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

MEDICAL FACULTY OF CHARLES UNIVERSITY
AND FACULTY HOSPITAL IN PILSEN

XXIst SYMPOSIUM OF BIOLOGY AND IMMUNOLOGY OF REPRODUCTION WITH INTERNATIONAL PARTICIPATION

in memory of Dr. Radslav Kinsky

PROGRAM AND ABSTRACTS

The Castle, Třešť, May 14 – 16, 2015

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XXIst Symposium of Biology and Immunology of Reproduction
with International Participation
The Castle, Třešť, May 14 – 16, 2015

PROGRAM

Thursday, MAY 14, 2015

Arrival and accommodation at the Castle Trest

19.00 DINNER

Friday, MAY 15, 2015

Breakfast from 7 am

09.30-09.40 OPENING CEREMONY:
Ulcova-Gallova Z., Peknicova J.

Chairpersons: Ulcova-Gallova Z., Jonak J.

09.40-10.00 **Chaouat G.** (France): T regs and eutherian pregnancy: reconsidering the Medawar paradigm.
10.00-10.20 **Rashev P.** (Bulgaria): Correlation of Mas1 and CD10 expression and localization with the breast cancer grading.
10.20-10.40 **Antalikova J.** (Slovakia): Dynamics of CD9 molecule during sperm capacitation in cattle.
10.40-11.00 **Jankovicova J.** (Slovakia): Characterization of the bull CD9 molecule during the acrosome reaction.

11.00-11.30 COFFEE BREAK

Chairpersons: Peknicova J., Madar J.

11.30-11.50 **Liska F.:** Transcriptomic profiling of spermatozoa of infertile men with astheno/teratozooaspermia.
11.50-12.10 **Děd L.:** Fluorescent analysis of the differential protein expression in normozoospermic and asthenozoospermic sperm samples.
12.10-12.30 **Dvorakova-Hortova K.:** Dynamics of mouse sperm capacitation and acrosome reaction.
12.30-12.50 **Sebkova N.:** CD46 and beta1 integrin interaction in mouse sperm head.

12.50-13.10 **Frolikova M.:** Visualisation of CD46 and beta1 integrins in mouse sperm head by super-resolution stimulated emission depletion (STED) microscopy.

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13.15-14.30 LUNCH

Chairpersons: Dvorakova-Hortova K., Jonakova V.

14.30-14.50 **Manaskova-Postlerova P.:** Biochemical methods as tool for study of reproductive proteins.
14.50-15.10 **Pohlova A.:** Sperm protein profiles of different mammalian species.
15.10-15.30 **Margaryan H.:** Possible role of spermatogenic protein glyceraldehyde-3-phosphate dehydrogenase (GAPDHS) in mammalian sperm.
15.30-15.50 **Valaskova E.:** Diabetes mellitus negatively affects male reproductive parameters *in vivo*.

15.50-16.20 COFFEE BREAK

Chairpersons: Sedmikova M., Liska F.

16.20-16.40 **Sedmikova M.:** Gasotransmitters protect porcine oocyte against aging.
16.40-17.00 **Zborilova B.:** Prevalence of HPV infection in assisted reproduction.
17.00-17.20 **Sokolova K.:** Anti-Müllerian hormone (AMH) serum levels – comparison of chemiluminescent and Elisa method.

Relaxation – Walk around the garden

19.00 DINNER (RAUT)

Saturday, MAY 16, 2015

Breakfast from 7 am

Chairpersons: Novakova D., Zidkova J.

9.00-9.20 **Jungvirtova J.:** The effect of contraceptive pills on secretion of anti-Müllerian hormone.
9.20-9.40 **Preislerova M.:** Serum and ovulatory cervical mucus levels of anti-Müllerian hormone (AMH) in infertile women.
9.40-10.00 **Bendova B.:** Monitoring of antiphospholipid antibodies (APA) during the treatment of antiphospholipid syndrome in some infertile women.
10.00-10.20 **Zidkova J.:** Effect of selenium, vitamin E and zinc on antioxidative enzymes in human seminal plasma.
10.20-10.40 **BIO-RAD:** New products

10.40-11.00 COFFEE BREAK

Chairpersons: Malickova K., Sebkova N.

11.00-11.20 **Dzurillova Z.** (Slovakia): Treatment with intravenous lipid emulsion for recurrent pregnancy loss.
11.20-11.40 **Malickova K.:** Can intravenous immunoglobulins improve pregnancy outcomes in patients with prior failed *in vitro* fertilization?
11.40-12.00 **Novakova D.:** There is a relationship between infertility treatment and the risk of thyroid cancer and gynecological cancers?

12.00-12.10 **CLOSING OF SYMPOSIUM**
Peknicova J., Ulcova-Gallova Z.

12.15-13.30 **LUNCH**

XXIst Symposium of Biology and Immunology of Reproduction
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ABSTRACTS

