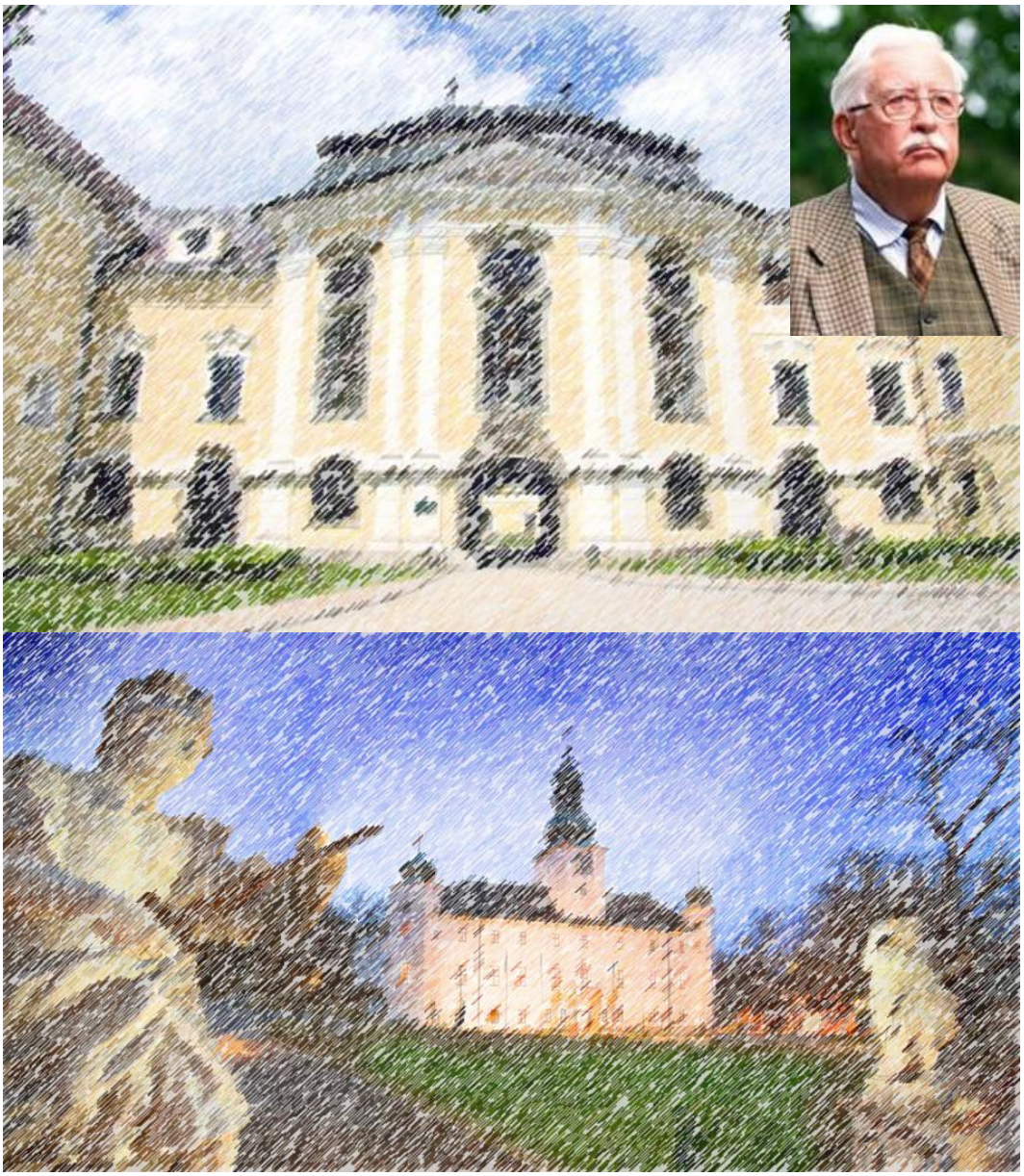

XXth SYMPOSIUM

OF BIOLOGY AND IMMUNOLOGY OF REPRODUCTION

WITH INTERNATIONAL PARTICIPATION

The Castle, Třešť
May 22 – 24, 2014

BIOTECHNOLOGICKÝ ÚSTAV
AKADEMIE VĚD ČESKÉ REPUBLIKY, v. v. i., PRAHA

LÉKAŘSKÁ FAKULTA KARLOVY UNIVERSITY
A FAKULTNÍ NEMOCNICE, PLZEŇ

XX. SYMPOSIUM
BIOLOGIE A IMUNOLOGIE REPRODUKCE
S **MEZINÁRODNÍ ÚČASTÍ**

věnováno památce Dr. Radslava Kinského

Zámek, Třešť, 22.5.- 24.5.2014

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

MEDICAL FACULTY OF CHARLES UNIVERSITY
AND FACULTY HOSPITAL IN PILSEN

XXth SYMPOSIUM OF BIOLOGY AND IMMUNOLOGY OF REPRODUCTION WITH INTERNATIONAL PARTICIPATION

in memory of Dr. Radslav Kinsky

The Castle, Třešť, May 22 – 24, 2014

XXth Symposium of Biology and Immunology of Reproduction
with International Participation
The Castle, Třešť, May 22 – 24, 2014

PROGRAMME

Thursday, MAY 22, 2014

Arrival and accommodation at the Castle Trest

19.00 DINNER

Friday, MAY 23, 2014

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): The Medawar paradox in the 21st century.
10.00-10.20 Mestecky J. (U.S.A., CZ): Concordant and discordant functions of HIV-specific antibodies of IgG and IgA isotypes in the systemic, genital and intestinal mucosal compartments.
10.20-10.40 Shibahara H. (Japan): Role of anti-sperm antibodies in immunologically infertile women.
10.40-11.00 Sargent I. (England): Syncytiotrophoblast vesicles and immunomodulation in pregnancy.

11.00-11.30 COFFEE BREAK

Chairpersons: Peknicova J., Madar J.

