other proteins which are likely to interact with ERM belong to the tetraspanin family. These proteins are essential for successful fertilization as major coordinators of a molecular network which consists of receptors or fusogens. Specifically, CD9 and CD81 are the key tetraspanins during the fusion process, but they have been studied mainly on oocytes. CD9 as the irreplaceable egg fusion protein was localized in microvilli with strong influence on their morphology. CD81 is complementary to CD9 and their interaction with ERM throughout a family of four cell-surface immunoglobulin superfamily proteins, sharing a conserved glutamine-tryptophan-isoleucine (EWI) motif,

and lining them with actin core is described in leukocytes. Moreover, latest research revealed a presence of CD81 on mouse and bull sperm head plasma membrane. Based on our ERM immune-localization in sperm, we hypothesise that these proteins could interact with aforementioned tetraspanins and play important part during sperm-egg interaction and fusion. We further aim to characterise the interaction between ERM and fusion relevant molecules which could help to reveal unknown reasons connected to idiopathic infertility issues.

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TRANSGENIC CENTROBIN OVEREXPRESSION DOES NOT CONFIRM THE ROLE OF CENTROBIN IN SPERMATID HEAD SHAPING

Liska F.[1], Gosele C.[2], Rivkin E.[3], Popova E.[2], Cardoso M.C.[2], Chylikova B.[1], Domaing P.[2], Krejci E.[1], Snajdr P.[1], Krenova D.[1], Kren V.[1], Bader M.[2], Tres L.L.[3], Hubner N.[2], Kierszenbaum A.L.[3]3

[1]1st Faculty of Medicine, Charles University in Prague, Prague, Czech Republic
[2]Max-Delbrück-Center for Molecular Medicine (MDC), Berlin, Germany
[3]Department of Pathobiology, The Sophie Davis School of Biomedical Education, The City University of New York, New York, USA

Rat hypodactyly (*hd*) is characterized by abnormal spermatogenesis and sperm decapitation, limb malformation (missing digits II and III) and growth retardation. *hd* mutants carry an insertion of an endogenous retrovirus into intron of *Cntrob*, disrupting splicing of *Cntrob* transcripts and resulting in the expression of a truncated protein. Centrobin localizes to the manchette, centrosome and the marginal ring of the spermatid acroplaxome, where it interacts with keratin 5-containing intermediate filaments. Mutant spermatids show a defective acroplaxome marginal ring and centrosome separated from the nucleus. We carried out a transgenic rescue experiment with full-length cDNA linked to a ubiquitous strong promoter/enhancer. The construct inserted to a chromosome 16 region devoid of known genes and was expressed in all tissues tested, including testis. Transgenic animals show normal body weight, limb morphology, weight of testis and epididymis. Yet, abnormal spermiogenesis persisted in the transgenic animals. Western blotting showed a coexistence of full-length and truncated/degraded centrobin in sperm of the transgenic males. We postulate that the presence of truncated centrobin together with incorrect timing of transgene expression may hamper the rescue of fertility in *hd* male rats.

ANALYSIS OF NORMOSPERMIOGRAMS IN THE YEARS 2015-2016

Kusova M.[1], Subrtova N.[1], Bibkova K.[2], Micanova Z.[2], Losan P.[2], Ulcova-Gallova Z.[1,2], Peknicova J.[3]

[1] Department of Gynecology and Obstetrics, Charles University Pilsen, Czech Republic
[2] Genetics Pilsen, Czech Republic
[3]Laboratory of Reproductive Biology, Institute of Biotechnology CAS, v.v.i., Biocev Vestec, Czech Republic

Theme: Reduce fertility of a couple is a reason for immunological testing. We have analysed spermiograms of randomly selected patients from The special consultation for reproductive immunology in years 2015-2016.
Purpose: The aim of our research was analysis of normospermiograms by immunological parameters.
Methodic: We focused on the results of spermiograms of overall 1191 patients in the age of 26-54 years (average 40 years). We evaluate the semen by WHO standards and reproductive immunology (volume of ejaculate, concentration and vitality of sperms, speed and quality of motion, morphology of sperms, other cells additive, pH, antispermatic antibodies by direct MAR test and values of intraacrosomal enzymes by immunofluorescence using monoclonal antibodies Hs8 and Hs14).