Friday, MAY 15, 2015

T REGS AND EUTHERIAN PREGNANCY: RECONSIDERING THE MEDAWAR PARADIGM

Chaouat G

INSERM, Hopital Saint Louis, Paris, France

Medawar's paradox sustaining pregnancy Reproductive Immunology since 1953 speakig immunological and endocrinological problems raised by the evolution of viviparity in vertebrates " assumes that appearing placental viparity (l) confronted a pre-existing " Burnett type" adaptive immunity. We will surprise some, but we know now that this is doubly false, even if this old version of the conflict will surely persist a few years yet in publications (articles and even books). 1st placentas, albeit primitive, exist in (very) early invertebrates, eg. Bryozoa, and onychophora (Cambrian) are placental as, close to 'us', fishes (Materpiscis, Devonian... and sharks) and then dinosaurs, snakes, lizards... But since dinosaurs, the Adaptive immune system had considerably developed... Whilr the marsupiams leve uterus just before an immune rejection... eutherians "regulate" the immune system, by a variety of circuits, including suppressor T "resurrected " (Waldmann) as Tregs (and their removal seriously compromises an allopregnany in animals, and the male foetus gestation in synpregnancy. A critical role of CNS1, present only in eutherian mammals, is demonstrated. Deficient Tregs were found during cases of implantation failure or abortions and recalling the work of Marie Petitbarat / Nathalie Ledée, what they can regulate, including the otherwise necessary inflammation pre and peri implantation, and cytotoxic potential functions of the NK cells. We will discuss if Tregs are alloantigen specific or self specific, or …. Both… We finish pointing that there are adaptive cytokine circuits that they cannot regulate, such as complement, and in cooperation with F Tedesco, we will present data on C' regulation.

CORRELATION OF MAS1 AND CD10 EXPRESSION AND LOCALIZATION WITH THE BREAST CANCER GRADING

[1] Ankova D, [1,2] Pupaki D, [3] Metodiev D, [4] Donat H, [1] Rashev P

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Breast cancer is the most frequent spontaneous malignancy diagnoses in women in the world. In the promotion and progression of carcinogenesis, the angiogenesis plays an important role as an essential component of the metastatic pathway. Local expression of several components of the renin angiotensin system (RAS) are involved in the control of cell growth and vascular permeability and has been shown in various cancer cells and tissues.

The aim of our study is to investigate the gene expression and cell-specific localization of Mas1 and CD10 in the pathogenesis of breast cancer and their relation to stage of disease.

Samples from tumours and adjacent normal tissue from 33 patients were used in this study. Samples were subjected to mRNA isolation, reverse transcribed and qPCR was performed for evaluation mRNA expression. The results were normalized to the expression of housekeeping gene *rplp0*. The cellular localization of Mas1 and CD10 in samples from different types, stages and grades of breast carcinomas were evaluated by immunohistochemistry.

It was found that Mas1 and CD10 are co-located in myoepithelial cells in normal tissue with high intensity of the reaction. The staining for CD10 was weaker in ductal carcinoma in situ and almost absent in invasive carcinoma. As for Mas1 expression, stronger reaction was found in low differentiated tumors.

The dynamics in the expression of CD10 and Mas1 in different histological types and grades of breast carcinomas suggests that they may be useful prognostic markers for the degree of malignancy.

This study was partly supported by the grants ReProForce FP-7- REGPOT-2009-1, DKOF7RP02/17 /DSN№D01-4787 and №BG051PO001-3.3.06-0059.

DYNAMICS OF CD9 MOLECULE DURING SPERM CAPACITATION IN CATTLE

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

Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Ivanka pri Dunaji, Slovak Republic

The presence of CD9 has been confirmed on gametes and tissues of various mammals as mouse, rat, pig and cattle, including the man. Although its function has been studied by various approaches, obtained results differ notably. Moreover, the oocyte CD9 has been studied preferentially, so it is difficult to hypothesize about the precise role of CD9 on sperm.

Prior to the successful fertilization, mammal spermatozoa undergo the capacitation process accompanied by rearrangement of sperm surface proteins acquired from seminal plasma as well as epididymal secretion. The presence of CD9 exclusively on plasma membrane of the bovine sperm was confirmed by immunofluorescence analysis and by Western blot analysis of the protein fractions after the discontinuous sucrose gradient fractionation of the bull sperm but no data is available regarding the dynamics of CD9 molecule during capacitation. To inspect the pattern of CD9 during the capacitation process we analysed the freshly ejaculated, frozen-thawed (capacitated-like) sperm and also the sperm during the capacitation in vitro using anti-CD9 antibodies. The chlortetracycline fluorescence analyses was applied to detect the portion of capacitated sperm, simultaneously, acrosomal status was assessed by PNA-lectin. When frozen-thawed (capacitated-like) as well as freshly ejaculated sperm capacitated for 4 h were analysed, comparable results were observed. In all tested samples, the IVA-50 reactivity exceeded 77 %. Taken all together, the capacitation process did not change the pattern of CD9 molecule on freshly ejaculated and frozen-thawed sperm. Based on facts, that CD9 is present on the bull spermatozoa before the contact with oocyte, and localization of sperm CD9 does not change during the capacitation, we supposed that CD9 molecule

“is queueing” for the right moment to participate in some of the subsequent events in the fertilization process.

This work was supported by grants VEGA 2/0006/12 and APVV/0137/10.

CHARACTERIZATION OF THE BULL CD9 MOLECULE DURING THE ACROSOME REACTION

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Prior to the fusion, recognition and primary binding of gametes, leading to acrosome reaction, is an important step in mammalian fertilization process. The sperm proteins participating in these events are usually located on apical region of sperm head. Recently, plasma membrane surface localization of CD9 protein has been described on bull sperm. Distribution of CD9 molecule appeared to be uniform during the whole capacitation process. In our study, the possible involvement of bull sperm CD9 in the acrosome reaction has been examined. Our experiments showed that CD9 molecule is loosing from the sperm surface after 40 or 60 minutes of stimulation of an acrosome reaction; independently to used inductor of an acrosome reaction (1 μM; 10 μM calcium ionophore or zona pellucida-intact-oocytes).

IVA-50 (anti-CD9 monoclonal antibody) influenced neither the portion of acrosome-reacted sperm nor the reaction pattern of CD9 in comparison with the sperm stimulated by calcium ionophore. On the other hand, when the IVA-50 has been added to the suspension of capacitated sperm, migration of CD9 molecule through the entire sperm head to the postacrosomal region has been observed. These findings lead us to suppose that although IVA-50 did not directly cause the inhibition of acrosome reaction, the possibility of CD9 involvement in sperm membrane proteins organization during the fertilization machinery is not excluded.