11.30-11.50 Madar J.: Role of reproductive immunology in *in vitro* fertilisation.
11.50-12.05 Antalikova J. (Slovakia): Sperm treatment with anti-bovine CD9 monoclonal antibody reduced the fertilization rate *in vitro.*
12.05-12.20 Dvorakova-Hortova K.: One more drop for decreasing reproduction.
12.20-12.35 Sebkova N.: Dynamics of CD46 and β1 integrin proteins in the sperm head during capacitation and acrosome reaction.

12.35-12.50 Jonakova V.: The ubiquitin-proteasome system is involved in the regulation of activity of spermadhesin AQN1 and acrosin inhibitor, the two sperm surface proteins, during porcine fertilization.

12.50-13.05 Ded L.: Endocrine disruptors induce transgenerational alterations of the male reproductive parameters and miRNA expression profiles in mouse primordial germ cells.

13.15-14.30 LUNCH

Chairpersons: Dvorakova-Hortova K., Jonakova V.

14.30-14.45 Zatecka E.: The effect of tetrabromobisphenol A on protamination and DNA quality of mouse sperm.

14.45-15.00 Dorosh A. (Ukraine): Effect of exposure to bisphenol A on gene expression in testicular tissue in male mice.

15.00-15.15 Manaskova-Postlerova P.: Detection of mannosidase in the porcine urogenital tract – study of the sperm releasing from oviductal reservoir.

15.15-15.30 Davidova-Cozlova N.: Enzymatic and inhibiting activity in boar epididymal fluid.

15.30-16.00 COFFEE BREAK

Chairpersons: Tlaskalova-Hogenova H., Sebkova N.

16.00-16.15 Dostalova P.: Expression of estrogen receptor beta (ER β) in murine male reproductive tract and sperm.

16.15-16.30 Zigo M. (Slovakia): Panel of monoclonal antibodies – alternative tool for monitoring of sperm-zona pellucida receptors localization and identification.

16.30-16.40 Ulcova-Gallova Z.: Significance of screening tests in systemic lupus erythematosus.

16.40-16.55 Tibenska E. (Slovakia): The role of vitamin D in the reproductive system.

16.55-17.15 Tlaskalova-Hogenova H.: Microbiome and mucosal barrier function in pathogenesis of inflammatory diseases.

17.15-17.30 BIO-RAD: New product – S3™ Cell sorter.

17.40-18.30 Accordionists – concert

19.00 DINNER (RAUT)

Saturday, MAY 24, 2014

Breakfast from 7 am

Chairpersons: Novakova D., Zidkova J.

9.00-9.15 Capkova J.: Flow cytometry (FCM) sperm assessment in normozoospermic and asthenozoospermic men using monoclonal antibodies against sperm proteins.
9.15-9.30 Zidkova J.: Study of immunological properties of sperm and seminal plasma antigens.
9.30-9.45 Bobak L. (Slovakia): Serological immunoblotting diagnostic of chlamydial infection in infertile men and its association with selected immunological parameters (pilot study).
9.45-10.00 Malickova K.: Diagnostic and prognostic value of presepsin in preterm deliveries.
10.00-10.15 Novakova D.: The impact of thyroid disorders on fertility and impact of assisted reproduction on the thyroid gland.
10.15-10.30 Ondryasova H.: Prevalence of HPV infection in infertile women and oocyte donors.

10.30-11.00 COFFEE BREAK

Chairpersons: Malickova K., Bobak L.

11.00-11.15 Cupperova P. (Slovakia): Developmental potential of bovine oocytes cultivated *in vitro* is affected by mineral oil exposed to UV light.
11.15-11.30 Krejcova T.: Effect of carbon monoxide on porcine oocytes meiotic maturation.
11.30-11.45 Dvorakova M.: S-allyl cysteine but not alliin influences meiotic maturation of porcine oocytes.
11.45-12.00 Weingartova I.: Hydrogen sulfide: a signal molecule in oocyte maturation and cumulus expansion.
12.00-12.15 Mala E.: Doctor,do everything. Although I do not know I want.
12.15-12.35 Landova V.: Some aspects of frustration.
12.35-12.45 Domorazkova E.: Maternal hope foundation.

12.45-13.00 CLOSING OF SYMPOSIUM
Peknicova J., Ulcova-Gallova Z.

13.15-14.30 LUNCH

XXth Symposium of Biology and Immunology of Reproduction
with International Participation
The Castle, Třešť, May 22 – 24, 2014

ABSTRACTS

XXth Symposium of Biology and Immunology of Reproduction
with International Participation
The Castle, Třešť, May 22 – 24, 2014

Friday, MAY 23, 2014

THE MEDAWAR PARADOX IN THE 21ST CENTURY

Chaouat G

INSERM, Hopital Saint Louis, Paris, France

We will first briefly recall that the classical view of the immune system is challenged from every aspect, the danger theory being the 1st attack, but not the sole one. In our opinion, it is important to recall into what context Medawar enunciated his “citation classic”. The discovery of innate immunity and the work carried out on emergence of adaptive immune system has not been fully integrated in a vision of when and how pregnancy did appear. It is incidentally interesting that eutherian placentation required … integration of a retrovirus … More important, placentation is not restricted to mammals, and if an advantage in evolution, has anyhow appeared, disappeared, reappeared at various stages , even within a phylum. The first placentae appeared in velvet worms, which show graft rejection and a (not so) primitive MHC. Placental fishes (materpisces, sharks) appeared at “molecular genetic clock” time when there is not yet a fully adaptive immunity. Dinosaurs are unknown, but one may assume that placental reptiles nowadays mimic them. In all those cases, placenta seems to evolve very well when cohabiting with NK cells. The mammals have first been oviparous, and the development of the marsupial pouch show that the by then existing placental and uterine controls of adaptive immunity were not as efficient as to obliterate the need for a quick out of uterus escape before rejection. In that context, if is interesting to note that very few immunoregulatory mechanisms described as suppressing maternal immune response are so important that their neutralization causes pregnancy loss. Amongst those demonstrated to be of such importance are RTF and Tregs. It is interesting to note that, just as for NK where uNK are the cells dedicated to pregnancy, only pTregs appear to be fully required for successful allopregnancy. Thus, CNS1 integration in FoxP3 appears crucial. We will then discuss evidence for T regs being “self specific” vs. T regs being alloantigen specific, and will propose a solution to solve the conflict paused by these 2 apparently opposed propositions and data, even within our own set of data.