Results:

Tab.1: Overall analysis of spermiograms

Total	1191	100 %
Astenospermatics	762	64 %
Oligospermatics	212	18 %
Normospermatics	160	13 %
Azoospermatics	57	5 %

Tab.2: Analysis of normospermiograms

Normospermatics	160	100 %
Pathol. of acrosome (Hs 8)	25	15,6 %
Pathol. of acrosome (Hs 14)	12	7,5 %
Pathol.– both (Hs 8 and Hs 14)	11	6,9 %
Positive sperm antibodies	22	13,75 %
Additive of leukocytes	6	3,8 %
Without pathology	103	64 %

Conclusion: 103 (64%) patients from 160 normospermatic men were without pathology. But from total count of all spermiograms (1191) it is only 9%.

ANALYSIS OF FEMALE FERTILITY OF NORMOSPERM PARTNERS

Subrtova N., Kusova M., Ulcova-Gallova Z.

Genetics Pilsen and Department of Gynecology and Obstetrics, Charles University Pilsen, Czech Republic

Problem: Almost 15% of couples suffer from reduced fertility. Infertility may affect men and/or women or both at the same time.

Purpose: The aim of our research was to continue on previous study "Analysis of normospermatics in time of 2015-2016" and focus only on a fertility of their female partners, if we exclude andrologic factor as a main cause of infertility.

Methods: We studied gynecological and obstetrical histories and medical documentation of examined factors in reproductive immunology from randomly selected 160 patients in the age of 20-44 (average 32) years.

Results: The following table shows important anamnestic data of our female patients.

Tab.1. Important anamnestic data

	N	%
Steril I	34	23,3
Previous delivery	33	22,6
Spont. abortions two and more	56	38,4
IVF 1x	30	20,5
IVF 2x	14	9,6
IVF 3x and more	20	13,7
Other dg. (endometriosis, ut.myom., PCO, obesity depression etc.)	32	22
APA syndrome	22	15%

Conclusion: We concentrate more on female fertility in case that partner in infertile couple has got normospermiogramme with excellent immunological properties. Detailed anamnestical data show multifactorial causes of infertility in some women. For example by screening in immunology of reproduction we found 22 infertile patients with antiphospholipid syndrome (APA syndrom), who are or have been treated. Today, 9 patients successfully gave a birth of their babies, 19 are in different phases of their pregnancy.

EFFECT OF PATIENTS' TREATMENT WITH SELENIUM, VITAMIN E, AND ZINC ON THE LEVEL OF SELENOPROTEINS AND OTHER ANTIOXIDATIVE ENZYMES IN HUMAN SEMINAL PLASMA

Zidkova J.[1], Melcova M.[1], Rayova D.[1], Bibkova K.[2], Micanova Z.[2], Kizek R.[3], Ulcova-Gallova Z.[2]

[1]*Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Czech Republic*
[2]*Counseling and Laboratory for Reproductive Immunology, Genetics Pilsen, Czech Republic*
[3]*Pharmaceutical Faculty, Veterinary and Pharmaceutical University, Brno, Czech Republic*

Selenium (Se) and selenoproteins act as antioxidants and have multiple and complex effects on human health. They play many important roles in human body, including the regulation of several hormones. Increasing evidence suggests that Se is essential for the normal growth, development and reproduction of animals and humans.
We focused on the characterization of seminal fluid proteins, especially antioxidant enzymes, to illustrate in detail the local responses of infertile males. We used 26 control patients with fertility problems (age 24-43, average age 33.6) and the group of 34 patients (age 26-58, average age 37.2) treated with Se and Zn preparation and vitamin E to enhance their antioxidative stress response. The daily treatment lasted from two to four months.
After the treatment, glutathione peroxidase showed an increasing activity in seminal plasma, the increase was seen in majority of patients. Glutathione S-transferase also exhibited an increasing activity in several patients. Thioredoxin reductase was the most affected enzyme, its activity dramatically increased in almost every patient in comparison with the control seminal plasma.