This work was supported by grants VEGA 2/0006/12 and APVV/0137/10

TRANSCRIPTOMIC PROFILING OF SPERMATOZOA OF INFERTILE MEN WITH ASTHENO/TERATOSPERMIA

[1] Chylíková B, [1] Semyakina A, [1] Hodúlová M, [2] Ariagno JI, [1] Šeda O, [2] Mendeluk G, [3] Kierszenbaum AL, [1] Liška F

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The pathogenesis of male infertility is mostly unknown. Specifically, the proportion of genetic and environmental factors has been hard to discriminate. However, transcriptomic or proteomic approaches can identify common deregulated genetic pathways in sperm that are significant for their function, regardless the primary etiological factor.

We analyzed the transcriptome of 12 sperm samples using Affymetrix HuGene2.1ST microarrays. 4 participants were fertile donors, 4 samples were from infertile patients that presented low sperm motility (asthenospermia), and 4 samples with low motility as well as abnormal morphology (asthenoteratospermia).

When comparing the asthenoteratospermic to controls samples, most of the 11 included meaningful differentially controlled genetic networks involved in regulation of development and morphology. Comparison of asthenospermic to controls samples revealed partial overlap with the former. However, there were more genes associated with intermediate metabolism, consistent with a more "functional" impairment of the asthenic sperm.

Remarkably, a comparison of the infertile groups showed marked upregulation of ribosomal protein genes and translation initiation factors in athenoteratospermia compared to asthenospermia groups. That may reflect anomalous ribosome processing in morphologically abnormal sperm.

Supported by University of Buenos Aires, Science and Technology, no. UBACYT-CB06; and Ministry of Health of the Czech Republic no. NT12269-5.

FLUORESCENT ANALYSIS OF THE DIFFERENTIAL PROTEIN EXPRESSION IN NORMOZOOSPERMIC AND ASTHENOZOOSPERMIC SPERM SAMPLES

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Asthenozoospermia (low percentage of motile sperm in an ejaculate) is one of the main seminal pathologies underlying male infertility. Previous proteomic studies have demonstrated the significant differences in the protein profiles between normozoospermic and asthenozoospermic sperm samples. Since these studies were primarily focused on the identification of differentially expressed proteins by mass spectrometry, we aimed to evaluate the ability of our diagnostic antibodies to detect the differential expression of selected protein markers by fluorescent microscopy and flow cytometry techniques. Therefore, we analyzed sperm samples from 30 men with normal spermiograms (>40% motile spermatozoa, average viability 78%) and 30 men with asthenozoospermia (<40% motile spermatozoa, average viability 74%) by the panel of our diagnostic anti-human sperm (Hs) antibodies. These antibodies were prepared in our laboratory and are used in clinical practice as a tool for the differential diagnosis of various sperm pathologies. Fluorescent microscopy and flow cytometry analysis revealed quantitative differences in the protein abundances between normozoospermic and asthenozoospermic sperm samples, namely, in GAPDHs (glyceraldehyde phosphate dehydrogenase), evaluated with Hs-8 MoAb, VCP (valosin-containing protein), evaluated with Hs-14 MoAb, and ATP synthase (cAMP-dependent protein kinase II, PRKAR2A), evaluated with MoAb Hs-36. On the other hand no statistically significant differences were found in the expression of the sperm surface protein clusterin, evaluated with Hs-3 MoAb, and semenogelin, evaluated with Hs-9 MoAb. From the methodological point of view, we observed very high correlation between the data obtained by fluorescent microscopy and flow cytometry techniques (for example using Hs-8 antibody, r = 0.938, p

≤ 0.001) and therefore both methods are useful for evaluation of protein differences associated with asthenozoospermia. From the clinical point of view, we observed the strong association of the low sperm motility in the sample with the expression of proteins, playing an important role in sperm energy metabolism (expected), but also with the expression of all tested intra-acrosomal proteins. These findings further demonstrate asthenozoospermia as a complex semen disorder frequently associated with other semen pathologies, which are not diagnosed by basic semen analysis, and the possibility to use monoclonal antibodies as a tool for diagnosis of protein associated sperm pathologies in the semen with the low sperm motility.

This work was supported by the Grant Agency of the Czech Republic, grant No. 349 P503/12/1834, and P502-14-05547S and by BIOCEV project CZ.1.05/1.1.00/02.0109 from 350 the ERDF.

DYNAMICS OF MOUSE SPERM CAPACITATION AND ACROSOME REACTION

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Capacitation followed by the acrosome reaction (AR), is a very complex event of molecular changes, including acrosome matrix rearrangement and actin polymerization, which mammalian sperm must undergo in the female reproductive tract in order to obtain the ability to penetrate and fertilize the egg. CD46 and β1-integrin belong to specific proteins, which are predicted to interact during molecular reorganization of capacitating sperm. The IZUMO1 as the primary fusion protein of the mammalian sperm is also involved in this dynamic network. We investigated the relationship between the Izumo, CD46 and β1 integrin relocation in the sperm head during the capacitation and AR *in vitro*. We have already successfully monitored by immunofluorescent labelling the dynamics of proteins CD46 and β1-integrin. The changes in the localization of these proteins associated with the AR and their mutual co-localization was observed. The original β1-integrin location in the freshly released epididymal sperm is in the acrosome and it relocates during the AR further through the sperm head compartments into the equatorial segment and over the whole sperm head. Its density over the equatorial segment is decreasing with the extended time of the AR. Also its presence in the perforatorium of the mouse sperm head is very prominent. The pattern for protein CD46 is extremely similar if not identical in both aspects such as compartment localization and time progress during capacitation and AR *in vitro*. The molecular interaction of CD46 and β1-integrin was investigated using the Proximity Ligation Assay and Super resolution microscopy STED. The data were statistically analysed. The newly obtained results from CD46 and β1-integrin relocation are in correlation with IZUMO1 dynamics and giving a

substantial knowledge on the studied protein network rearrangement during capacitation and AR in mouse spermatozoa.