CONCORDANT AND DISCORDANT FUNCTIONS OF HIV–SPECIFIC ANTIBODIES OF IgG AND IgA ISOTYPES IN THE SYSTEMIC, GENITAL AND INTESTINAL MUCOSAL COMPARTMENTS

Mestecky J

Department of Microbiology, University of Alabama at Birmingham, U.S.A; Institute of Immunology and Microbiology, 1st School of Medicine, Charles University, Prague, Czech Republic

Humoral responses of individuals infected with HIV or immunized with experimental HIV vaccines are dominated in all body fluid examined by IgG-associated antibodies irrespective of the route of HIV acquisition or immunization. In contrast to other bacterial and viral mucosally-acquired infections, in which specific antibodies in external secretions are associated with the IgA isotype, HIV-specific antibodies in all external secretions, even those with high levels of total IgA (e.g., saliva, tears, intestinal fluid, milk), are found overwhelmingly in the IgG1 and IgG3 isotypes. Although extremely low levels of HIV-specific IgA antibodies are present in the majority of infected individuals, HIV-vaccine systemically immunized volunteers who respond by the production of high levels of IgA HIV-specific serum antibodies, display a higher rate of HIV infection than those who do not have prominent serum IgA responses. In contrast, HIV-specific antibodies of the secretory IgA isotype generated by various molecular-genetic approaches effectively prevented and blocked the penetration of HIV through the mucosal barrier *in vitro* as well as *in vivo* experiments. Systemically or locally (intra-rectally or vaginally) administered monoclonal HIV-specific antibodies of the IgG isotype prevented the infection of rectally or vaginally applied SHIV in the macaque model. However, the most recent results suggest that the protective effect of IgG antibodies in the female genital tract mucosa is pH-dependent. Free or HIV-complexed IgG interacts at the acid pH with the FcRn receptor that recognizes the Fc region of IgG and is expressed on the surface human vaginal epithelia and consequently augments transcytosis of HIV across the epithelial cells. Both HIV-neutralizing and non-neutralizing antibodies may be effective in this process. All of the above described experiments have

been performed in the presence of HIV-specific antibodies of either IgG or IgA isotype but never in physiologically more relevant settings including HIV-antigen-specific neutralizing and non-neutralizing antibodies of both IgG and IgA isotypes mixed with HIV at variable molar proportion, temporal sequences, and different molecular forms (e.g., monomeric/polymeric IgA, secretory IgA; IgG of various subclasses and glycosylation patterns etc.). All of these factors and parameters may profoundly influence the protective or enhancing function of HIV-specific antibodies of IgG or IgA isotypes and should be considered in the design and routes of delivery of HIV vaccines.

ROLE OF ANTI–SPERM ANTIBODIES IN IMMUNOLOGICALLY INFERTILE WOMEN

Shibahara H

Department of Obstetrics and Gynaecology, Hyogo College of Medicine, Hyogo, Japan

Half a century has passed since the relationship between antisperm antibody and sterility was identified in female guinea pigs by Isojima et al.[1] Several assay methods have been developed to detect antisperm antibodies. The most important consideration in this field is the selection of the method for detection. Amongst them, the 'sperm immobilization test' which detects sperm-immobilizing antibodies, has been shown to be the most reliable assay for detecting antisperm antibodies linked to female infertility.[2,3] In this presentation, I would like to focus on the role of anti-sperm antibodies in immunologically infertile women

The incidence of sperm-immobilizing antibodies detected by the quantitative sperm immobilization test[4] in the sera of infertile women has been shown to be 3%.[5] A follow-up study has shown that the antibody titers (SI_{50}) were found to be unstable and to fluctuate over a period of several months within each patient.[6] It is important to assess the undulation patterns of individual patient's SI_{50} titers to propose a strategy for the treatment of infertile women with the antibodies.[7]

Infertile women having higher SI_{50} titers of sperm-immobilizing antibodies can be refractory to conventional treatments such as timed intercourse (TI) or intrauterine insemination (IUI) because the antibodies secreted in the female reproductive tract might impair sperm passage,[8-10] inhibit fertilization,[11-14] and prevent normal post-fertilization processes.[14,15] Therefore, manipulation of gametes and embryos from patients with sperm-immobilizing antibodies should be carried out with additional care to avoid fertilization failure resulting from the presence of antibodies during in vitro fertilization (IVF).[16,17]

The reasons why majority of women do not develop sperm-immobilizing antibodies on exposure to sperm is not clear. The production of sperm-immobilizing antibodies is likely

to occur in women with particular HLA haplotypes[18] after repeated exposure to sperm.[19,20] Characterization of sperm-immobilizing antibodies may help in the identification and characterization of sperm specific antigens that can be used as candidate antigens for the development of sperm based contraceptive vaccines.[21,22]