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γH2AX AS THE REFLECTION OF DNA DAMAGE IN HUMAN SPERM

Stiavnicka M.[1], Garcia-Alvarez O.[1], Abril-Parreno L.[2], Nevoral J.[1], Ulcova-Gallova Z.[3,4], Kralickova M.[1]

[1] *Charles University, Faculty of Medicine in Pilsen, Biomedical Center and Department of Histology and Embryology, Pilsen, Czech Republic*
[2] *University of Valencia, Valencia, Spain*
[3] *Genetics Pilsen, Czech Republic*
[4] *Department of Gynecology and Obstetrics, Charles University, Pilsen, Czech Republic*

Acquaintance of the DNA status in sperm belongs to one of the most important parameter that could be evaluated directly or through other indicating marker, mainly epigenetic.1 One of them is phosphorylated histone γH2AX whose abundancy is related to DNA damage and reparative processes of DNA. Qualified estimation of sperm quality is required for adequate success of assisted reproductive technologies (ART). The aim of our study was to assess if human sperm samples with different levels of DNA fragmentation also show different γH2AX level.

In accordance with the Ethical Committee of Faculty of Medicine in Pilsen in cooperation with laboratory Genetika Pilsen Ltd., semen samples from males with different sperm quality were collected and processed routinely according to World Health Organisation (WHO) criteria. Subsequently there were made analysis of DNA fragmentation by Sperm Chromatin Structure Assay (SCSA®) and measurement of the γH2AX level by flow cytometry. The same samples were also prepared for γH2AX analysis by flow cytometry using monoclonal mouse anti-gamma H2AX antibody (Abcam, UK). There was prepared isotype and positive control (using H2O2 to induce DNA damage) for each sample. The mean γH2AX antibody staining intensity relative to the isotype control was calculated based on mean fluorescence. Statistical analyses were performed using the SPSS statistical software package version 22.0 (SPSS Inc., USA). A Linear Mixed Model was performed and P-value ≤0.05 was considered as statistical significance. In addition, correlation between %DFI and γH2AX signal was studied.

Based on the SCSA results, samples were divided into three categories with different level of DNA damage: samples with high %DFI (>27%), medium %DFI (15% - 27%) and low %DFI (<15%). After comparison of γH2AX signal in all mentioned group, the higher γH2AX signal was observed in the group with DFI>27% (59.9 ± 11.1 versus 7.70 ± 4.9, respectively). A significant positive correlation ($r = 0.830$; $p = 0.011$) between %DFI and γH2AX signal was also found.

Our results confirm the relationship between γH2AX and DNA damage. Based on the results we could expect that γH2AX signal as the indicator of sperm DNA integrity. However, further studies focused on further candidate markers of fertilization ability of human sperm, are required to improvement of successful ART.

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PREVALENCE OF HPV INFECTION IN SPERM DONORS AND MEN TREATED FOR INFERTILITY: A PROSPECTIVE STUDY

Jaworek H.[1], Hladikova B.[2,3], Oborna I.[2,4,5], Brezinova J.[3,4,6], Koudelakova V.[1], Vrbkova J.[1], Hajduch M.[1]

[1]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry Palacky University Olomouc, Czech Republic
[2] Fertimed, Olomouc, Czech Republic
[3]Dept. of Biology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic
[4]Sperm Bank International, Olomouc, Czech Republic
[5]Department of Obstetrics and Gynaecology, University Hospital Olomouc, Czech Republic
[6]Arleta IVF, Kostelec nad Orlicí, Czech Republic

Introduction: Human papillomavirus (HPV) infection is a cause of many cancers but may also affect human fertility. HPV infection estimated to be associated with poor semen quality including sperm DNA fragmentation and sperm morphology. HPV examination is not included into the obligatory panel of infection for both, donors and infertile couples because the association between infertility and HPV is still uncertain.