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CD46 AND β1INTEGRIN INTERACTION IN MOUSE SPERM HEAD

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CD46 protein plays an important role during fertilization and its role is associated with acrosome stability but the exact mechanism is yet unclear. CD46 is probably involved in signalling pathways triggering the acrosome reaction. Failure of CD46 expression may alter intracellular signalling and disrupt the acrosome region. It also associates, through membrane integrins, with specific MAP kinases involved in acrosome reaction. Integrins interact with many cytoskeletal proteins such as actin, therefore changes in the actin cytoskeleton before and after AR may lead to changes in the association and localization of CD46 and β1integrin.The interaction of these two proteins in sperm can be predicted, however it still has not been shown. Our aim was to monitor mutual CD46 and β1integrin interaction. *In situ* interactions were detected by the proximity ligation assay (PLA) kit Duolink. *In situ* PLA is a technology capable of detecting protein interactions with high specificity and sensitivity. This new technology couples antibody recognition with the amplification of DNA surrogate of the protein. It generates a localized, discrete signal in a form of spots revealing the exact position of the recognition event. CD46 and β1integrin interaction was study in freshly released sperm and sperm during the calcium ionophore induced acrosome reaction, during which there is a gradual relocation of these proteins towards the equatorial segment and the whole sperm head. Proteins α and β tubulin were used as a positive control, α tubulin and β1 integrin as a negative control. *In situ* PLA showed a distinct spotted signal indicating the mutual interaction of CD46 and β1integrin. A positive response was demonstrated not only in freshly released sperm but also in sperm during the acrosome reaction. Freshly released sperm were distinctively

labelled in the acrosome region and the neck, similarly to the positive control. Sperm during the acrosome reaction showed the signal across the whole sperm head region. No signal or sporadic nonspecific staining was detected in the case of the negative control. In summary, our results deliver new information that proteins CD46 and β1 integrin interact with each other. These results suppose the theory that β1 integrin can mediate a connection between CD46 and sperm cytoskeleton thereby molecules of signalling pathways leading to activation of the acrosome reaction.

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.

VISUALISATION OF CD46 AND B1 INTEGRINS IN MOUSE SPERM HEAD BY SUPER-RESOLUTION STIMULATED EMISSION DEPLETION (STED) MICROSCOPY

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Protein CD46 is present on the acrosomal membrane of mouse sperm head and experiments with CD46-deficient mouse established the theory that CD46 participates in its stabilization. However, our recent findings show that CD46 is relocalizated during capacitation and acrosome reaction across the sperm head. This fact suggested that its role does not end by the loss of the acrosome. CD46 is probably a component of large multiprotein complex on sperm membrane that is responsible for sperm-egg interaction. In parallel, similar assembly of molecular network done by β1 integrins and the ability of β1 integrins to associate with CD46 is known from somatic cells. β1 integrins were detected in acrosomal area of mouse sperm head and they are relocalized during capacitation and acrosome reaction similarly to CD46 (unpublished data). These facts suggest the existence of interaction between CD46 and β1 integrins on sperm. Our aim was, therefore, to investigate in detail the mutual position of CD46 and β1 integrins in mouse sperm head. Dual imunnofluorescent labelling was used and position of studied proteins was investigated by super-resolution Stimulated Emission Depletion (STED) microscopy. STED enables to gain high quality images of studied molecules and provides imaging of fluorescent subjects with resolution approximately 60nm. At present, detailed localization of CD46 and β1 integrins was visualised on freshly released sperm with intact acrosome. CD46 is localized on the sperm acrosomal membrane with intact acrosome and it is present in its both compartments called outer and inner acrosomal membrane. Contrary to that, β1 integrins were detected only on the outer acrosomal

membrane and in an area of apical hook of sperm with intact acrosome. In the outer acrosomal membrane, CD46 and β1 integrins were localized closely together. This finding suggested that in mouse sperm head the interaction between CD46 and β1 integrins could exist. We confirmed this interaction by using the proximity ligation assay (PLA) kit Duolink. In near future, we would like to follow up with CD46 and β1 integrins detection during mouse sperm capacitation and acrosome reaction.

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.

BIOCHEMICAL METHODS AS TOOL FOR STUDY OF REPRODUCTIVE PROTEINS

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Study of molecular mechanisms in reproduction is essential for the understanding of this outstanding process. Our lab studies proteins secreted by reproductive organs and sperm using various biochemical methods for a long time. We have expertise in protein extraction from spermatic cells using different approaches, and by kits for proteins from the sperm surface and distinct subcellular compartments. The proteins of reproductive organ fluids are separated by chromatographic methods, such as size exclusion chromatography, high-performance liquid chromatography with reverse phase (RP-HPLC) and affinity chromatography on matrices with various ligands. Proteins are subjected to SDS- or 2D-electrophoresis for their characterization and comparison of various extraction methods, different mammalian species, and sperm in different functional development. Electrophoretically separated proteins may be transferred onto nitrocellulose membrane (Western blot) for antibody detection or binding studies with lectin-labelled ligands (lectins, polysaccharides, *zona pellucida* glycoproteins). We use immunoprecipitation method with specific antibody for protein determination followed by the MALDI identification. Proteins are localized by immunofluorescent techniques on/in spermatic cells and tissue sections of reproductive organs. Isolation of proteins from reproductive tissues and fluids, and the antibody detection is crucial for the studying of reproductive protein origin.

