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MEDICAL FACULTY OF CHARLES UNIVERSITY
AND FACULTY HOSPITAL IN PILSEN

XXIInd SYMPOSIUM OF IMMUNOLOGY AND BIOLOGY OF REPRODUCTION WITH INTERNATIONAL PARTICIPATION

in memory of Dr. Radslav Kinsky

PROGRAM AND ABSTRACTS

The Castle, Třešť, May 26 – 28, 2016

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XXIInd Symposium of Immunology and Biology of Reproduction
with International Participation
The Castle, Třešť, May 26 – 28, 2016

PROGRAM

Thursday, MAY 26, 2016

Arrival and accommodation at the Castle Trest

~19.00 DINNER

Friday, MAY 27, 2016

Breakfast from 7 am

9.30-9.40 OPENING CEREMONY:
Ulcova-Gallova Z., Peknicova J.

Chairpersons: Ulcova-Gallova Z., Jonak J.

9.40-10.00 Chaouat G. (France): Critical role and therapeutic control of the lectin pathway of complement activation/prone mouse mating.
10.00-10.20 Moore H. (U.K.): Identifying new ligands of human sperm-egg interaction.
10.20-10.40 Sebkova N. (CZ): Distribution of CD46 and β1 integrin molecules with respect to different membrane structures of the sperm head.
10.40-11.00 Frolikova M. (CZ): Super-resolution Stimulated Emission Depletion (STED) microscopy imaging of selected integrins in mouse acrosome intact sperm.

11.00-11.30 COFFEE BREAK

Chairpersons: Peknicova J., Madar J.

11.30-11.50 Di Simone N. (Italy): Idiopathic recurrent pregnancy losses: new diagnostic tools.
11.50-12.10 Dostalova P. (CZ): Estrogen receptor beta in testicular cells and sperm.
12.10-12.30 Jankovicova J. (Slovakia): Detection of tyrosine phosphorylated proteins on bull sperm during epididymal maturation.
12.30-12.50 Postlerova P. (CZ): Epididymal maturation – a crucial step in the post-testicular sperm development.
12.50-13.10 Antalikova J. (Slovakia): Capacitation state evaluation of sperm.
13.10-13.30 Liska F. (CZ): Splicing mutation in *Sbf1* causes nonsyndromic male infertility in the rat.

13.30-14.30 LUNCH

Chairpersons: Jonakova V., Nagyova E.

14.30-14.50 Lipcseyova L. (Slovakia): Anti-phosphotyrosine assay as a tool for evaluation of bull sperm capacitation.
14.50-15.10 Stiavnicka M. (CZ): New approaches in sperm evaluation: heterogeneity of ejaculates and non-invasive tehniques.
15.10-15.30 Pohlova A. (CZ): Study of sperm proteins in different mammalian species.
15.30-15.50 Zatecka E. (CZ): The effect of tetrabrombisphenol A on Sertoli cells: *in vivo* and *in vitro* approach.
15.50-16.10 Paal D. (Slovakia): Taurine maintains sperm motility *in vitro*.

16.10-16.40 Christening of the book: "Imunology and immunopathology of human reproduction"

COFFEE BREAK

Chairpersons: Dvorakova-Hortova K., Antalikova J.

16.40-17.00 Petelak A. (CZ): Cryopreservation of FACS sorted boar spermatozoa based on the extracellular ubiquitination.
17.00-17.20 Nagyova E. (CZ): Versican expression in porcine ovarian follicle.
17.20-17.40 Dorosh A. (Ukraine/CZ): Single cell expression analysis of genes with potential mRNA gradient in mouse oocytes.
17.40-18.00 Nevoral J. (CZ): Histone code in zygote: its establishment, regulation and involvement in embryonic development.

18.00-18.20 Pasculli R.: Presentation of firm ACCELA

19.30 DINNER (RAUT)

Saturday, MAY 28, 2016

Breakfast from 7 am

Chairpersons: Sedmikova M., Zidkova J.

9.00-9.20 Prokesova S. (CZ): Meiotic maturation of porcine oocytes *in vitro* – the effect of endocrine disruptors bisphenol S.

9.20-9.40 Pavlinkova G. (CZ): Diabetic embryopathy.

9.40-10.00 Valaskova E. (CZ): The effect of diabetes mellitus on reproductive parameters and expression of selected testicular genes in streptozotocin-induced diabetic mice.

10.00-10.20 Tibenska E. (Slovakia): The effect of treatment – the oral methylprednisolone – on the values of pro-inflammatory cytokines in the fertility problems treatment.

10.20-10.40 Tvaruzkova P. (CZ): Polycystic ovary syndrome (PCOS) and the serum level of anti-Müllerian hormone (AMH).

10.40-11.00 COFFEE BREAK

Chairpersons: Sebkova N., Dorosh A.

11.00-11.20 Benesova E. (CZ): Serum levels of anti-Müllerian hormon in women with endometriosis.

11.20-11.40 Zidkova J. (CZ): Antioxidative enzymes in human sera and seminal plasma after treatment with selenium, vitamin E, and zinc.

11.40-12.00 Dzurilova Z. (Slovakia): Intravenous immunoglobulin treatment for immunologically based reproductive disorders.

12.00-12.20 Mala E. (CZ): I have the right to make decisions about my own health.

12.20-12.40 Lestan M. (Slovakia): Recurrent pregnancy loss on the basis of autoimmunity? ... or woman is a mysterious creature. Case report.

12.40-13.00 Polakova M. (CZ): Treatment of rare intrauterine adenomyosis at the patient with primary sterility. Case report.

13.00 CLOSING OF SYMPOSIUM
Peknicova J., Ulcova-Gallova Z.

13.10-14.00 LUNCH

14.10 VISITING OF KINSKY PRIVATE GARDENS AND MUSEUM, **ŽĎAR NAD SÁZAVOU** - BUS

XXII[nd] Symposium of Immunology and Biology of Reproduction
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ABSTRACTS

Friday, MAY 27, 2016

CRITICAL ROLE AND THERAPEUTIC CONTROL OF THE LECTIN PATHWAY O COMPLEMENT ACTIVATION IN AN ABORTION-PRONE MOUSE MATING

Chaouat G, Petitbarat M, Durigutto P, Macor P, Bulla R, Palmioli A, Bernardi A, De Simoni M-G, Ledee N, Tedesco F

I will briefly recall the characteristics of the abortion-prone mating combination CBA/J x DBA/2 and the kinetics of events during the “window of resorption window”. This model, thanks to Guillermina Girardi, now is known to be as well as has been recognized a model of preeclampsia, Complement activation as well as Ts have been implicated in the high rate of pregnancy loss observed in CBA/J mice. We have analyzed the implantation sites collected from DBA/2-mated CBA/J mice for the deposition of the complement recognition molecules using CBA/J mated with BALB/c mice as a control group. MBL-A was observed in the implantation sites of CBA/J 3 DBA/2 combination in the absence of MBL-C and was undetectable in BALB/c-mated CBA/J mice. Conversely, C1q was present in both mating combinations. Searching for other complement components localized at the implantation sites of CBA/J 3 DBA/2, we found C4 and C3, but we failed to reveal C1r. These data suggest that complement is activated through the lectin pathway and proceeds to completion of the activation sequence as revealed by C9 deposition. MBL-A was detected as early as 3.5 d of pregnancy, and MBL-A deficiency prevented pregnancy loss in the abortion-prone mating combination. The contribution of the terminal complex to miscarriage was supported by the finding that pregnancy failure was largely inhibited by the administration of neutralizing Ab to C5. Treatment of DBA/2-mated CBA/J mice with Polyman2 that binds to MBL-A with high affinity proved to be highly effective in controlling the activation of the lectin pathway and in preventing fetal loss. We will present hypothesis on how to link complement activation and Ts deficiency.