Materials and methods: Semen samples and penile swabs were obtained from 100 potential sperm donors and 329 men from infertile couples. Semen samples were analysed for presence of HPV using cobas® 4800 system (Roche) and PapilloCheck® HPV-Screening system (Greiner bio-one). Penile swabs were analysed only by PapilloCheck® HPV- Screening system. All study participants fulfilled a questionnaire focused especially on sexual life and fertility for statistical evaluation.

Results: Thirty seven out of 100 (37%) potential sperm donors were HPV positive; of them 28 were hrHPV positive. While only two potential donors (2%) had HPV positive semen sample, 30 (30%) had HPV positive penile swab and 5 (5%) donors had HPV positive both semen sample and penile swab. HPV infection was detected in 143 out of 329 (43.5%) men from infertile couples; of them 117 (35.6%) were hrHPV. Only six (1.8%) men had their semen sample HPV positive, 95 (28.9%) had the penile swab HPV positive, and only 42 (12.8%) men had both semen sample and penile swab HPV positive.

Conclusion: In a group of 429 asymptomatic men 42% were HPV positive. Men from infertile couples were more frequently HPV positive than potential sperm donors (43.5% vs. 37%). HPV infection was more frequently detected in penile swab then in semen sample (40.1% vs. 12.8%).

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ROLE OF THYROID ANTIBODIES IN SPONTANEOUS MISCARRIAGES IN EUTHYROID WOMEN

Cibulka J.

Genetics Pilsen, Czech Republic

KEYWORDS: antithyroid autoantibodies, miscarriages, levothyroxine.

It is well known fact that the proper function of thyroid gland is essential for succesfull pregnancy outcome. However, there are studies suggesting that the "euthyroid" state itself may not be a sufficient presumption. In women with normal thyroid function and thyroid autoantibodies (aTA) the risk of spontaneous miscarriage is more than tripled and the risk of preterm birth is doubled. The prevalence of aTA is even higher in women with a history of recurrent pregnancy loss. The role of aTA in miscarriages is still not satisfactory explained and there are these hypotheses being generally presented: 1. aTA may be considered as a marker of generalized autoimmune dysfunction; 2. aTA positive euthyroid women before pregnancy are more prone to develop hypothyroidism during pregnancy; 3. age factor - women with aTA are older than those without; 4. cross reactivity of aTA with embryonic tissues.

Since there is a proof of the benefit of levothyroxine treatment on abortion in these aTA positive euthyroid women, we estimate that their presence leads to a hypothyroidism during pregnancy. Based on these findings, we tend to test these antibodies in women with recurrent miscarriages and, in the case of positivity, to preventive supplementation with levothyroxine.

REGULATORY T CELLS AND THEIR ROLE IN REPRODUCTIVE PROCESSES

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

[1]*Medirex, Bratislava, Slovak Republic*
[2]*Imunovital, Bratislava, Slovak Republic*

Regulatory T cells (Tregs) are a distinct population of T cells, which play a role in immune tolerance. Disturbance of their population has been linked to multiple immunopathologies, including allergies, autoimmunity diseases and cancer. Human Tregs have also been identified in the process of maintaining the pregnancy. Successful pregnancy is a challenge for the immune system of a mother, as tolerance mechanisms have to protect the semiallogeneic fetus against immune attacks from the mother. Among these factors providing tolerance during pregnancy, Tregs may play an important role. Several functional studies have shown that unexplained infertility, miscarriage and preeclampsia are often related to deficit in Treg cell number and function. However, successful pregnancy in humans is associated with increased numbers of Treg cells, at least locally in decidua, whereas Treg cells changes in blood have recently been questioned. Many studies suggest their importance in peripheral blood and therefore also in fetal tolerance. Our aim was to measure Treg cells in peripheral blood throughout the menstrual cycle in order to determine their importance in reproductive health.

EFFECT OF CARBON MONOXIDE ON AGING OF PORCINE OOCYTES

Nemecek D., Dvorakova M., Heroutova I., Chmelikova E., Sedmikova M.