This work was supported by Nos. P503/12/1834 and P502/14/05547S of the GA CR and by the project BIOCEV CZ.1.05/1.1.00/02.0109 from the ERDF.

SPERM PROTEIN PROFILES OF DIFFERENT MAMMALIAN SPECIES

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Proteins are a substantial equipment of the spermatic cell; therefore, the characterization of sperm proteins is crucial for explanation of molecular mechanisms in the reproduction process. We isolated sperm proteins from different mammalian species - pig, bull, human, mouse, dog and cat. Extracted proteins were separated by SDS-electrophoresis and protein/glycoprotein profiles from epididymal or ejaculated sperm were compared. Additionally, we tested cross-reactivity of antibodies prepared to sperm boar proteins on spermatozoa of other mammalian species using immunofluorescent technique. Our future plan is to compare the protein profiles of sperm during their functional development (epididymal, ejaculated, capacitated) in various mammalian species and identify species-specific sperm proteins with *zona pellucida* binding activity.

This work was supported by Nos. P503/12/1834 and P502/14/05547S of the GA CR and by the project BIOCEV CZ.1.05/1.1.00/02.0109 from the ERDF.

POSSIBLE ROLE OF SPERMATOGENIC PROTEIN GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDHS) IN MAMMALIAN SPERM

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Sperm proteins are important for the structure and function of these specific, highly differentiated cells. Certain of these proteins play a role in sperm-egg recognition during primary or secondary binding at *zona pellucida* glycoprotein matrix. The aim of this study was to characterize the acrosomal sperm protein recognized by a monoclonal antibody (MoAb) Hs-8, prepared in our laboratory by immunization of BALB/c mice with human ejaculated sperm, and to test the possible role of this protein in gamete interaction. MoAb Hs-8 specifically labelled a 45 kDa protein from the sperm extract in the immunoblotting test. Sequence analysis identified this Hs-8 protein as GAPDHS. In order to perform a control tests, a commercial mouse anti-GAPDHS MoAb was applied. Both antibodies showed similar staining patterns using immunofluorescence labelling, transmission electron microscopy and immunoblot analysis. Moreover, both Hs-8 and commercial anti-GAPDHS antibodies blocked the secondary sperm-*zona pellucida* binding.

Generally, GAPDHS was considered mainly as sperm-specific glycolytic enzyme involved in energy production during spermatogenesis and sperm motility and its role in the sperm head was unknown. In this study, we confirmed the potential additional role of GAPDHS as a binding protein that is involved in the sperm-*zona pellucida* interaction.

This study was supported by grant No. P503/12/1834 from the Grant Agency of the Czech Republic and by project BIOCEV CZ.1.05/1.1.00/02.0109 from the ERDF.

DIABETES MELLITUS NEGATIVELY AFFECTS MALE REPRODUCTIVE PARAMETERS *IN VIVO*

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

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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. This could be caused by 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 reproductive parameters and sperm quality, using mouse model.

DM (type 1) was induced by Streptozotocin in FVB inbred mouse strain. Mice with blood sugar levels higher than 13.9 mmol/L were considered diabetic. After 4 weeks of diabetes exposure, diabetic males were bred with wild type females and transgenerational effect of DM was assessed. Selected morphological, cellular, and molecular parameters of diabetic males and their male offspring were compared to appropriate controls. There was an increased in sperm fragmentation and abnormalities of sperm morphology in diabetic mice in both generations. An increased staining with apoptotic marker annexin V was also detected in the diabetic groups. Furthermore, a presence of protamines as major sperm nuclear proteins was analysed. Protamine 1 to protamine 2 ratio (P1/P2), a marker of male fertility, was altered in sperms of experimental diabetic animals in both generations.

Our findings indicate that DM type 1 negatively affects sperm quality and P1/P2 ratio and this negative effect is transmitted to the progeny.

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GASOTRANSMITTERS PROTECT PORCINE OOCYTE AGAINST AGING

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Porcine oocytes can be fertilized in the MII stage of meiotic maturation. If oocytes are not fertilized shortly after the completion of meiotic maturation, then they undergo a number complex undesirable changes called aging (Petrová et al., 2009). The quality of aged oocytes and its capacity to undergo proper further embryonic development after fertilization rapidly decrease, because aging is associated with abnormalities of chromosomes, defects of the meiotic spindle, mitochondrial disorders, partial exocytosis of cortical granules and hardening of the *zona pellucida* etc. (Miao et al., 2009; Mammucari and Rizzuto, 2010 and others). Oocyte aging is partly due to changes in M-phase promoting factor (MPF) and mitogen-activated protein kinase (MAPK) activity, which are necessary to maintain meiotic arrest in metaphase II (Whitaker, 1996). Hydrogen sulfide (H_2S) as a gaseous mediator produced by three enzymes (cystathionine-β-synthase - CBS, cystathionine-γ-lyase - CSE, and 3-mercaptopyruvate sulfurtransferase - MPST) is one of the upstream factors that control MAPK activity (Li et al., 2011; Šmelcová et Tichovská, 2011). Carbon monoxide (CO) as a gas signal molecule produced by heme oxygenase (HO) can regulate MAPK signaling too (Peers et al., 2015). Both gasotransmiters (H_2S and CO) participate by regulating ion channels and kinase activities in the regulation of apoptosis, in somatic cells. They can suppress formation of pro-apoptotic mediators (eg. caspases) and induce production of anti-apoptotic factors (Yang and Wang, 2007; Li et al., 2013 and others).