References

1. *Isojima, S., et al., Science 129: 44, 1959*
2. *Isojima, S., et al., Am. J. Obstet. Gynecol. 101: 677-683, 1968.*
3. *Isojima, S., et al., Am. J. Obstet. Gynecol. 112: 199-207, 1972.*
4. *Isojima, S., Koyama, K., Excerpta Med. Int. Congr. Ser. 370: 10-15, 1976.*
5. *Shibahara, H., et al., J. Reprod. Immunol. 83: 139-144, 2009.*
6. *Koyama, K. et al., Int. J. Fertil. 33: 201-206, 1988.*
7. *Kobayashi, S., et al., Fertil. Steril. 54: 1107-1113, 1990.*
8. *Koyama, K., et al., Excerpta Med. Int. Congr. Ser. 512: 705-708, 1908.*
9. *Shibahara, H., et al., Am. J. Reprod. Immunol. 34: 120-124, 1995.*
10. *Shibahara, H., et al., Am. J. Reprod. Immunol. 57: 142-146, 2007.*
11. *Shibahara, H., et al., Acta Obstet. Gynaecol. Jpn. 43: 237-238, 1991.*
12. *Shibahara, H., et al., Fertil. Steril. 60: 533-539, 1993.*
13. *Shibahara, H., et al., Hum. Reprod. 11: 2595-2599, 1996.*
14. *Taneichi, A., et al., Am. J. Reprod. Immunol. 47: 46-51, 2002.*
15. *Koyama, K., et al., Gamete Res. 10: 143-152, 1984.*
16. *Shibahara, H., et al., Am. J. Reprod. Immunol. 36: 96-100, 1996.*
17. *Shibahara, H., et al., Reprod. Med. Biol. 5: 137-143, 2006.*
18. *Tsuji, Y., et al., J. Reprod. Immunol. 46: 31-38, 2000.*
19. *Shibahara, H., et al., Am. J. Reprod. Immunol. 35: 57-60, 1996.*
20. *Shibahara, H., et al., Reprod. Med. Biol. 2: 105-108, 2003.*
21. *Koyama, K., et al., Fertil. Steril. 56: 954-959, 1991.*
22. *Shibahara, H., et al., J. Reprod. Immunol. 53: 1-12, 2002*

SYNCYTIOTROPHOBLAST VESICLES AND IMMUNOMODULATION IN PREGNANCY

Sargent I

Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Oxford, Great Britain

The syncytiotrophoblast of the human placenta sheds a variety of cellular debris into the maternal circulation throughout pregnancy. This debris ranges in size from syncytial sprouts or knots (20-200μm), to microvesicles (100nm-1μm) and exosomes (50-100nm). Both microvesicles and exosomes are involved in cell–cell signalling and there is growing evidence that those derived from the syncytiotrophoblast play an important role in suppressing the maternal immune response during normal pregnancy. In contrast, the maternal syndrome of pre-eclampsia is characterised by an excessive inflammatory response associated with endothelial dysfunction, brought about by the release of multiple factors from the placenta into the maternal circulation. While some of these factors are released as soluble molecules it is now apparent that many of them are associated with syncytiotrophoblast microvesicles and exosomes (collectively termed STBM), which are present in increased amounts in the circulation of women with pre-eclampsia. STBM have proinflammatory, anti-endothelial and procoagulant activity *in vitro*, all of which are features of the maternal syndrome. We propose that the different effects of STBM result from different types of vesicles within the preparation, the smaller exosomes being immunoregulatory and the larger microvesicles being proinflammatory, with a shift to the latter in pre-eclampsia. In support of this, we have demonstrated using a novel technique, Nanoparticle Tracking Analysis, that pre-eclampsia STBM are indeed larger than those from normal placentas. Furthermore, the range and types of factors they carry (and hence their functions) may differ in pre-eclampsia, where the syncytiotrophoblast is subjected to oxidative and inflammatory stress. We have carried out proteomic analysis on vesicles prepared from normal and pre-eclampsia placentas by perfusion and have identified differences in the repertoire of molecules they carry.

Candidates include immunoregulatory molecules (B7-H1, CD200 and Galectin 1), complement and complement regulatory molecules (C1q, C3, CD55, CD59 and vitronectin), proinflammatory molecules (HSP70, HMGB1, Galectin 3 and Synctin 1), anti-angiogenic molecules (CD49e, CD51, CD26, Flt-1 and endoglin) and procoagulant molecules (tissue factor and phosphatidylserine). Characterising the molecular cargos of the STBM may lead to the discovery of new biomarkers for pre-eclampsia and inform future treatments.

ROLE OF REPRODUCTIVE IMMUNOLOGY IN *IN VITRO* FERTILISATION

[1] Madar J, [2] Nováková D, [3] **Mardesić T**

[1]Institute for the Care of Mother and Child, Prague, Czech Republic
[2]Departmen of Nuclear Medicine and Endocrinology, 2nd Faculty of Medicine Charles University Prague and University Hospital Motol, Prague, Czech Republic
[3]Pronatal Sanatorium, Prague, Czech Republic

In this year we celebrate 20 years of reproductive immunology symposium in Czech Republic. Therefore we look at our past work in this field. Our retrospect leads us to thinking about the future prospects of reproductive immunology and its role in assisted reproduction.

We would like, together with gynaecological society, to be involved in the organization of the concept of immunological tests that have become a standard part of the diagnostic schema for patients with fertility problems. We follow scientific articles that were recently published on this issue.

Our aim in future is to pass comprehensive information about the importance of immune disorders in infertility, disorders of implantation and pregnancy losses, to gynaecologists and specialists in the field of assisted reproduction.

SPERM TREATMENT WITH ANTI-BOVINE CD9 MONOCLONAL ANTIBODY REDUCED THE FERTILIZATION RATE *IN VITRO*

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 essential role of the oocyte CD9 tetraspanin in the gamete fusion was demonstrated in mice more than ten years ago by the inability of CD9 knock-out mouse oocytes to fuse with sperm and by the ability of anti-CD9 antibodies to inhibit this fusion in normal mouse. Reduction of sperm binding and fusion after oocyte antibody treatment was also reported in pig and cattle. The CD9 molecule on sperm was detected later in mouse and boar; we suggested the expression of CD9 on plasma membrane of bovine sperm, too. However, the relevance of CD9 molecule on spermatozoa in sperm-egg fusion is still unclear. The aim of this study was to investigate whether the anti-CD9 antibody (IVA-50) treatment of bovine sperm influences the sperm-oocyte interaction during *in vitro* fertilization. Frozen-thawed sperm before *in vitro* fertilization were incubated with TL medium containing IVA-50 or DMEM as a control and number of fertilized oocytes after gametes co-incubation was evaluated. The sperm treatment with IVA-50 significantly reduced the number of fertilized oocytes (69%) compared to control group (97%) (Mann-Whitney, $P \leq 0.001$; $n = 100$). It seems that sperm CD9 play a role in fertilization process. It is probably not essential for sperm egg fusion but it may facilitate the interaction between sperm CD9 and egg CD9 molecules or possibly, CD9 is important for formation of complexes with other sperm proteins required for gamete fusion.