Department of Veterinary Sciences, Czech University of Life Sciences, Prague, Czech Republic

Oocyte aging, a time-dependent decrease of quality, takes place, if the fertilization of mature oocyte (in MII stage) does not occur. The negative effect of oocyte aging includes the decrease of the fertilizing capability, increase of polyspermy, parthenogenesis, and structural changes of the zone pellucida. Oxidative stress during the aging contributes to the formation of negative effects of aging and relates to the disruption in functions of the mitochondria. It finally leads to lytic or, more often, apoptotic cell death of aged oocytes. Carbon monoxide (CO), a signal molecule produced by the heme oxygenase enzyme (HO), possesses cytoprotective and anti-apoptotic effects that have been described in somatic cells. However, the effects of CO in oocytes have yet to be researched.

There have been proof that both isoforms of HO are present in MII and aged porcine oocytes. We found that the inhibition of HO by Zn-protoporphyrin IX (2.5; 5; 25 μM) leads to an increase in the number of apoptotic oocytes and decrease in the number of intact oocytes in aging oocytes. Contrarily, the presence of a CO donor, CORM-2 (5; 25; 50; 100 μM) significantly decreases the number of apoptotic oocytes while increasing the number of intact oocytes. We also determined that CO donor significantly decreases the of caspase-3 activity.

Our experiments have shown that the CO donor suppresses negative signs of aging in porcine oocytes and inhibits apoptosis. It can be assumed that the HO/CO system contributes to sustaining the viability of oocytes and regulates the programmed cell death of oocytes.

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IN IMMUNOLOGICALLY BASED REPRODUCTIVE DISORDERS

Dzurillova Z.[1,2], Dzurilla M.[1,2]

[1]Departmen of Reproductive Immunology, Medicentrum Dzurilla, Nitra, Slovak Republic
[2]Laboratory of Clinical Immunology and Allergology, Medicentrum Dzurilla, Nitra, Slovak Republic

During implantation, maternal immunoactivation and tolerance are not only limited to the decidua but are also observed in the periphery, predominantly affecting the innate immune system. Since unexplained female infertility, as well as recurrent spontaneous abortion and implantation failure, are thought to be associated with pathological maternal immunotolerance mechanisms.

NKT cells are a subpopulation of thymus- dependent (T) cells that are primarely $CD4^+$ but express NK lineage surface markers $CD56^+$ $CD16^+$. They have the ability to rapidly produce cytokines, particularly interleukin (IL)-4, tumour necrosis factor-α (TNF- α) and interferon- γ (IFN- γ) without need for priming or clonal expansion unlike classical T cells. The population of uterine NKT cells is likewise enriched after ovulation and during pregnancy. The rapid ability of uterine NKT cells to produce either IFN-γ or IL-4 led to speculate that may be response in the uterus to either Th1 (pro- inflammatory pregnancy failure pathway) or Th-2 (pregnancy protective pathway).

We determined NKT cells in peripheral blood by flow cytometry and correlate with pregnancy outcome in two groups. One group is women with recurrent miscarriage as defined by three or more spontaneous abortions until 14^{th} gestation week. The other group is women with recurrent implantation failure (RIF) can be considered after three or more *in vitro* fertilization- embryo transfer (IVF- ET) attempts.

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SIRT1 IMPROVES THE QUALITY OF MATURED OOCYTES DUE TO ITS EPIGENETIC AND NON-EPIGENETIC TARGETS

Nevoral J.[1,2], Landsmann L.[1,3], Stiavnicka M.[1,2], Kralickova M.[1,2]

[1]Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[2]Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[3]Faculty of Science, Charles University, Prague, Czech Republic