Inhibition of H_2S-producing enzymes accelerates signs of aging in pig oocytes and significantly increases the ratio of fragmented oocytes. The presence of exogenous H_2S from a donor ($Na_2S.9H_2O$) significantly suppressed the manifestations of aging, reversed the effects of inhibitors and resulted in the complete suppression of oocyte fragmentation

– apoptosis (Krejčová et al., 2015). CO donor had similar effect and it suppressed pig oocyte fragmentation during aging.

This work was supported by the NAZV QJ1510138 and CIGA 20142049.

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PREVALENCE OF HPV INFECTION IN ASSISTED REPRODUCTION

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Introduction: HPV infection could play a role in human fertility like other sexually transmitted diseases (STDs). Higher risk of spontaneous abortion and possible mother-fetus transmission has been also described in HPV positive women. Moreover, *in vitro* studies demonstrated increased apoptosis and delayed development in HPV positive embryos. Unlike other STDs, HPV is not tested obligatorily for gamete donors or infertile couples, although it could significantly affect fertility, pregnancy or the fetus itself.

Is there any correlation between HPV status, childlessness and female infertility?

Material and methods: A prospective laboratory based study wherecervical smears of oocyte donors (n=158) and women treated for infertility (n=610) were collected between April 2013 and October 2014. All participants filled a questionnaire focused on their health status and sexual behavior.

Cervical smears were analyzed for presence of 14 high-risk (hrHPV) genotypes by Cobas 4800 HPV system (Roche) and PapilloCheck HPV-Screening system (Greiner Bio-One) detecting 18 hrHPV. Data from questionnaires, clinical data and HPV screening results were analysed.

Results: Forty-one (26%) out of 158 oocyte donors were HPV+. Childlessness in HPV+ oocyte donors was more frequent than in the HPV- group (39% vs. 20%; p=0.016). The average age was 25.6 in HPV+ vs. 27.4 in HPV- (p=0.023).

HPV infection was detected in 90 (15%) women out of 610 women from infertile couples and increased with the number of sexual partners (median 4 vs. 5; p=0.002). Interestingly, women treated for infertility ≤6 months were more frequently HPV+ than

women treated ≥ 48 months (32.4% vs. 7.5%, p=0.001). The prevalence of HPV was twice as high within oocyte donors as in infertile women (26% vs. 15%), which could be related to the lower age of oocyte donors (27.0 vs. 32.7; p<0.001).

Conclusions: The significantly higher prevalence of HPV infection in the group of oocyte donors is disconcerting. According to the literature, HPV positivity is a risk factor for pregnancy. HPV positive oocyte donors may be therefore a risk for the oocyte recipient and for the further development of the fetus.

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ANTI-MÜLLERIAN HORMON (AMH) SERUM LEVELS - COMPARISON OF CHEMILUMINESCENT AND ELISA METHOD

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Introduction: Anti-Müllerian hormone (AMH) is a dimeric glycoprotein which belongs to the TGF-β growth factors family. The molecular weight of the entire glycoprotein is 140 kDa. AMH is a product of granulosa cells in ovarian follicles. It´s an important marker of ovarian reserve. AMH serum levels can be used as an indicator of the individual number of follicles.

Approach of this study was to compare manual imunoassay method ELISA, which represents routine method used for many years, and automatic chemiluminescent method, which is available from the end of a year 2014.

Materials and metods: Our group of patients consisted of 133 females with a median age 32 years (minimum 22 years, maximum 43 years). Serum samples of each woman was collected and stored at -20°C until the examination of AMH. For detection of AMH we used kit ELISA AMH Gen II (Beckman Coulter, USA) and chemiluminescent method with kit ACCESS AMH (Beckman Coulter, USA). The chemiluminescent assay(determination) was performed on the device Dxl (Beckman Coulter, USA). Both of these methods determine levels of AMH by using two monoclonal antibodies. These antibodies are identical for both of these methods.

Results: Serum levels measured by the chemiluminescent method are lower that levels determined by the ELISA method. Achieved correlation coefficient is very good (R= 0.9899). The mean difference during the whole calibration curve (0.16 – 22.00 ng/ml) is less than 15%.

Conclusions: Both of these immunoassays, the ELISA method and the chemiluminescent method are reliable for determining serum levels of AMH. It is not necessary to change recommended reference ranges for individual indications.

This work has been supported by the Charles University Research Fund (project number P36).

Saturday, MAY 16, 2015

THE EFFECT OF CONTRACEPTIVE PILLS ON THE SECRETION OF ANTI-MÜLLERIAN HORMONE

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Background: Anti-Müllerian hormone (AMH) is a glycoprotein that ranks among the superfamily of growth factors TGF-β. AMH is produced by granular cells of ovarian follicles in women. Its level in serum reflects the total number of follicles in both ovaries. According to this fact we are able to make a use of AMH as a marker of ovarian reserve.

Approach: The assessment on the effect of long term use of hormonal contraception (HC) on the secretion of AMH in women whose age is up to 35 years. All of the women stopped using HC at least one year before the sera were taken from them.

Material and Methods: We examined 149 women divided into two groups. The first one ranks 105 women that had used HC in the period between 10 to 17 years and they stopped using HC one and more years before the sera examination. The second (control) group is comprised of 44 women never used HC before. The serum samples were taken from venous blood. The separation took no longer than two hours after the blood draw. The samples were 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 DxI (Beckman Coulter, USA). The levels of AMH in serum vary by age. We have assessed a concordance in the age of both groups. The results were determined by Wilcoxon test with the level of statistical significance $p \leq 0.05$.

Results: The middle value of the age was 32 years in both of the groups. The middle value of AMH in sera of women that had used the HC was lower (2.89 ng/ml) in comparison to those who had not used it before ($p=0.3261$).