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

ONE MORE DROP FOR DECREASING REPRODUCTION

[1] **Dvořáková**-Hortová K, [1] Šídlová A, [2] **Děd L,** [1] Hladovcová D, [3] Vieweg M, [3] Weidner W, [3] Steger K, [1] Stopka P, [3] Paradowska-Dogan A

[1]Biocev Group, Department of Zoology, Faculty of Science, Charles University in Prague, Prague, Czech Republic
[2]Laboratory of Reproductive Biology, Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic
[3]Department of Urology, Pediatric Urology and Andrology, Justus Liebig University of Giessen, Giessen, Germany

Toxoplasma gondii is a common protozoan parasite that infects warm-blooded animals throughout the world, including mice and humans. During infection, both, the parasite and the host, utilize various mechanisms to maximize their own reproductive success. Mice and humans are both the intermediate hosts for *Toxoplasma gondii*, which forms specialized vacuoles containing reproductive cysts in the formers' tissue. As half of the human population is infected, developing a disease called toxoplasmosis, along with an ever-growing number of couples suffering with idiopathic infertility, it is therefore surprising that there is a lack of research on how *Toxoplasma gondii* can alter reproductive parameters. In this study, a detailed histometric screening of the testicular function along with the levels of the pituitary luteinizing hormone (LH) were analysed in infected mice. Data on relative testis and epididymis weight, and sperm count were also collected. Based on the results obtained, the level of LH in the urine of *Toxoplasma gondii* infected mice was lower compared to the control. In direct correlation with the hormone level, testicular function and sperm production was also significantly lower in *Toxoplasma gondii* positive group using sperm count and histometric analysis as a marker. Not only were the number of leptotene primary spermatocytes and spermatids lowered, but the number of Sertoli cells and the tubule diameter were elevated. In parallel, a pilot epigenetic study on global testicular methylation, and specific methylation of Crem, Creb1 and Hspa1genes essential for successfully ongoing spermatogenesis was performed. Global methylation was elevated in *Toxoplasma* infected mice, and differences in the DNA methylation of selected genes were detected between the

Toxoplasma positive and control group. These findings demonstrate a direct relation between *Toxoplasma gondii* infection and the decrease of male reproductive fitness in mice, which may contribute to an increase of idiopathic infertility in humans.

The presentation is supported by the Grant Agency of the Czech Republic GACR No. 14-05547S , No. P503/12/1834, and by project AVOZ 50520701 awarded by the Academy of Sciences of the Czech Republic, and by the project BIOCEV CZ.1.05/1.1.00/02.0109 from the ERDF.

DYNAMICS OF CD46 AND β1 INTEGRIN PROTEINS IN THE SPERM HEAD DURING CAPACITATION AND ACROSOME REACTION

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

[1]Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic
[2]Department of Cell Biology, Faculty of Science, Charles University, Prague, Czech Republic

CD46 (membrane cofactor protein) plays an important role during fertilization. It is localized in sperm only in the acrosome membrane and its role is associated with acrosomal stability. CD46 is probably involved in signalling pathways triggering the acrosome reaction (AR). It also associates, through membrane proteins integrins, with specific MAP kinases involved in AR. Disturbed CD46 expression may, therefore, lead to altering an intracellular signalling and disrupting the acrosome region due to affecting a distribution of integrins (such as β1) and lately cytoskeletal protein actin. Faulty restructuring of these proteins throughout capacitation may therefore, result in unsuccessful fusion of membranes at the beginning of the acrosome reaction.

The aim of our work is to study the molecular mechanisms of CD46 and β1 integrin binding and their link to the cytoskeleton, namely actin.

The first question was whether CD46 and β1 integrin changes their localization during capacitation and subsequent AR. The dynamics of these proteins was monitored. CD46 relocates from the acrosome membrane towards the equatorial segment, but it has not been detected further in the postacrosome region. β1 integrin localization exhibits extended changes. Similarly, to CD46, β1integrin was observed across the apical acrosome and the equatorial segment. However, its relocation carries on further to the postacrosome region of the sperm head and its movement is similar to one described previously in primary fusion protein Izumo. There is also expected to be actin cytoskeleton involved.

The mutual molecular co-localization of CD46 and β1 integrin or probably actin will be further investigated using the FRET method and Proximity Ligation Assay Duolink®.

The work was supported by the Grant Agency of the Czech Republic GACR No. 14-05547S.

THE UBIQUITIN–PROTEASOME SYSTEM IS INVOLVED IN THE REGULATION OF ACTIVITY OF SPERMADHESIN AQN1 AND ACROSIN INHIBITOR, THE TWO SPERM SURFACE PROTEINS, DURING PORCINE FERTILIZATION

[1] Jonáková V, [2,3] Yi YJ, [2] Sutovsky P, [1] Postlerová P, [1] **Pěknicová J**

[1]Laboratory of Reproductive Biology, Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic
[2]Division of Animal Science and Department of Obstetrics, Gynaecology and Women's Health, University of Missouri-Columbia, Columbia, USA
[3]Division of Biotechnology, Chonbuk National University, Iksan-si, Jeonbuk, Korea