SIRT1, a NAD^+-dependent protein deacetylase, is capable to modulate epigenom via histone deacetylation as well as through the regulation of non-epigenetic cytoplasmic targets. A few substrates, essential for correct chromosome alignment and chromatin stability, are considered. We hypothesized that modulation of SIRT1 activity lead to positive changes, including epigenetic and non-epigenetic markers, in oocytes.
Oocytes were isolated from PMSG-stimulated ICR mice and *in vitro* matured for 16 hrs in M16 medium supplemented with 0.125, 0.25 or 0.5 μM BML-278, selective SIRT1 activator. Oocytes were subjected to immunocytochemical staining of di-/trimethylation of histone H3 on lysine K9 (H3K9me2/3), followed by image analysis. Concurrenty, TUNEL assay was perfomed for DNA damage measurement.
We observed signifficant SIRT1-derived suppression of DNA damage for 52.9 and 63.8 % after treatment with 0.125 and 0.25μM BML-278, respectively. In addition to chromatine stability, H3K9me2/3-derived heterochromatin was observed when BML-278 induced enlargement of the area with H3K9me2/3 positive signal. Taken together, our observations confirm that SIRT1 is an important player in the course of oocyte maturation and it represents promising factor for human assisted reproductive technologies.

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BISFENOL S NEGATIVELY EFFECTS THE QUALITY OF MICE OVARIES

Kochova K.[2], Benesova K.[1,2], Kolinko Y.[1,2], Nevoral J.[1,2], Klein P.[1], Tonar Z.[1,2], Kralickova M.[1,2]

[1]Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[2]Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic

Bisphenols are common component of plastics and are so-called endocrine disruptors, that act in extremely low concentrations. The noxiousness of bisphenol A has already been demonstrated and has therefore been replaced by bisphenol S (BPS). Although the BPS was considered to be harmless, the latest findings indicate its negative effect on reproduction health. The aim was to verify the thesis that BPS negatively affects the quality of mouse ovaries.
Four-week-old mice of the ICR strain (n = 36) were used for experiments. BPS was administered for 28 days through drinking water in the following doses: BPS1=0.001, BPS2=0.1, BPS3=10 and BPS3=100 ng . g bw^{-1} . day^{-1}. At the termination of the experiment, ovaries were collected and an analysis of the primary, preantral and antral follicles has been performed.
Our findings shown that BPS3 and BPS4 decrease amount of primary, preantral and antral follicles in analyzed ovaries. In addition to their amount, the volume of antral follicles is affected. Our observation underlines a jeopardy of recently widely-used BPS for mammalian reproduction including human health. Astoundingly, extremely low doses of BPS, even comparing with daily tolerable intake of BPA, show significant biological effects with non-linear response.

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EXTREMELY LOW DOSES OF BISPHENOL S AFFECTS QUALITY OF MATURED MOUSE OOCYTES

Ghaibour K.[1,2], Nevoral J.[1], Prokesova S.[3], Stiavnicka M.[1], Hoskova K.[3], Zalmanova T.[3], Petr J.[4], Kralickova M.[4]

[1]*Charles University, Faculty of Medicine in Pilsen, Biomedical Center and Department of Histology and Embryology, Pilsen, Czech Republic*
[2]*Université Lille1- Sciences et Technologies, Team Régulation des Signaux de Division, Villeneuve-d'Ascq, France*
[3]*Czech University of Life Sciences, Faculty of Agriculture, Food and Natural Resources, Prague, Czech Republic*
[4]*Institute of Animal Science, Department of Reproductive Biology, Prague, Czech Republic*

The negative effect of bisphenol A (BPA) on female reproduction has been described. Therefore, BPA has been substituted by BPS which has become widely used in plastic and other materials. Mankind is noticeably exposed by unregulated BPS usage although its biological effects remains unknown.
We simulated BPS-affected follicle wave using 8-week-old ICR mice (n=25), treated with BPS in four different concentrations (0, 0.001, 0.1, 10, 100 ng BPS. g BW-1 . day-1) for 7 days per os. Immature oocytes were isolated and in vitro matured. Thereafter, oocytes were subjected to TUNEL assay or spindle α-tubulin immunolabeling, followed by analysis under confocal microscope (Olympus, Germany) and ImageJ software (NIH, USA).
BPS treatment of mice negatively affected DNA stability of in vitro matured oocytes. We observed 4.7, 7.0 and 5.8-fold TUNEL signal intensity in metaphase chromosomes after 0.001, 0.1, 100 ng BPS. g BW-1. day-1 treatment, respectively. In accordance with previous experiment, BPS shown significantly increased incidence of malformed spindle microtubules and chromosome misalignment.
Our findings highlight the risk of ubiquitinous BPS for oocyte maturation, with warrantable consideration of negative impact to human reproductive health.