Conclusion: We have not proved the negative impact of HC on the level of AMH in serum in the group of women examined one and more b years after the HC use.

This work has been supported by the Charles University Research Fund (project number P36).

SERUM AND OVULATORY CERVICAL MUCUS LEVELS OF ANTI-MÜLLERIAN HORMONE (AMH) IN INFERTILE WOMEN

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Background: AMH is a glycoprotein that belongs to the transforming growth factor-B (TGF-β). Two monomers of molecular weight about 72kDa form AMH. In females, AMH is produced in granulosa cells of early growing, preantral and small antral follicles. AMH concetration is directly proportional to the number of antral follicles, therefore serum levels may be used as a marker for ovarian reserve.

Approach: AMH comparison of serum and of unusual biological material - ovulatory cervical mucus (OCM).

Material and Methods: Our group of patients consisted of 133 females (mean age of 32 years, minimum 22 years and maximum 43 years) with regular ovulatory cycles but with infertility. Each serum sample was kept at -20°C until examinatiopn. OCM was taken from cervical area by special syringe adapted to capillary sampling during ovulation under ultrasonografic controlls of ovaries. We used 0.25% of bromelain to liquefy OCM samples. We also diluted 3 serum samples of well-known concentration with bromelain, to make sure, that bromelain does not affect the change of AMH levels or/and a process of chemilunescent reaction. We determined levels of AMH using chemiluminescent kit ACCESS AMH (Beckman Coulter, USA, set DxI -Beckman Coulter, USA).

Results: The AMH serum levels were between 0,12 - 22,12 ng/ml. We found zero concentration of AMH in OCM. Only in 5 infertile women we detected very low concentration on border of detectability. Serum samples diluted with bromelain show after multiplying with dilution factor same concentration as original samples.

Conclusions: AMH is not a origin part of the OCM. Very low concentration of AMH probably get into mucus by transudation from the blood. Bromelain, which we used for liquefaction of ovulatory samples does not affect a AMH molecule or a process of chemiluminescent reaction.

This work has been supported by the Charles University Research Fund (project number P36).

MONITORING OF ANTIPHOSPHOLIPID ANTIBODIES (APA) DURING THE TREATMENT OF ANTIPHOSPHOLIPID SYNDROME IN SOME INFERTILE WOMEN

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Background: Antiphospholipid syndrome (APA sy) I and II is an autoimmune disease which is characterized by the presence of organ non-specific autoantibodies. It is the most common cause of acquired thrombophilia. Any organ or blood vessel may be affected considering the lack of antibody specificities. The most common clinical symptoms are thrombocytopenia or arterial or venous thrombosis, recurrent fertility failures. The presence of APA in women with reduced fertility (infertility, abortions and other complications of pregnancy) also causes microthrombotization between mother and fetus. Such pregnancy then most often ends such as spontaneous pregnancy loss.

Approach: Monitoring of antiphospholipid antibodies in women with APA sy in relation to their treatment.

Material and Methods: APA-IgG, IgM against phosphatidic acid, ph-serine, ph-ethanolamine, ph-glycerol, ph-inositol, β2-glycoprotein I (beta2-GPI), annexin V and cardiolipin were proven using the classic ELISA method. We selected a random group of 32 women with APA syndrome I and one patient APA sy II (rheumatoid arthritis), (mean age 32.5, ranging from 23 to 40 years) from special consultation for reproductive immunology. Blood samples were taken at the first examination and after 3 months of the treatment. Patiens were given non-steroidal anti-inflammatory, and immunosuppressive, and B-group vitamins.

Results: Sera of most patients were positive in 3 - 6 different APA. IgG antibodies against ph-inositol (24), ph-serine (23), ph-glycerol (16), ph-ethanolamine (8), beta2-GP I (4), cardiolipin (3) and annexin V (3) were prevalent. The levels of these antibodies

predominantly decreased during the treatment. In our presentations we show graphic changes in antibodies and the effect of the treatment.

Conclusion: Monitoring of APA levels is signifiant for the adjustment of the treatment. Five patients successfully gave a birth of their babies, others are still under strict medical supervision.

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EFFECT OF SELENIUM, VITAMIN E, AND ZINC ON ANTIOXIDATIVE ENZYMES IN HUMAN SEMINAL PLASMA

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Selenium (Se), an essential trace element, has multiple and complex effects on human health, mostly because of its antioxidant activity and the role in the balance of several hormones. Increasing evidence suggests that this mineral plays an important role in normal growth, development and reproduction in animals and humans. The Czech Republic belongs to the areas with low content of selenium in soil resulting 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 viability of sperm cells, namely progressive motility and acrosomal reaction of spermatozoa.

We focused on the characterization of seminal fluid proteins, especially antioxidant enzymes, to illustrate in detail the local responses of infertile males. The content of selenium 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 seminal plasma and sera. We use 12 control patients with fertility problems and group of 13 patients treated with selenium and zinc preparation and vitamin E to enhance their antioxidative stress response. The daily treatment of patients lasted from two to four months with dose of selenium 50 μg per capsule, zinc 72 mg per capsule and vitamin E 200 mg per capsule.

Glutathione peroxidase showed an increasing activity in seminal plasma after treatment, the increase was seen in eight of the thirteen patients. Glutathione S-transferase also exhibited an increasing activity in seminal plasma after selenium and vitamin E treatment 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 patients and with the levels of selenium and zinc in seminal plasma.