The spermadhesin AQN1and acrosin inhibitor (AI/SPINK2) proteins bind to the sperm plasma membrane at ejaculation. The AQN1 has been implicated in sperm binding to zona pellucida (ZP) of the oocyte as well as in sperm interactions with the epithelium of the oviductal sperm reservoir. The SPINK2 protects spermatozoa from proteolytic degradation during their trip up the female genital tract toward the oocyte. This study examined the role of two components of the 19S proteasome regulatory complex, the ubiquitin C-terminal hydrolase UCHL3 and PSMD8 in the AQN1-mediated boar sperm binding to zona pellucida. Interaction of PSMD4 subunit with the acrosomal surface-associated acrosin inhibitor AI/SPINK2 provided another line of evidence for the presence of 26S proteasomes on the sperm surface. Detection of the ubiquitinated forms of SPINK2 supports the hypothesis that SPINK2 activity is controlled by ubiquitin-proteasome system (UPS). The activity of the porcine AQN1, and thus the efficiency of sperm-oocyte recognition/binding, may be controlled by elements of the sperm surface-bound UPS, in particular by UCHL3, and by proteasomal regulatory complex subunit PSMD8. Ubiquitinated isoforms of AQN1 were also detected in boar sperm extracts. The UCHL inhibitor ubiquitin aldehyde and the antibodies against UCHL3 or PSMD8 increased the rate of sperm-ZP penetration and polyspermy during *porcine in vitro* fertilization (IVF). In contrast, the addition of recombinant UCHL3 to fertilization medium significantly reduced polyspermy rates, while maintaining satisfactory rate of

monospermic fertilization (~50%). These results are significant for production agriculture. The high level of polyspermy that hinders porcine IVF for commercial embryo transfer could be mitigated by the modulation of the UCHL3 and/or PSMD8 activity.

This work was supported by grant No. P503/12/1834 and No. P502/14/05547S of the Grant Agency of the Czech Republic, and by the project BIOCEV CZ.1.05/1.1.00/02.0109 from the ERDF, and by National Research Initiative Competitive Grant no. 2011-67015-20025 from the USDA National Institute of Food and Agriculture, and by the Food for the 21st Century Program of the University of Missouri-Columbia.

ENDOCRINE DISRUPTORS INDUCE TRANSGENERATIONAL ALTERATIONS OF THE MALE REPRODUCTIVE PARAMETERS AND miRNA EXPRESSION PROFILES IN MOUSE PRIMORDIAL GERM CELLS

[1] **Děd L**, [2] Brieño-Enríquez M A, [2] García-López J, [2] Cárdenas D. B, [3] Guibert S, [2] Hourcade JdD, [1] **Pěknicová J,** [2] Weber M, [2] del Mazo J

[1]Laboratory of Reproductive Biology, Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic
[2]Centro de Investigaciones Biológicas, C.I.B. (CSIC), Madrid, Spain
[3]University of Strasbourg/CNRS, Illkirch cedex, France

Primordial germ cells (PGCs) are the embryonic precursors of the germ cell linage, which are restricted to form only sperm and oocytes following their specification from pluripotent cells. PGC precursors are specified in the epiblast around 6.25 days post coitum (dpc), and around 7.25 dpc become identifiable in a 40 cell-cluster. Thereafter, PGCs migrates through hindgut endoderm and colonize the genital ridges at day 10.5. PGCs specification depends on interactions among various molecular factors including different microRNA and microRNA-regulated molecules.

In the present study, we used a mouse model to evaluate the trans-generational (F1-F3) effects of vinclozolin (VCZ) administrated in two doses (1mg/kg/bw/day and 100mg/kg/bw/day) on male reproductive parameters. We observed decreased fertility rate, higher apoptotic rate and histopathologic alterations in adult testis, PGC number reduction, increments of PGCs apoptosis and changes in PGCs gene expression among all three generations.

In the attempt to clarify the trans-generational transmition of the altered phenotypes, we performed the microRNA expression and DNA methylation analysis. We observed the significant alteration in the expression of multiple microRNA and microRNA-regulated genes which are important for PGCs specification, including LIN28, *let-7* and BLIMP1. In the absence of microRNA-binding protein LIN28, microRNA *let-7* binds to the 3´UTR of the *Blimp1* mRNA to block its translation and prevent PGCs from developing. LIN28 binds to the *let-7* family pri-miRNA loop to prevent processing of these precursor forms into the mature *let-7* miRNA, allowing BLIMP1 translation, and permitting PGC

specification. Therefore, trans-generational deregulation in the expression of factors involved in the Lin28-let-7-Blimp1 pathway can lead to specific VCZ-induced phenotype observed in our study. The possible mechanisms responsible for the trans-generational transmission of altered microRNA expression patterns can involve paramutations and/or altered DNA methylation. The analysis of DNA methylation patterns in PGCs from control and experimental groups is now in progress.

This work was supported by grant No. P503/12/1834 of the Grant Agency of the Czech Republic, and by project AVOZ 50520701 awarded by the Academy of Sciences of the Czech Republic and by BIOCEV project CZ.1.05/1.1.00/02.0109 from the ERDF.

THE EFFECT OF TETRABROMOBISPHENOL A ON PROTAMINATION AND DNA QUALITY OF MOUSE SPERM

[1] Žatecká E, [2] Castillo J, [1] Elzeinová F, [1] Kubátová A, [1] **Děd** L, [1] **Pěknicová** J, [2] Oliva R

[1]Laboratory of Reproductive Biology, Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic
[2]Human Genetics Research Group, IDIBAPS, Faculty of Medicine, University of Barcelona, Barcelona, Spain

Tetrabromobisphenol (TBBPA) is a widely used brominated flame retardant, currently its consumption is 210,000 tons / year and is still growing. In our previous multigenerational *in vivo* study we have demonstrated that TBBPA is able to induce apoptosis of testicular cells and changes in the expression of genes important for proper spermatogenesis. However the potential effect of TBBPA on epididymal spermatozoa had not yet been investigated. Therefore, we performed further study to evaluate the effect of on sperm DNA integrity and on the protamines as the major nuclear proteins. C57Bl/6J mice pups (n=10) were exposed to TBBPA (experimental group) during the gestation, lactation, pre-pubertal and pubertal periods up to the age of 70 days and compared control mice pups (n= 10) which were not exposed. Our results demonstrate that TBBPA treatment results in a significantly decreased P1/P2 ratio, increased total protamine/DNA ratio and increased DNA fragmentation observed between TBBPA and control mice, respectively. Protamines have recently been connected to the epigenetic marking of sperm chromatin in human and mouse spermatozoa. Thus, our findings suggest that TBBPA exposure, in addition to result in increased sperm DNA damage, may also alter the epigenetic marking of sperm chromatin.