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CRYOPRESERVATION OF HUMAN OOCYTES – RESULTS 2015 – 2016 IN ISCARE IVF PRAGUE

Tepla O., Hulvert J.

ISCARE IVF Prague, Czech Republic

Oocyte cryopreservation is a medically recognized treatment option for women at risk of losing their fertility potential, including those needing to undergo cancer therapy and those with premature ovarian insufficiency. It is also increasingly being used by women who, for the purpose of education, health, career, or other reasons, desire to postpone childbearing. Freezing of oocyte is practise in cases, when we have after OPU oocytes, but no sperm. Cryopreservation of donor oocytes through 'oocyte banking' may be a more practical alternative to fresh oocyte donation.

We were frozen 1322 oocytes in 152 cycles. There were 594 oocytes of oncological patiens and social freezing. We offered in program 'oocyte banking' oocytes from 63 donors. It is important to test often the quality of freezing procedure of oocytes. Many centers of assisted reproduction propose freezing of oocytes, but they have little experience with a quality of freeze- thawed oocytes.

We evaluated 45 recipients in program egg donation, where we used frozen - thawed oocytes. We achieved 47 % (21/45) pregnancy rate, 38 % (17/45) clinical pregnancy rate. When we thawed oocytes in 9 standarded cycles, we achieved 44 % (4/9) pregnancy rate and 30% (3/9) clinical pregnancy rate.

Oocyte cryopreservation is an established component of ART. Increasing numbers of women undergo oocyte cryopreservation for both medical and social reasons. It is important to continue auditing outcomes.

POST THAW DISTRIBUTION OF SPERM SUBPOPULATIONS IN THE OLD KLADRUBER STALLIONS IS AFFECTED BY EXTENDER TYPE AND PACKAGING SYSTEM

Bubenickova F., Nehasilova A., Simonik O., Sichtar J.

Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic

The endangered Old Kladruber horse breed is the only indigenous breed of horses in the Czech Republic, which is bred continually on the territory of this country for more than 400 years and became important gene resource with unique characteristics and high cultural and historical value. The genome of these endangered stallions is preserved via freezing of semen. Variable response to freezing methods in stallions is responsible for a constant effort to increase the efficiency of cryopreservation. Even though some stallions can have problems with cryotolerance it is only a matter of finding a suitable freezing extenders and methods to produce fertile frozen insemination dose. It is clear that one of the most utilized tests of potential fertilizing ability of frozen-thawed semen is evaluation of motility, which is the manifestation of structural and functional competence. Moreover, andrological scientific community has recently accepted the existence of heterogeneous sperm populations in mammalian ejaculate. Thus data obtained by computer assisted sperm analysis should be evaluated on level of different subpopulations occurrence. Consequence of this finding is that using mean values for describing motility characteristics of spermatozoa is highly inaccurate and gives misleading results. According to aforementioned information we evaluated the presence of slow, medium a rapid sperm subpopulation in thawed sperm samples frozen either in 5 ml aluminium tubes or 0.5 ml straws and Gent and Lactose freezing extenders to determine the most appropriate combinations for cryopreservation of Old Kladruber stallions' semen. For motility evaluation, k-means cluster analysis was used to classify sperm into subpopulations. We found out that the extender and packaging system used significantly affected percentage of sperms belonging to slow, moderate and rapid subpopulations ($P < 0.05$). In all evaluated incubations times the combination of Lactose extender and 5 ml aluminium tube yielded significantly higher percentage of slow sperms compared to the rest of combinations ($P < 0.05$). The percentage of sperms belonging to moderate subpopulation was highest within the whole incubation time in combination of extender Gent and 5 ml aluminium tubes ($P < 0.05$). The highest percentage of rapid subpopulation within the

whole incubation period of 60 minutes was in Gent 0.5 ml samples when compared to rest of the combinations ($P < 0.05$).

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