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TREATMENT WITH INTRAVENOUS LIPID EMULSIONS FOR RECURRENT PREGNANCY LOSS

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Commercially available intravenous lipid emulsions (IVLE) are well known as the part of parenteral nutrition mainly in critically ill and intensive care patients. These emulsions contain soybean oil, glycerine and egg phospholipids. Degradation takes place at extrahepatic endothelial sites by lipoprotein lipase-mediated hydrolysis. First therapeutic use of IVLE for recurrent pregnancy loss dates back to early 1990's (Johnson 1991, Clark 1994). Immunosupressive (and immunomodulatory) effects of IVLE are executed mostly through NK cells inhibition, thus reducing their cytotoxicity. Mechanisms appear to be related to omega-6 fatty acids (linoleic acid), omega-3 (α-linoleic acid) and PGE2 (linoleic acid metabolite). Various studies demonstrated, that supression of uNK by IVLE seems to be comparable to its effect on peripheral NK cells. Fatty acids (FA) are incorporated into cytoplasmatic membranes and alter their fluidity. They act as direct ligands for nuclear receptors (e.g. peroxisome proliferator-activated receptors - PPARs and G-protein-coupled receptors - GPCRs), thus affecting intracellular signaling pathways. Failures of embryo implantation have been also associated with abnormal angiogenesis/arteriogenesis and NK cells probably participate in spiral artery arteries remodeling and uterine blood flow.

Many papers point out, that there is more to IVLE than just influencing NK cells response. Naive T-lymphocytes show balanced glucose and lipid oxidation metabolism, whereas effector T-cells (Th1, Th2, Th17) use predominantly aerobic glycolysis. Contrary to this observation, Treg cells use lipid oxidation for their metabolic needs. Exogenous FA promote Treg cells differentiation, thus helping to protect semi-allogenic embryo.

However, IVLE show also potential adverse events and drug interactions, which seem to affect chiefly higher risk patients with obesity, insulin resistence, diabetes mellitus and LPL polymorphisms. IVLE may also affect vitamin D3 and some heparin/LMWH therapeutic properties.

Future work on IVLE should concentrate in several directions towards controlled larger scale clinical trials, comparative studies between various IVLE formulas, effective dosage determination and role in angiogenesis during pregnancy to name just a few of potential research targets.

CAN INTRAVENOUS IMMUNOGLOBULINS IMPROVE PREGNANCY OUTCOMES IN PATIENTS WITH PRIOR FAILED *IN VITRO* FERTILIZATION?

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Background: Among current options for the immunomodulatory treatment of reproductive failures, intravenous immunoglobulins (IVIGs) are highly expensive, may pose serious side effects, and require strict treatment criteria for clinical practice. Nevertheless, dozens of large, well-designed studies have suggested that IVIGs may improve the success of in vitro fertilization (IVF) and embryo transfer in patients with prior IVF failures, as well as the preconception treatment of primary recurrent abortion patients, though other clinical trials offer conflicting results.

Aims: The aims of this study were twofold: to determine whether any group of patients benefits from IVIG in IVF and, if so, whether the group can be identified by preconception blood testing.

Methods: Our cohort consisted of 31 women, each with a normal oocyte reserve, whose infertility was independent of male infertility and who had experienced at least three failed IVF or embryo transfer procedures. Sixteen women had high serum levels of antiphospholipid antibodies (APLA, group A), eight women had high counts of circulating natural killer (NK, CD3-16+56+) cells along with an expressed Th1 phenotype of T-lymphocyte cytokine production (group B), and seven women did not show any laboratory immunopathology prior to IVF (group C). All patients received 150 mg/kg IVIG per IVF cycle during three infusions: 1–7 d prior to embryo transfer, 1–7 d following embryo transfer, and immediately after the positive pregnancy test.

Results: Fourteen (88%) APLA-positive women from group A became pregnant, 10 of whom (63%) achieved live birth. In group B, five (63%) women tested positive for

pregnancy following embryo transfer, achieving a live birth rate of 38%. In group C, only one patient (14%) achieved live birth (p = 0.0024).

Conclusions: IVF outcomes improve significantly when IVIGs are administered to women with repeat IVF failures showing humoral and/or cellular immunopathology prior to IVF. However, immunologically healthy women do not seem to benefit from such treatment. Careful discussion about IVIG indications in reproductive immunology is thus necessary in the Czech Republic.

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THERE IS A RELATIONSHIP BETWEEN INFERTILITY TREATMENT AND THE RISK OF THYROID CANCER AND GYNECOLOGICAL CANCERS?

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Infertility affects approximately 15% of the population of Czech Republic. Majority of infertile couples are trying to seek health care. After infertility is diagnosed, patients are treated with assisted reproductive techniques: using methods of intrauterine insemination or in vitro fertilization. In the Czech Republic in recent years, approximately 3% of all live births are born by means of assisted reproduction techniques.

Combination of hormones, such as agonists or antagonists of gonadotropin releasing hormone (GnRH) in combination with gonadotropins (FSH, LH), progesterone, or clomifene are commonly used with assisted reproduction methods.

The relationship between hormonal stimulation and the risk of some cancers has been addressed by many studies since the eighties. The studies focused primarily on relationship with gynecological tumors. A relation between hormonal stimulation and thyroid carcinoma was also observed, particularly because in this tumor hormonal factors are one of the risk factors for developing the disease.

Results of some studies have shown some increased risk of different types of tumors, in the context of assisted reproduction. In most studies, there was no proof of increased risk. Some of the published the results for some types of cancer were included multicenteric studies. It is interesting that the risks of the studied tumors are related to the diagnosis of infertility itself. The authors of the studies agree that for a proper evaluation of the long-term impact of fertility treatment on women's health, it is necessary to conduct more population studies after a longer period after the treatment.

The aim of our opresentation is to report on patients of the Clinic of Nuclear Medicine and Endocrinology that underwent fertility treatment before being diagnosed with thyroid cancer. The presentation is also devoted to an overview of new studies on this topich, recently published in the scientific literat

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