IDENTIFYING NEW LIGANDS OF HUMAN SPERM-EGG INTERACTIONS

[1] Moore H, [1] Gregory S, [2] Klinovska K, [2,3] Frolikova M, [3] Dorosh A, [2,3] Dvorakova-Hortova K

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Understanding the molecular mechanisms of human fertilization is important for developing new methods of assisted conception. Although some of the main receptors of mammalian fertilization have been identified (i.e Izumo on the sperm and Juno on the egg), it is considered that other factors will also play major roles in bringing about sperm-egg fusion and activation of the egg and have species specific aspects.
Detection methods that are unbiased are important when attempting to identify candidate molecules involved in fertilization processes. In drug discovery, a bead library where every bead in the library detects a different moiety (peptide sequence) – the so called one bead one compound (OBOC) method is often used. We adapted this approach for use with human spermatozoa *in vitro* and based on sperm attachment to just a few beads (out of many thousands) identified 16 candidate peptide sequences that might be involved in fertilization processes. Further sperm incubations in the presence and absence of calcium ranked these peptides for potential importance in sperm-egg fusion. Informatics analysis of the lead peptide sequence indicated it was of an immunoglobulin or FC receptor-like molecule (FCRL3). Since it had been previously hypothesized that immunoglobulin super family (IgSF) like molecules may be involved as sperm receptors on the egg, we have investigated this candidate in more detail. FCRL3 is expressed on human and mouse eggs and during mouse fertilization FCRL3 and Izumo co-localise on the egg membrane. Monoclonal antibody to FCRL3 blocks sperm attachment to failed-to-fertilize human eggs. Recently we have transfected CHO cells (that do not express FCRL3) with an FCRL3 transgene. These CHO cells transiently express FCRL3 and bind human sperm *in vitro* to a much greater extent than non-transfected control cells. I will summarise our latest findings and propose a model of the sperm-egg fusion process.

This research was partially supported by the GACR No. P502-14-05547S, by the project BIOCEV CZ.1.05/1.1.00/02.0109 from the European Regional Development Fund, and by the Institutional support of the Institute of Biotechnology RVO: 86652036.

DISTRIBUTION OF **CD46 AND B1 INTEGRIN MOLECULES** WITH RESPECT TO DIFFERENT MEMBRANE STRUCTURES OF THE SPERM HEAD

[1,3] Šebková N, [1,2] Frolíková M, [1] **Děd L,** [1,2] **Dvořáková**-Hortová K

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CD46 protein plays an important role during fertilization and its role is associated with acrosome stability. CD46 is probably involved in signalling pathways triggering the acrosome reaction (AR). It also associates, through membrane integrins, with specific MAP kinases involved in the AR.

Our aim was to monitor possible dynamics of relocation and movement of CD46 and β1 integrin during sperm maturation and its preparation for the fertilization. The possible dependence of this localization changes on the dynamic of actin cytoskeleton was studied. Our results show changes in the localization of these proteins associated with the AR and their mutual co-localization was observed using proximity ligation kit Duolink. After the acrosome reaction, CD46 and β1integrin spreads across the sperm head, entering the post-acrosomal compartment, and permeates the borders of different domains. The quantitative analysis of protein relocation dynamics was performed using the fluorescent intensities of CD46 and β1integrin dual staining. The redistribution of fluorescent signals was showed among the individual segments of the sperm head.

It was shown previously that actin dynamics is necessary for acrosome reaction-associated translocation of Izumo1 protein that is required for sperm-egg fusion. The question rose whether CD46 and β1 integrin proteins are also affected by actin dynamic. Cytochalasin B, a toxin binding to actin filaments and blocking its polymerization, was used during sperm incubation. This toxin significantly shifted the ability of sperm to relocate both the CD46 and β1 integrin when the AR was induced by calcium ionophore. The co-incubation of capacitated sperm with Cytochalasin B lead to a dramatic decrease of the percentages of sperm, which express relocation pattern after induced AR for both CD46 (~30%) and β1 integrin (~40%) compared to the control group with relocation pattern of both proteins in 90% for sperm.

3D models and visualizations of potential membrane processes responsible for the relocation of the proteins from the acrosomal area to the other compartments of the sperm head were prepared.

In summary, our results deliver new information that proteins CD46 and β1 integrin undergo dynamic relocation towards the sites of sperm-egg fusion during the AR *in vitro*. We have also shown that inhibitor of actin dynamics abrogate significantly the acrosome-reaction-associated changes in CD46 and β1 integrin localization. We speculate that this relocation is of importance for the successful sperm-egg interaction, adhesion and subsequent gamete fusion. The new 3D models represent highly important and efficient visual representation of proteins rearrangements essential for the process of fertilization.

This research was supported by the GACR No. P502-14-05547S, by the project BIOCEV CZ.1.05/1.1.00/02.0109 from the European Regional Development Fund, and by the Institutional support of the Institute of Biotechnology RVO: 86652036.

SUPER-RESOLUTION STIMULATED EMISSION DEPLETION (STED) MICROSCOPY IMAGING OF SELECTED INTEGRINS IN MOUSE ACROSOME INTACT SPERM

[1,2] Frolíková M, [1,2,3] Šebková N, [1] **Děd L,** [1,2] **Dvořáková**-Hortová K

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The integrins are transmembrane proteins that participate in many cell-cell and cell-extracelular matrix interaction. These membrane receptors are important part of signalling pathway and they have ability to transmit signals in both directions – into and out of the cell. The integrins are able to associate with other membrane receptors in multi-molecular complexes that participate in cell activation. Integrins proteins consist of two different subunits α and β. β subunit is capable of binding to actin and controlling of its remodeling. To present, 18 α and 6 β integrin subunits and 24 their heterodimers combinations have been described in mammals. Recently, there have been certain integrins described to be expressed also on sperm. Importantly, in some cases of man infertility, there was detected reduced expression of integrins and positive correlation between expression of integrins and fertilizing ability of human spermatozoa was shown. For this reason integrins were proposed as a potential clinical marker to evaluate man sperm quality. However, up to date, there is lack of information about distribution of individual integrins heterodimers and their function on mature sperm. Therefore, we set the aim to investigate and characterize the presence of selected integrins subunits and their accurate position in acrosome intact sperm using mouse model for a start. Dual fluorescent labeling and STED super resolution microscopy was used for localization of the studied proteins. So far we have described the expression of tree subunits α3, α6 and β1 on acrosome intact mouse sperm. The presence of α3 was detected on outer acrosomal membrane and plasma membrane of acrosomal cap area. β1 subunit was present in same structures and even in plasma membrane of the apical hook. α6 was found in plasma membrane of apical hook and equatorial segment. Taking together previously described integrins subunit affinity in somatic cells, and our findings, we suggest presence of tree functional heterodimers such as α3β1, α6β1 and

α6β4 in mouse sperm. To confirm these conclusions, a detection and characterization of β4 subunit localization will be our next step.

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 Microscopy Centre, IMG ASCR, Prague, Czech Republic for their support with obtaining STED super-resolution microscopy data presented in this paper.

IDIOPATHIC RECURRENT PREGNANCY LOSSES: NEW DIAGNOSTIC TOOLS

Di Simone N

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Spontaneous pregnancy loss is the most common complication of pregnancy, occurring in 15% of clinically recognized pregnancies. Approximately 1-5% of all women suffer from recurrent pregnancy loss (RPL). Although RPL has been associated to various hematologic, anatomic, hormonal, immune and genetic defects, in about 30-40% of the cases, screening tests included in the RPL workup may result negative. Thus, the identification of factors involved in RPL and a clearer understanding of its causes are urgently needed.