This work was supported by grant No. P503/12/1834 of the Grant Agency of the Czech Republic, and by project AVOZ 50520701 awarded by the Academy of Sciences of the Czech Republic and by BIOCEV project CZ.1.05/1.1.00/02.0109 from the ERDF.

EFFECT OF EXPOSURE TO BISPHENOL A ON GENE EXPRESSION IN THE TESTICULAR TISSUE IN MALE MICE

Dorosh A, **Elzeinová F, Žatecká E, Kubátová A, Pěknicová J**

Laboratory of Reproductive Biology, Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic

Bisphenol A (BPA) is a synthetic, endocrine-disrupting compound able to directly bind estrogen receptors. Free BPA has been detected in human samples indicating that humans are internally exposed to BPA.

The purpose of this study was to analyse the effect of BPA on the male reproductive system and testicular gene expression in germ cells. We studied the influence of long-term low concentration BPA exposition on male fertility *in vivo* in a two-generation study in C57BL/6 mouse strain. In this work, BPA was added with water at two environmentally relevant concentrations: 0, 4 and 4 μg/l. We measured the reproductive organs weight and sperm cells morphology and quality. Expression of genes involved in endocrine regulation and energy metabolism in testis was analysed after BPA exposure.

Next, the epigenetic mechanisms of gene expression and regulation during the germ cell differentiation and effect of BPA will be analysed.

This work was supported by grant No. P503/12/1834 of the Grant Agency of the Czech Republic, and by project AVOZ 50520701 awarded by the Academy of Sciences of the Czech Republic and by BIOCEV project CZ.1.05/1.1.00/02.0109 from the ERDF.

DETECTION OF MANNOSIDASE IN THE PORCINE UROGENITAL TRACT – STUDY OF THE SPERM RELEASING FROM OVIDUCTAL RESERVOIR

[1] **Maňásková**-Postlerová P, [2] Ren Š, [1] Jelínková J, [3] **Krejčová T,** [2] Liberda J

[1]Laboratory of Reproductive Biology, Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic
[2]Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic
[3]Department of Veterinary Sciences, University of Life Sciences in Prague, Prague, Czech Republic

One of the most important steps of reproduction process is the meeting of sperm with oocyte. Binding of sperm with oviductal cells maintains spermatozoa in fertile state. The beginning of sperm capacitation is associated with oocyte ovulation resulting in the sperm release from oviductal reservoir. Hormonal changes after ovulation probably induce distinct oviductal secretion leading to disruption of the sperm protein binding with oviductal saccharide moieties. Another eventuality of the sperm releasing from oviductal reservoir is a change of oviductal environment caused by components of follicular fluid transported with oocyte after ovulation. In the pig, previous studies indicate lectin-type interaction of sperm protein receptors by mannose structures on the surface of oviductal cells. Our study was focused on enzymatic activity of mannosidase and its detection in porcine oviduct (fluid and tissue) and follicular fluid during hormonal cycle.

In fluid from follicles in early and late hormonal stages, we measured mannosidase activity by colorimetric methods at physiological and acidic pH. Expression of secreted mannosidase was studied by specific antibody in follicular and oviductal fluids, and oviductal tissues during hormonal cycle. Clearly increased enzymatic activity of secreted mannosidase was found as specific-species in porcine fluid from follicles in late stage of hormonal cycle. On the other hand, detection of secreted mannosidase in follicular fluid as well as in oviductal fluid did not shown any significant differences during hormonal cycle. In oviductal isthmic tissue, we detected decreased protein expression of secreted mannosidase at middle and late follicular phases. These results suggest possible role of follicular mannosidase rather than oviductal one in the sperm releasing from oviductal

reservoir in the pig. The additional study of the gene expression of secreted form of mannosidase in oviductal tissue during hormonal cycle should be necessary.

This work was supported by GACR P503/12/1834, GACR P502/14/05547S, UNCE204025/2012, RVO61388971, NAZV QI101A166, CIGA CZU20142049 and by BIOCEV CZ.1.05/1.1.00/02.0109 from the ERDF.

ENZYMATIC AND INHIBITING ACTIVITY IN BOAR EPIDIDYMAL FLUID

[1] Davidová-Cozlová N, [2] Ren Š, [2] Liberda J, [1] Jonáková V, [1] **Maňásková**-Postlerová P

[1]Laboratory of Reproductive Biology, Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic
[2]Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic

Sperm maturation, taking place during the transit of spermatozoa through the epididymis, represents a key step in the reproduction process. Spermatozoa, particularly the plasma membrane, are exposed to epididymal fluid components representing the natural environment essential for their post-testicular maturation. Changes in the sperm membrane proteins are influenced by proteolytic and glycosidic enzymes present in the epididymal fluid. Accordingly, the occurrence of inhibitors in this reproductive organ is very important for the regulation of sperm membrane protein processing. In present study, we monitored protease and glycosidase activities, and inhibitors of metallo- and serine proteinases in boar epididymal fluid. Additionally, we studied acrosin inhibitor in fluid, spermatozoa and tissue along the epididymis.

We chromatographically separated boar epididymal fluid into several fractions. These fractions were subjected to SDS-electrophoresis and the separated proteins were either studied by zymographic methods or transferred to nitrocellulose membranes for detection of metallo- and serine proteinases and their inhibitors, and acrosin inhibitor by specific antibody, respectively. Acrosin inhibitor was monitored also in the sperm and tissue of the boar epididymis.