The establishment and maintenance of pregnancy occurs through the interaction of maternal endometrial and trophoblast tissues, so the pathogenic mechanisms underlying pregnancy loss must directly or indirectly affect this interaction. Recently, we noticed that abnormal endometrial inflammosome activation, in absence of detectable infectious causes, might be one of the molecular mechanisms involved in establishing an unreceptive endometrium, potentially leading to early fetal loss. The detection of this inflammatory response in human endometrium might lead the way to the identification of immunomodulatory therapies in order to improve endometrial receptivity.

Over the last few years, medical scholars have reported the significant association between RPL and celiac disease (CD). The pathogenic mechanism underlying the pregnancy failure in CD is not well understood: the ability of anti-transglutaminase antibodies to impair trophoblast invasiveness and endometrial endothelial cells differentiation has been suggested as a possible mechanism able to disrupt early placentation. CD shows a complex non-Mendelian pattern of inheritance, involving major histocompatibility complex (MHC) genes. The strongest effects are mapped to the classical HLA-DQA1 and HLA-DQB1 genes. Recently, we observed a significantly increased prevalence of HLA-DQ2/DQ8 positivity in RPL population compared to control women. HLA DQ2/DQ8 haplotypes codify for the DQ2/DQ8 proteins, HLA class II molecules with an important role in the presentation of disease-related peptides to T lymphocytes. In particular, in CD, both proteins are responsible for the presentation of immunogenic gluten peptides to DQ2/DQ8-restricted CD4+ T cells. Once activated, CD4+ T enhance a complex

immune response that involves both the innate and the adaptive immune system, with increased production of interferon (IFN) γ, tumor necrosis factor (TNF) α, and autoantibodies like anti-transglutaminase, -endomysium, and –gliadin antibodies.

These data would suggest that both inflammosome and HLA DQ2/DQ8 might represent a novel family of markers and/or therapeutic targets in RPL. Their use would be of great promise for i) better understanding of the process of both normal and abnormal implantation and ii) providing clues to the causes and therapy of a subgroup of early pregnancy losses and/or unexplained infertility.

ESTROGEN RECEPTOR BETA (ERβ) IN TESTICULAR CELLS AND SPERM

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Estrogen is a steroid hormone that plays an important role during sperm development in the male and female reproductive tract. Estrogen signalling is a complex process that depends on cell milieu and presence of receptors. Thanks to the steroid nature of estrogens, they can pass through the plasmatic membrane and bind to the intracellular estrogen receptors (ERs). Within the cell, there are several pools of ERs. One of them is localized to the cell nucleus and their activation leads to direct or indirect binding to DNA and ultimately to alternation in gene expression (genomic pathway). Other pools of ERs are associated with plasma membrane or are located in cytosol. Activation of membrane associated ERs leads to rapid non-genomic responses. Nowadays, two classical estrogen receptors are known – ERα and ERβ. Since ERβ is a predominant variant in testes, we focused our study on expression of ERβ variants in murine testes and sperm. We detected two variants of ERβ at mRNA level in both, testes and sperm. These variants differ in 54 nucleotids within the ligand binding domain and this variability results in different affinity to estrogens. Further, we analyzed individual testicular cell types (spermatogonia, spermatocytes, spermatids, and Sertoli cells) by RT-qPCR. Our results suggest that both ERβ variants are coexpressed in the same cell type and may therefore interact together. This may have consequences in mediating of estrogen signalling. Moreover, ERβ is expressed more in the later stages of spermatogenesis suggesting the role of ERβ in these stages or alternatively in spermatozoa alone. At the protein level, we detected ERβ in nuclear, membrane and cytosolic fraction prepared from testicular tissue suggesting the involvement of both, genomic and non-genomic, pathways of estrogen signaling in testes. In sperm, anti-ERβ antibodies localized ERβ in acrosome region and tail which is in accordance with the known role of estrogen on capacitation, acrosome reaction and motility.

This work was supported by the Grant Agency of Charles University No. 618812 and by BIOCEV project from the ERDF (CZ.1.05/1.1.00/02.0109) and by the Grant Agency of the Czech Republic P502-14-055 47S.

DETECTION OF TYROSINE PHOSPHORYLATED PROTEINS ON BULL SPERM DURING EPIDIDYMAL MATURATION

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The mammalian spermatozoa released from seminiferous tubules of testes mature in epididymis to acquire the ability to fertilize the egg. Epididymal maturation of sperm is founded on secretion of hundreds of proteins and other molecules by epididymal epithelium into the luminal fluid and their direct or indirect interactions with spermatozoa. Epididymis is characterized by specific segment-dependent production of proteins with different participation in physiological maturation of sperm. The changes in protein spectrum of spermatozoa include an addition of new proteins, deletion or translocation and modification of some proteins. Tyrosine phosphorylation of sperm proteins is thought to be a critical point to approach the capacitation state of spermatozoa, including the hyperactivated movement. Whereas significant interspecies differences regarding impact of epididymal maturation on the tyrosine phosphorylation of sperm proteins were described, in our study, fluorescent patterns representing localization of tyrosine phosphorylated proteins of bull sperm during the transit through the epididymis were inspected. In our experiments, the spermatozoa isolated form *caput, corpus* and *cauda* as well as cryosections of epididymis segments and testes were tested in indirect immunofluorescence assay using anti-phosphotyrosine antibody P-Tyr-01. Four different patterns of P-Tyr-01 antibody were distinguished on sperm smears and their portion in population of sperm was epididymal segment-dependent. Generally, we observed decrease in number P-Tyr-01 negative sperm /from *caput* to *cauda*/ and on the other hand, number of sperm positively stained for tyrosine phosphorylated proteins increased. On the tissue sections, tyrosine phosphorylated proteins were detected on sperm in lumen of *caput, corpus* as well as *cauda* of epididymis. No tyrosine phosphorylated proteins were detectable on sperm cells in testes sections.

Supported by grants VEGA 2/0037/16; APVV- 0137-10, SAV-AV ČR 15-05.

EPIDIDYMAL MATURATION – A CRUCIAL STEP IN THE POST-TESTICULAR SPERM DEVELOPMENT

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Mammalian spermatozoa after their development in testis undergo the post-testicular maturation in epididymis where acquire their fertilization ability and competence of movement. The epididymis is tissue with very active fluid-absorbing and fluid-secreting activity. Epididymal fluid contains ions and small molecules, proteins, glycoproteins and enzymes. The surface of spermatozoa is exposed directly to the epididymal fluid, and the sperm plasma membrane is significantly changed. Some testicular proteins are altered, masked, or replaced by new proteins/glycoproteins of epididymal origin. Several proteins produced by epididymis have been described in various mammalian species and shown to be associated with spermatozoa suggesting a role in the sperm maturation and/or sperm-egg binding and fusion.

We isolated proteins from fluid, tissue and sperm of boar epididymis, and separated them by chromatographic and electrophoretic methods. We searched for known proteins using panel of antibodies and tested proteins of epididymal fluid for binding abilities. In the epididymis, we found proteins described as proteins of seminal plasma and associated with the sperm surface, such as spermadhesins, beta-microseminoprotein and acrosin inhibitor. These proteins were detected in epididymal sperm, fluid and tissue. We showed that some epididymal proteins may bind the spermatozoa and change the binding sites on the sperm surface. We determined and identified some proteins from boar epididymal fluid with affinity to heparin, hyaluronan and zona pellucida glycoproteins. These phenomena indicate that epididymal fluid proteins bind to the sperm surface during epididymal maturation and might subsequently play role in the sperm capacitation or sperm-zona pellucida binding.

This work was supported by GA CR P502/14/05547S and by the project BIOCEV CZ.1.05/1.1.00/02.0109 from the ERDF.

CAPACITATION STATE EVALUATION OF SPERM

Antalíková J, **Jankovičová J,** Cupperová P, Michalková K,
Lipcseyová D**, Horovská Ľ, Simon M**

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Ejaculated mammalian sperm must undergo a series of biochemical and physiological modifications, collectively called capacitation, normally occurred in the female genital tract, however, it can also be achieved *in vitro*. Sperm capacitation is associated also with the surface protein profile changes. We study these changes on *in vitro* capacitated bovine sperm using a set of monoclonal antibodies by immunofluorescent assay and electrophoretic analysis. To verify the ratio of capacitated sperm population, it is necessary to use reliable detection method of sperm capacitation state applicable for cattle. In some species (mice, boar), epididymal or freshly ejaculated sperm are subjected to capacitation process and the methods applied for their evaluation are based on detection of biochemical changes associated with the capacitation process, like CTC-chlortetracycline, phalloidin and anti-phosphotyrosine antibody fluorescence assays. Whereas cryopreservation is an important tool for assisted reproduction in cattle, we simultaneously analysed freshly ejaculated and cryopreserved sperm. *In vitro* capacitation of freshly ejaculated sperm lasts for 4 h, while frozen-thawed bovine spermatozoa were referred to as capacitated or able to capacitate very easily. The evaluation of capacitation state or frozen-thawed sperm using CTC or phalloidine assays shows massive population of bull sperm cells with a non-specific or intermediate signal status. It was also reported, that the profile of phosphotyrosine-containing protein in freshly ejaculated-capacitated and frozen-thawed sperm differs. Based on these facts it seems, that detection method using anti-phosphotyrosine antibody fluorescence assays could reliably evaluate the capacitation status of bull sperm.

Supported by grants VEGA 2/0037/16; APVV- 0137-10, SAV-AV ČR 15-05.

SPLICING MUTATION IN SBF1 CAUSES NONSYNDROMIC MALE INFERTILITY IN THE RAT

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In the inbred spontaneously hypertensive rat (SHR/OlaIpcv) colony, we identified males with small testicles and inability to reproduce. By selectively breeding their parents we revealed the infertility to segregate as an autosomal recessive mendelian character. No other phenotype was observed in males and females were completely normal. By linkage using a backcross with Brown Norway (BN/Cub), we mapped the locus to a 1,2 Mbp segment on chromosome 7, harboring 35 genes. Sequencing of candidate genes revealed a G to A substitution in a canonical "AG" splice site of intron 37 in *Sbf1* (SET binding factor 1, alias myotubularin-related protein 5). This leads either to skipping exon 38 or shifting splicing one base downstream, invariantly resulting in frameshift, premature stop codon and truncation of the protein. Western blotting using a C-terminal anti-Sbf1 antibody revealed absence of the full-length protein in the mutant testis. Testicles of the mutant males were significantly smaller compared to SHR from 4 weeks, peaked at 84% wild-type weight at 6 weeks and declined afterwards to 28%, reflecting massive germ cell loss. Histological examination revealed lower germ cell number, latest observed germ cell stage were round spermatids. This resulted in absence of sperm in the epididymis (azoospermia). Transcriptome profiling of juvenile males (6 weeks) using Affymetrix microarrays reflected the germ cell loss in mutants by a large drop in spermatid-specific gene expression, while Sertoli cell specific genes were slightly upregulated. Spermatogonia and spermatocyte genes did not show significant differences between the mutant and wild-type males. Sbf1 is a member of a phosphatase family lacking the catalytical activity. It probably modulates the activity of a phosphoinositol phosphatase MTMR2. MTMR2 is specific for position 3 in phosphatidylinositol-3-monophosphate (PtdIns3P) and phosphatidylinositol-3,5-bisphosphate (PtdIns3,5P2). PtdIns3P is in turn concentrated in early endosomes and is an important regulator of endosome trafficking. Human homozygotes or compound heterozygotes for missense SBF1 mutations exhibit Charcot-Marie-Tooth disease (manifested mainly as progressive neuropathy), while a single mouse knock-out reported in the literature identified male infertility as the only phenotype manifestation.

ANTI-PHOSPHOTYROSINE ANTIBODY FLUORESCENCE ASSAY AS A TOOL FOR EVALUATION OF BULL SPERM CAPACITATION

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Capacitation is a multiple event process which is required prior to fertilization, as only capacitated sperm has an exclusive ability to undergo acrosome reaction and fertilize the oocyte. This biological phenomenon includes a series of sequential or parallel biochemical changes in the sperm leading to its activation. The cholesterol efflux alters the permeability of the sperm plasma membrane leading to high influxes of HCO3- and Ca2+ ions. They can facilitate capacitation by activating adenylate cyclase regulating cAMP metabolism which stimulates protein kinase A (PKA) signalling cascade. Thus, PKA triggers the phosphorylation of various target proteins that are presumed to initiate several signalling pathways. To assess the functional status of bull sperm incubated *in vitro* we designed an immunofluorescence labelling assay which uses an anti-phosphotyrosine P-Tyr-01 antibody whereas massive phosphorylation on tyrosine residues is required for capacitation. We aimed to compare changes of the P-Tyr-01 reaction pattern in freshly ejaculated sperm with spermatozoa incubated in TL medium for sperm cell capacitation at 39 °C with 5 % CO2 for 4 hours. In contrast to ejaculated, the capacitated sperm showed the intensive fluorescent signal representing sperm phosphorylation status. These results suggest that this method is applicable for evaluation the bull sperm capacitation state.

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NEW APPROACHES IN SPERM EVALUATION: HETEROGENEITY OF EJACULATES AND NON-INVASIVE TEHNIQUES

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Sperm is the most diverse cell type known and it is considered that this diversity reflects differences in sperm function. However, how assess these differences between sperm belonging to the same ejaculate and most importantly, how to select in real-time that spermatozoa with a particular trait remains incompletely described.

Hitherto, many sperm selection techniques have been developed, obtaining different sperm subpopulations based on shape, motility or membrane composition. Additionally, most of these techniques are time-consuming and in some cases require that sperm samples are centrifuged which may be harmful to the cell.

Furthermore, we should take into account that in current assisted reproductive technologies (ART) such as intracytoplasmic sperm injection (ICSI) all natural selection barriers are bypassed. Thus, most of routinely assessed sperm parameters do not provide relevant information due to the spermatozoa is injected directly into the oocyte. However, since each single sperm used in ICSI does not result in a child, new sperm quality markers have to be discovered.

Many evidences suggest that both genetic and epigenetic abnormalities, which are not manifested microscopically, may affect the outcome of ART. The use of powerful tools such as flow cytometry in combination with antibodies will help initially to establish those sperm characteristics at molecular level to identify sperm subpopulations with higher fertilization ability. However, the potential of flow cytometry is limited since sperm could be damaged by this procedure (incubation with fluorescence dye, high dilution rate), although its objectivity, speed, replicability and the large number of evaluated cells cannot be overlooked neither equaled by any other technique, allowing us to obtain robust conclusions.

The preliminary results obtained by means of flow cytometry should be the basis for future studies aimed at exploring non-invasive techniques for sperm evaluation and selection. In this line, the combination of novel sperm markers with Raman spectroscopy is presented as a reliable approach for selecting the healthiest gametes ensuring a successful

fertilization and embryo development. This techniques has been employed in various medical fields and its application in andrology has just emerged and appears promising for improvement of *in vitro* ART outcomes.

STUDY OF SPERM PROTEINS IN DIFFERENT MAMMALIAN SPECIES

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Reproduction is an essential feature of all animals and a fundamental step to produce new generations. Study of sperm proteins is crucial for understanding of the sperm-egg recognition. We searched out sperm surface proteins involving in the *zona pellucida* (ZP) binding and studied whether these proteins are preserved throughout mammalian species. Indirect immunofluorescent technique was used to test a panel of monoclonal antibodies prepared against boar sperm surface proteins on spermatozoa of bull and mice. We found a cross-reactivity of some antibodies against boar sperm with bull ejaculated and mouse epididymal spermatozoa. Further, we isolated sperm proteins from different mammalian species, such as pig, bull, dog, cat, mouse and human. Proteins were separated by SDS-electrophoresis and protein/glycoprotein profiles from epididymal, ejaculated and *in vitro* capacitated sperm were compared. The interaction of sperm with ZP was studied on electrophoretically-separated sperm surface proteins from pig and bull with biotin-labeled ZP glycoproteins using Far Western blot. Antibodies, which reacted with boar sperm surface proteins with ZP-binding activity, therefore could be potential egg-binding receptors, were used for monitoring of the sperm protein origin in reproductive fluids and tissues.

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THE EFFECT OF TETRABROMOBISPHENOL A ON SERTOLI CELLS: *IN VIVO* AND *IN VITRO* APPROACH

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Tetrabromobisphenol A (TBBPA) belongs to the most widely used flame retardants. It is found in the environment and can also be detected in the human body. Several studies have shown that TBBPA is able to negatively influenced reproductive parameters in vivo. Moreover, it has been proven that TBBPA has negative effect on different mammalian cell lines in vitro and it is able to interfere with the thyroid hormone system as well as it might be able to affect the androgen/estrogen hormone system.

In this study, we decided to examine the potential effect of TBBPA on Sertoli cells (SC) *in vivo* as well as *in vitro*. In our *in vivo* study, animals were exposed to TBBPA (35 ug/kg b.w.) continuously during life. In 10 weeks old animals, we showed that TBBPA is able to induce apoptosis of testicular cells, which involved mainly the diploid cell population (including SC). We also analysed expression of Sox 9 gene (SC marker), which was significantly lower in experimental group. Moreover, histological analysis revealed significantly lower thickness of seminiferous epithelium in exposed animals. Further, we focused on 4 week old (pre-pubertal) animals in order to analyse SC and their maturation status. In these animals we detected higher number of TUNEL positive cells as well as less Wt1 (SC marker) positive cells. Furthermore, we detected significantly decreased expression of genes for Wt1 and Thyroid hormone receptor, which is sensitive indicator of SC maturational state. Taken together our results suggest that TBBPA causes premature maturation of SC, which in turn leads to apoptosis of these cells. However, more experiments are needed to further support our hypothesis. Moreover, *in vitro* outcome of various TBBPA concentrations on SC cell line proliferation and apoptotic status showed negative concentration-dependent effect of TBBPA on proliferative activity of SC.

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TAURINE MAINTAINS RABBIT SPERM MOTILITY *IN VITRO*

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Artificial insemination in rabbits has been considerably growing over the last decades. Amino acids have been repeatedly shown to maintain mammalian sperm motility. This study was designed to examine the effect of taurine on the motility of rabbit sperm *in vitro*. Semen from five sexually mature and healthy New Zealand White bucks was pooled. Three pooled samples were treated with taurine at 0 (control), 1.5, 3, 6.25 and 12.5 mM and subsequently incubated at 5°C for 4 h. CASA analyses displayed no immediate effect of taurine on sperm kinetics represented by total motility (MOT), progressive motility (PRO), beat cross frequency (BCF), amplitude of lateral head displacement (ALH) and curvilinear velocity (VCL) ($p>0.05$). The continued incubation for 2 h resulted in higher MOT and PRO in the treatments with 1.5 mM taurine, and elevated VCL, ALH and BCF in all treatments ($p<0.05$). Taurine maintained MOT as well as PRO of the sperm treated with 6.25 mM taurine for 2 h. Here, an increase in VCL, ALH and BCF was observed for all samples containing taurine as well ($p<0.05$). A noticeable increase in MOT, PRO, VCL, ALH and BCF was observed for all treatments by 4 h of incubation ($p<0.05$). Taurine has been proved to maintain the motility of rabbit sperm *in vitro.*

CRYOPRESERVATION OF FACS SORTED BOAR SPERMATOZOA BASED ON THE EXTRACELLULAR UBIQUITINATION

Petelak A

Our work is focused on the methodology of fluorescence activated cell sorting (FACS) of spermatozoa stained by the antibody against extracellular surface marker ubiquitin (eUb) and subsequent protocol for their long term storage in liquid nitrogen (LN). High level of spermatozoa surface ubiquitination has been previously discussed in many articles as a negative quality marker. From general point of view any other outer membrane antigen would be compatible with our approach.

Regarding our experimental design we found that only those insemination doses with at least 40% of motile spermatozoa after freezing and thawing (F/T) in the egg-yolk medium with lactose are suitable for the subsequent antibody staining and FACS. The sorting rate was sufficient for the preparation up to 20 spermatozoa aliquots for intracytoplasmic sperm injections (ICSI). Two significantly different groups with good freezability were prepared and stored in LN (0.73% contamination of spermatozoa with high eUb level in non-ubiquitinated group and reversely 6.65% spermatozoa without eUb in highly ubiquitinated spermatozoa). Sperm viability after FACS variated from 11% to 28% regardless used media ($P = 0.15$). Required viability of F/T sorted spermatozoa was obtained by using extender Solusem® as a load and collection medium. In this case 12% of viable spermatozoa with progressive motility in low eUb level group and 7% in high eUb level ($P < 0.05$) was detected.

Our approach allows obtaining sufficient number of viable spermatozoa for subsequent artificial fertilization by ICSI. This procedure could be used for wide variety of spermatozoa sorting based on different surface markers.

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VERSICAN EXPRESSION IN PORCINE OVARIAN FOLLICLES

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Versican is a large extracellular matrix (ECM) proteoglycan. In rodent, versican V0/V1 expression increases about 4 h after hCG induction, while in bovine granulosa cells no increase was observed post hCG injection. The versican V1 cleaved product G1-DPEAA accumulates in the mouse cumulus matrix *in vivo* prior ovulation. We investigated the spatiotemporal expression of G1-DPEAA cleaved product in porcine follicles. Porcine oocyte cumulus complexes (OCCs) stimulated *in vivo* and OCCs and mural granulosa cells (MGCs) stimulated with FSH/LH were digested with either chondroitinase ABC or Streptomyces hyaluronidase. Total, matrix and cell pellet extracts were analyzed by Western blot by using a versican antibody recognizing the neoepitope DPEAAE. Both *in vivo* expanded and *in vitro* FSH/LH stimulated OCCs accumulated V1 versican cleaved form (~70 kDa) in the ECM. We suggest that the versican ~70 kDa cleaved product accumulated in the ECM with time, since it was barely visible at 26 h and became quite evident at 44 h of culture. The OCCs treated with hyaluronidase showed an additional band of about 75 kDa MW. This ~75 kDa form was quite evident at 26 h after stimulation, increased slightly at 44 h and was absent in MGCs. The identity of ~75 kDa band is under investigation.

SINGLE CELL EXPRESSION ANALYSIS OF GENES WITH POTENTIAL mRNA GRADIENT IN MOUSE OOCYTES

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In frogs, there are clearly visible differently pigmented animal and vegetal poles of the egg determined before fertilization and leading to asymmetrical divisions. Mammalian egg does not show any comparable differentiation and it has been generally accepted that even the individual blastomeres in 2-cell and 4-cell embryos are homogenous. However, recent findings suggest that those blastomeres display different gene expression patterns and might already possess some inclinations to specific cell lineages. We therefore raised a question, whether there could be any mRNA or protein gradients in pre-fertilization oocytes similar to a previously described amphibian egg one.
In mammalian eggs, there is a membrane region that is poor in microvilli, cortical granules are absent beneath plasma membrane and sperm cells generally do not bind to this location. This microvilli free region also covers the egg nucleus, and cytoskeleton localization differs markedly to the rest of the cortical space, forming actin –myosin II cortical cap/ring and is considered as animal pole. The purpose of this study was to determine gene products that can be detected at single cell level using qPCR and display gradient like distribution in mature oocytes. We checked expression of 12 selected genes in a pool of 10 oocytes and single mature oocytes. Then, we analysed gene expression in fixed intact oocytes and those undergoing laser capture microdissection procedure (LCMD).
Eventually, we have determined six candidate genes for the study of intracellular spatial gene expression in mature mammalian oocytes by subcellular qPCR and *in situ* hybridization.

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HISTONE CODE IN ZYGOTE: ITS ESTABLISHMENT, REGULATION AND INVOLVEMENT IN EMBRYONIC DEVELOPMENT

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The complex of post-translational modifications of individual nuclear core histones, called the histone code, is one of epigenetic phenomena. Histone code establishment is a dramatic process occuring immediately after fertilization, i. e., during the formation of male and female pronuclei in the zygote. The histone code undergoes further changes in parallel with embryonic cell division. Such changes occur differentially *in vitro* compared with *in vivo* conditions. Inadequate resetting of histone could be a reason for failure of embryonic development *in vitro*.

The histone code consists of various post-translational modifications, such as phosphorylation, ubiquitination, acetylation and/or methylation, mostly affecting lysine (K) residues of all four core histones. The histone modification pattern, often related to DNA methylation, is specific to cell type and differentiation level. Di- and/or tri-methylation of histone H3 (H3K9me2/3, H3K27me2) are the markers of heterochromatin establishment, associated with chromatin stabilisation and increased cell viability. On the other hand, heterochromatin does not allow transcription of DNA, and therefore gene silencing is observed accompanying cell differentiation.

Totipotent zygote, formed following the fusion of a spermatozoon with an oocyte, is predestined to enter cellular differentiation during ensuing embryonic development. Therefore, DNA demethylation and adequate histone code changes in the nascent pronuclei are essential for further success of embryonic cell differentiation. However, these chromatin changes bring a risk of increased DNA damage and higher incidence of embryo fragmentation. At present, the exact molecular mechanism of histone code regulation is still unknown and the proposed dual effect of heterochromatin formation – protection of

genome against gene silencing vs. transcriptional inactivation, is yet to be confirmed in mammalian zygotes and early embryos. Based on recent knowledge and our own observations, NAD+-dependent histone deacetylase SIRT1 and its signalling regulated by ubiquitin-proteasome system and histone methyl transferases seems to be a key factor for establishing correct histone modifications through early embryonic development. Therefore, broad experiments focused on histone code dynamics and their regulation is needed for the transfer of knowledge into routine of human assisted reproduction.

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MEIOTIC MATURATION OF PORCINE OOCYTES *IN VITRO* – THE EFFECT OF ENDOCRINE DISRUPTOR BISPHENOL S

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Meiotic maturation of mammalian oocytes is unique asymmetric division of female sex cells, during which occurs germinal vesicle breakdown, as well as to the formation of organizing centers of microtubules in functional indexing spindle, the movement of chromosomes necessary for their subsequent reduction and the establishment of full-fledged female gametes. A key role in the organization of chromosomes and other cellular organelles during meiotic maturation play components of the cytoskeleton, whose disruption leads to serious disturbances in vitro maturation of oocytes.

A complicated control mechanism of meiotic maturation may be negatively affected by substances from the environment, which mimic hormones action, called endocrine disruptors. Very extended group of endocrine disruptors are bisphenols, which are an integral part of industrially produced plastics. The most widely used representative of this group is bisphenol A (BPA), which according to recent studies extends into reproduction control and negatively affects the development of oocytes. Negative changes were observed in meiotic spindle organization, chromosomal aneuploidies, and more frequent occurrence during maturation of mammalian oocytes *in vitro* effect of BPA affected.

Therefore, a rapid replacing of harmful BPA in plastics other substances, among which is also its chemical analog - Bisphenol S (BPS). BPS is thus now becoming through replacing BPA widespread. It has been found that BPS is more chemically stable and less biodegradable than BPA. A limited number of studies also suggests that BPS is able to mimic the effects of hormones and interact with estrogen receptors. More detailed studies demonstrating effects of BPS on mammalian reproduction has not yet been carried out.

The objective of our study was to evaluate the progress of meiotic maturation of porcine oocytes exposed to the effect of BPS *in vitro* - changes in expression, cellular localization and distribution of selected cytoskeletal structure, essential component of microtubules - alpha tubulin.

Material and Methods: The porcine oocytes were cultured *in vitro* at 39 ° C in an air mixture of 5 % CO_2 for 24 and 48 hours in modified M199 medium supplemented with BPS concentration 300 nM. It was performed immunocytochemical localization of alpha tubulin followed by confocal microscopy and image analysis to evaluate the effect of BPS. The obtained data were statistically analyzed using SAS software 9.0. using t-test and ANOVA. Our results showed the effect of BPS on the course of meiotic maturation of porcine oocytes and meiotic spindle arrangement. Depending on the dose of BPS, it was significantly disrupted the structure and organization of microtubules. To successfully suppressed the effects of BPS on reproduction of mammals including humans, it is necessary to clarify its integration into the signaling pathways of oocytes. Our results help to clarify the mechanisms of endocrine-disrupting effects of BPS, which had not been described.

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DIABETIC EMBRYOPATHY

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Diabetic pregnancy is associated with a 4- to 10-fold increased incidence of congenital malformations compared with non-diabetic pregnancy. Diabetic embryopathy can affect any developing organ system, although cardiovascular malformations are most common. The same types of heart malformations that are associated with diabetic pregnancies in humans have been reported in animal models. Teratogenic effects of maternal diabetes are well documented, the causes remain elusive. Based on our findings that the embryonic expression of *Hif1a* is dysregulated by maternal diabetes, and that a loss of gene copy of HIF1A mutation is associated with ventricular septal defects, we hypothesized that maternal diabetes impairs HIF-1-controlled hypoxia-response pathways and that these pathways are critically involved in the susceptibility to heart defects observed in diabetic embryopathy. We tested this hypothesis in a genetic mouse model of partial HIF-1α deficiency by exposing *Hif1a*+/- embryos to maternal diabetes. We observed a decreased number of embryos per litter and increased incidence of heart malformations, including atrioventricular septal defects and reduced myocardial mass, in diabetes-exposed *Hif1a*+/- embryos as compared to *Wt* embryos. We also detected significant differences in expression of mRNAs in diabetes-exposed *Hif1a*+/- and *Wt* embryonic hearts, including those encoded by *Vegfa, Nkx2.5,* and *Tbx5*. Our data strongly suggest that HIF1-regulated pathways could be one of the key molecular pathways involved in the pathogenesis of the teratogenic insult of maternal diabetes.

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THE EFFECT OF DIABETES MELLITUS ON REPRODUCTIVE PARAMETERS AND EXPRESSION OF SELECTED TESTICULAR GENES IN STREPTOZOTOCIN-INDUCED DIABETIC MICE

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According to the World Health Organization (WHO), 15% of couples in reproductive age suffer from infertility problems, and up to 60% of cases are caused by male factor. Causes of this condition could be genetic background, environmental factors and various diseases, including diabetes mellitus (DM). However, the impact of DM on male fertility is not fully understood. The aim of this study was to investigate the effects of DM on reproduction and expression of selected testicular genes using FVB inbred mouse strain, suitable for inducing DM and subsequent reproductive parameters analysis. This mouse strain is characterized by vigorous reproductive performance and consistently large litters.

DM (type 1) was artificially induced in the age of 6 weeks by chemical substance streptozotocin, which causes destruction of pancreatic β cells. Mice with blood sugar levels higher than 13.9 mmol/L were considered as diabetic. These mice were exposed to diabetic condition for 6 weeks and then subjected to analysis.

Our results have shown that diabetic condition had an impact on body weight, weight of reproductive organs as well as kidneys and livers. We also evaluated sperm quality by analysing several sperm parameters (morphology, viability, concentration, apoptotic status). We observed decreased concentration and viability of diabetic sperm compared to control. We also noticed increased staining with apoptotic marker annexin V. Moreover, we evaluated the qualitative and quantitative changes of sperm nuclear proteins - protamines. In diabetic animals, we observed higher number of sperm with insufficient protamination. Nevertheless, protamine 1 to protamine 2 ratio (P1/P2), a marker of male fertility, was not altered in sperm of diabetic animals compared to controls. Regarding the testicular tissue, we observed impaired morphology of seminiferous tubules and increased number of apoptotic cells in testes of diabetic animals. We also analysed expression of several important testicular genes (*Vegfa, Sycp1, Sycp3, Ccna1, Meig1, Prm1, Prm2,*

Tnp1, Tnp2, Grth, Gja1, Wt1, Sox8, p21, p53, Bcl-2, Ar, Fshr, Star, p450scc), which are responsible for proper function of spermatocytes and spermatids.

Our findings indicate that DM affects sperm quality in terms of alterations in the morphology of testicular tissue. The metabolic disorders also caused changes in the expression of genes important during meiotic phase of spermatogenesis (*Sycp1* and *Sycp3*) and genes important during spermiogenesis (*Tnp1*, *Tnp1*, *Prm1 and Prm2*). Changes in gene expression could further contribute to impaired sperm quality of diabetic animals.

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THE EFFECT OF TREATMENT – THE ORAL METHYLPREDNISOLONE – ON THE VALUES OF PRO-INFLAMMATORY CYTOKINES IN THE FERTILITY PROBLEMS TREATMENT

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Fertility disorders are now becoming an increasing health problem and they affect the lives of the western and central Europe population. This indicates the need to emphasize the correct role of the problem. After excluding the genetic, hormonal, anatomical, infectious and hematologic cause of fertility disorders, the presence of pathological mechanisms of the immune system is tested . Those are responsible for up to 40-60% of unexplained fertility problem cases.

Immune fertility disorders are characterized by impaired regulation of immune effectors, which directs its effect against the fetus rather than induce its tolerance. Until the moment of fertilization it is physiological for the body, that the TH1 lymphocytes are primarily in the blood- they provide the fight against foreign antigens, which is necessary for maintaining the integrity of the body. After fertilization the ratio physiologically flips in favor of suppressive TH2 lymphocytes.

Immune fertility disorders can be caused by pathological autoimmune condition (the presence of antibodies against germinal tissues of men and women or the presence of antiphospholipid antibodies). It can be manifested by pathologically elevated levels of TH1 cytokines (IFN-**γ, TNF)** or by breaching of the TH1-/TH2-lymphocytes ratio which subsequently flips to suppressive TH2-lymphocytes side.

The aim of our study was to compare the values of pathologically elevated immunological parameters before the treatment and 6-12 weeks after the treatment initiation in patients with fertility problems using the oral methylprednisolone - Medrol.

We compared the values of pro-inflammatory TH1 cytokines, IFN-**γ and TNF**, which are reffered as embryotoxic. This reflects at high levels vasculature disruption of the fetus, the formation of local heart attacks or death of the fetus. Glucocorticoids inhibit the NFkB transcription regulator, which suppress the activity of several types of immunocompetent cells, including T-lymphocytes. This reduces the production of IFN-γ and TNF by TH1-lymphocytes. Our results show that treatment with methylprednisolone (Medrol) has a significant effect on reducing the levels of IFN-**γ, TNF**-a.

In these cases it is important for the treatment, besides the reduction of pro-inflammatory cytokines, not to result in more evident deepening of the suppressive TH2 cytokines deficit. It should be emphasized, that the aim is to minimize the TH1 cytokines, mainly because of their embryotoxic activity, which is related to pregnancy negatively.

POLYCYSTIC OVARY SYNDROME (PCOS) AND THE SERUM LEVEL OF ANTI-MÜLLERIAN HORMONE (AMH)

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Background: PCOS is characterized by the menstrual cycle disorder, infertility, hyperandrogenism, hirsutism and male-pattern alopecia. PCOS is found in 5% to 10% of women in their reproductive age. Women with this syndroma have up to six times more follicles in their ovary comparing to healthy women.
Approach: The observation of PCOS in infertile women and of the AMH serum levels.
Material and methods: We examinated 54 women aged between 24 to 40 years (the median was 31 years), 30/54 were never pregnant, 4/54 have never used contraceptive pills, the rest used it from 3 to 20 years , 19/54 of them 10 and more years. All patients were treated by three unsuccessful in vitro fertilizations (IVF).
We separated the serum samples from patient s venous blood and stored at -20°C until the laboratory processing. The AMH levels were measured by chemiluminiscent kit ACCESS AMH (Beckman Coulter, USA) and immunoassay system Unicell Dxl (Beckman Coulter, USA). The results of AMH were determinited by Wilcoxon test with the level of statistical significance $p \leq 0.05$.
Results: The values of AMH ranged from 0,17 to 37,6 ng/mL (the median was 8,34 ng/mL), the lowest values (0,17 a 0,6 ng/mL) were found only in two patients. By Wilcoxon test we found out significantly higher values of AMH in women with PCOS comparing to the control group.
Conclusion: 52/ 54 patients with PCOS have higher values of AMH, which clarifies diagnosis of this disease. We found no statistical significance correlation between AMH values and age, duration of use of contraceptive pills and the number of IVF stimulations.

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SERUM LEVELS OF ANTI-MÜLLERIAN HORMON (AMH) IN WOMEN WITH ENDOMETRIOSIS

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Background: Endometriosis (E) is ectopic, hormon - dependent endometrial mucose located outside the uterine cavity changing during the menstrual cycle. There are four stages (I. - IV.) of E according to ASRM score (evaluation of range and localization of ectopic bearing). AMH is a glycoprotein produced by granulosa cells of ovarian follicles and its levels can be used such as a marker of ovarian reserve.
Aim: We decided to observe serum levels of AMH in women with different stages of E and evaluate the impact of this disease to ovarian reserve and fertility.
Material and Methods: We included 141 women aged 24 - 41 (median 33) years. 38 patients had stage of E - I, 57 - II, 34 - III, and 12 women stage - IV. Anamnestic data of our infertile women before immunological examination were: 107 underwent in vitro fertilization (IVF), 37 had missed abortins, 6 spontaneous abortions, 12 gave a birth their babies, in 5 women an ectopic pregnancy occured and in 37 women the primary infertility was diagnosed. The serum samples of each patients was collected and stored at -20°C until the examination of AMH. We used chemiluminescent kit ACCESS AMH (Beckman Coulter, USA) to detect the levels of AMH.
Results: The levels of AMH were found between 0,28-23,46 ng/ml (minimal extension of E - stage I: 0,51-10,72 ng/ml, stage II 0,28-23,46, stage III 0,28-14,57 ng/ml and stage IV of 0,31-5,55 ng/ml. No statistical difference was found.
Conclusion: We have not proved the negative impact of various stages of E on the serum levels of AMH secretion. AMH depends on the age of a woman only.

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ANTIOXIDATIVE ENZYMES IN HUMAN SERA AND SEMINAL PLASMA AFTER TREATMENT WITH SELENIUM, VITAMIN E, AND ZINC

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Selenium (Se) and selenoproteins act as antioxidants and 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. The Czech Republic belongs to areas with a low Se content in soil which results in Se deficiency in plants and subsequently in animal and human organisms. Decreased fertility in men is the main reason for our study of some factors such as Se, vitamin E, and zinc (Zn) in seminal plasma, where they influence the viability of sperm cells, namely the progressive motility and acrosomal reaction of spermatozoa.
To illustrate in detail the local responses of infertile males, we focused on the characterization of seminal fluid proteins, especially antioxidant enzymes. The content of Se and selected heavy metals in seminal plasma was determined by atomic absorption spectrophotometry. The activities of selected selenoenzymes (glutathione peroxidase and thioredoxin reductase) and other antioxidative enzymes were measured spectrophotometrically in serum and seminal plasma. We used samples of control patients with fertility problems and of patients treated with Se and Zn preparation and vitamin E that should enhance their antioxidative stress response. The daily treatment of the patients lasted from two to four months with the dose of Se 50 μg per capsule, Zn 72 mg per capsule and vitamin E 200 mg per capsule.
After the treatment, glutathione peroxidase showed an increasing activity in seminal plasma, the increase was seen in eight of the thirteen patients. Glutathione S-transferase also exhibited an increasing activity in seminal plasma in several patients. Thioredoxin reductase was the most affected enzyme, its activity dramatically increased in seminal plasma in four patients. Our preliminary data has to be compared with the clinical state of the patients and with the levels of Se, Zn, and metallothioneins in seminal plasma.

This work was supported by the Grant Agency of the Czech Republic (GACR 13-04580S) and by specific university research (MSMT 20/2015).

INTRAVENOUS IMMUNOGLOBULIN TREATMENT FOR IMMUNOLOGICALLY BASED REPRODUCTIVE DISORDERS

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Immunomodulatory treatment in patients with recurrent spontaneous miscarriage or recurrent implantation failure has been already widely studied and accepted.
Intravenous immunoglobulins (IVIg) are one of the most prominent therapeutic choices. Their advantage lies in low adverse events rate, plausibility during pregnancy and no documented foetal malformations.
The first randomized trial of IVIg for idiopathic recurrent pregnancy losses was published in 1994 and then several studies have been followed. The metaanalysis results by Li and Chen published in 2013 strongly support that IVIg is a useful treatment option for women undergoing repeated IVF failure (based on PubMed, EMBASE and CNKI databases data).
Recurrent miscarriage is defined by three or more spontaneous abortions until 14th gestation week, 50% are of unexplained cause. Recurrent implantation failure (RIF) can be considered after two or more high-quality embryo transfers or at least two good quality blastocyst stage embryos transfers including also chemical pregnancies.
Various *in vitro* and *in vivo* studies have shown several mechanisms of action of IVIg.
IVIg lower count and cytotoxicity of peripheral NK cells (CD^{56dim} CD16+) mediated by FcγRIII receptor. IVIg also increase GM-CSF synthesis; an important Th2 cytokine with positive impact on chromosomal aberations reparation and endometrium perceptivity. Other mechanisms include lower expression of CD94/NKG2 inhibitory receptors on peripheral NK cells and effect on Th17 lymphocytes differentiation. IVIg treatment promotes Treg lymphocytes differentiation as well.
There is no international consensus, nor guidelines on IVIg dosage, timing and therapy duration regarding these two conditions. However, there are several national guidelines (e.g. Germany, Hungary) that are widely accepted by local immunological and gynaecological societies.
Studies published in recent years have shown clinically significant effects of IVIg treatment only in thoroughly selected groups of patients with proven immunologically based reproductive disorders. It is appropriate to consider this treatment after failure of other immunomodulatory approaches, where it can considerably improve successful pregnancy outcomes.

I HAVE THE RIGHT TO MAKE DECISIONS ABOUT MY OWN HEALTH

Mala E et al.

Institut of Clinical Immunology and Allergology, University Teaching Hospital Hradec Kralove, Czech Republic

The authentic overview of almost five year lasting stagnation without an adequate possibility to intervent impaired fertility in our patient - despite progressing comorbidities.
Overview of the unphysiologic findings, the development of health and complications, treatment and recommendations in the course of time
(2010-2015) - and in contrast to patient´s own decisions, attitudes and opinions.
Our presentation also includes the outcomes and consequences in health and diseases together with the reflection of therapeutic procedures and analysis.

RECURRENT PREGNANCY LOSS ON THE BASIS OF AUTOIMMUNITY? ... OR WOMAN IS A MYSTERIOUS CREATURE

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Case report of a 30-year-old woman with an anamnesis of 3 pregnancy losses (14th, 12th, 16th week of gravidity). In the introduction we are dealing with the courses of failed pregnancies with relatively uniform image of a sudden rise in CRP and uterine contractions after previously trouble free course of pregnancy and the subsequent histological diagnosis of chorioamnionitis without thrombus. Case report discusses in detail the course of the 4th pregnancy, laboratory and consultative examinations, diagnostic considerations and our immunointervention treatment. Despite our combined therapy (peroral corticosteroids, IVIG) the course of pregnancy is dramatic - requiring repeated hospitalizations and corticoid bolus treatment. Also, thanks to the immunointervention treatment, it is managed to maintain pregnancy to the 35th week of gravidity. Conclusion of the case study includes the clinical condition of the child in the first three years of its life. The paper briefly analyses the issue of corticosteroid treatment during pregnancy, its risks for the woman and child. The issue of autoimmune disorders in pregnancy is also mentioned, the focus is particularly on autoimmune subclinical prodromal stages and their impact on pregnancy loss. In conclusion, we discuss the possible cause of pregnancy loss in the case of our patient and prospects of immunointervention at her next scheduled pregnancy.

TREATMENT OF RARE INTRAUTERINE ADENOMYOSIS AT THE PATIENT WITH PRIMARY STERILITY -CASE REPORT

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Introduction: Intrauterine adhesions are mostly after surgery in the uterus. Incidence intrauterine adhesions without intrauterine surgery and in addition to that the patient is nuligravida is very rare (7,7%). In past we could find it in genital tuberculosis. One of the next cause it can be huge adenomyosis which destroys the lining between endometrium and myometrium. Basal layer of endometrium is disrupted, endometrial proliferation and secretion is dysregulated. As a result of this dysregulation we have dysfunctional endometrium with fibrous adhesions.

Method: Our case report describes patient with diagnosis primary sterility, endometriosis, fibroid uterus. Ultrasound finding: ovary endometriosis, multiple small fibroids, multiple adenomyosis, irregular, non-homogenous endometrium. Hysteroscopic finding: multiple adhesions between anterior and posterior wall. Neither fundus, neither ostia of both tubes are visualised. We removed adhesions and we repeatedly have applied hyaluronic gel to the uterus after operation. Since the cause of adhesions is not postoperative or post gestational, but as a result of adenomyosis, we gave the patient gestagen only treatment – dienogest 2mg daily for five months. At the end of this period we have performed ultrasound hysterocontrastsonography and hysteroscopy. The uterine cavity was totally free with regular mucous membrane and both tubes were patent. We continued the treatment fluently with a long agonist protocol IVF. After the transfer of one embryo there was singleton pregnancy ended by the delivery of healthy girl.

Discussion: Postoperative treatment with hyaluronic gel is widely used. Mostly this kind of treatment is combined with oestrogen therapy in higher dose to induce proliferation of endometrium. In this case the oestrogen therapy could worsen the effect, because adenomyosis is oestrogen dependent disease. That is why we combine hyaluronic gel treatment with modern gestagen pill and continue fluently to long IVF protocol.

Conclusion: Conservative treatment of endometriosis/adenomyosis is mostly at the patients with chronical abdominal pain. At the patient with diagnosis of sterility and

endometriosis we prefer IVF or operative treatment. We decide for conservative treatment of endometriosis/adenomyosis when the chronical abdominal pain after surgery is still dominant and we cannot continue with IVF treatment. The case report offers another situation when we decide for conservative treatment of endometriosis/adenomyosis before IVF cycle.

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