In boar epididymal fluid, several metallo- and serine proteinases with different molecular masses, and inhibitors of metalloproteinase MMP-9 and acrosin were found. We measured strong activity of mannosidase in this fluid. Using specific antibody, we registered the increasing signal of acrosin inhibitor from caput to cauda epididymis in the spermatozoa, fluid and also tissue.

Proteinases and their inhibitors in reproductive fluids may play a significant role in reproduction processes. Especially, acrosin inhibitor in the reproductive tract inactivates

prematurely released sperm acrosin and protects spermatozoa and reproductive epithelium against proteolytic degradation. High mannosidase activity in boar epididymal fluid suggests evident role of mannose structures in the sperm interaction during reproductive events.

This work was supported by GACR P503/12/1834 and P502/14/05547S, by Charles University in Prague UNCE204025/2012, and by BIOCEV CZ.1.05/1.1.00/02.0109 from the ERDF.

EXPRESSION **OF ESTROGEN RECEPTOR BETA (ERβ)** IN MURINE MALE REPRODUCTIVE TRACT AND SPERM

Dostálová P, **Děd L, Dorosh A, Elzeinová F, Pěknicová J**

Laboratory of Reproductive Biology, Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic

Estrogens are steroid hormones that play an important role in reproduction of both sexes. In male, the main source of estrogens are testes where both somatic and germ cells are responsible for testosteron conversion to estrogens. Estrogens are involved in control of spermatogenesis, fluid reabsorption in *rete testis* and epididymis, and in later maturation steps that sperm undergo in female genital tract (capacitation, acrosome reaction). Generally, estrogen action is mediated through binding to estrogen receptors (ERs) which than lead to classical genomic or rapid non-genomic signaling. Nowadays, two classical estrogen receptors are known – ERα and ERβ. ERβ is a predominant variant in testes, while ERα is more abundant in *rete testis* and initial segment of epididymis. In addition to classical ERs, several splice variants that can differ in their ligand- or DNA-binding properties were detected in different tissues and cell lines. ERs mostly work as a dimer (homo- and hetero-) and splice variants often „only" modulate function of classical full-length ERs. Therefore, estrogen action seems to be a very complex. To contribute to understanding of estrogen action in male, we detected ERβ and its potential splice variants in mice testis, epididymis and sperm. According to our results, two variants are present in all analysed tissues and cells. These variants differ in one exon in ligand binding domain which leads to different affinity for estrogens. To analyse these variants also at a protein level, we prepared specific monoclonal antibodies recognizing particular variant of ERβ. Both atibodies detected band(s) in protein extracts from testes or epididymis.

Taking together, there are at least two variants of ERβ in mice testes, epididymis and sperm and it seems that both variants are similar in abundance within the same organ or sperm.

This work was supported by the Grant Agency of Charles University No. 1510-243-253149 and by BIOCEV project from the ERDF (CZ.1.05/1.1.00/02.0109) and by the Grant Agency of the Czech Republic 14-055 47S.

PANEL OF MONOCLONAL ANTIBODIES – ALTERNATIVE TOOL FOR MONITORING OF SPERM–ZONA PELLUCIDA RECEPTORS LOCALIZATION AND IDENTIFICATION

[1,2] Zigo M, [1] Dorosh A, [1,2] Pohlová A, [1] Jonáková V, [2,3] Šulc M, [1] **Maňásková**-Postlerová P

[1]Laboratory of Reproductive Biology, Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic
[2]Department of Biochemistry, Faculty of Science, Charles University, Prague, Czech Republic
[3]Institute of Microbiology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic

Primary binding of the sperm to the *zona pellucida* (ZP) is one of the many steps necessary for successful fertilization in all sexually reproducing species. Sperm bind ZP by means of membrane receptors which recognize carbohydrate moieties on ZP glycoproteins according to a well-precised sequential process. Primary-binding receptors are localized throughout the acrosomal region of the sperm surface of which many have been disclosed in various mammals. For the monitoring sperm-zona pellucida receptors in terms of localization and characterization - panel of monoclonal antibodies against proteins from the sperm surface was prepared. Antibodies were screened by immunofluorescence and Western blotting for protein localizations and competence of antibodies, respectively. Antibodies recognizing proteins localized on the sperm head and simultaneously detected by Western blot were further studied by means of immunolocalization in reproductive tissues and fluids, binding to ZP, immunoprecipitation and protein identification using MS analysis. Out of 17 prepared antibodies, 8 antibodies were simultaneously recognizing proteins localized on the sperm head and detecting proteins of interest by Western blotting. Further only 3 antibodies recognized proteins which also coincided in binding to ZP. These 3 antibodies were used for immunoprecipitation, and further protein identification of immunoprecipitates revealed that the antibodies distinguish acrosin precursor, RAB2A protein, and lactadherin P47. Acrosin and lactadherin P47 have been already detected on the sperm surface and their

physiological functions in reproduction have been proposed. To our knowledge, this is the first time RAB2A has been found on the surface of sperm and its physiological function in the process of fertilization remains undisclosed.

This work was supported by GACR P503/12/1834, P502/14/05547S, UNCE204025/2012, RVO61388971, and by BIOCEV CZ.1.05/1.1.00/02.0109 from the ERDF.

SIGNIFICANCE OF SCREENING TESTS IN SYSTEMIC LUPUS ERYTHEMATOSUS

[1,2] **Ulčová**-Gallová Z, [3] Mocková A, [1] Bibková K, [1] **Mičanová Z**

[1]Department of Gynecology and Obstetrics, Charles University and Faculty Hospital, Pilsen, Czech Republic
[2]Genetica-Pilsen, Czech Republic
[3]Department of Neonatology, Charles University and Faculty Hospital, Pilsen, Czech Republic

Objective: Women with system lupus erythematosus (SLE) have often a problem in their reproduction (anovulation, or early spontaneous miscarriage or intrauterine death of foetus). The present work was undertaken to investigate the occurence of autoantibodies to *zona pellucida*, to eight various phospholipids and of iso-antibodies to sperm cells in 52 patients with SLE remission and planning of their reproduction.

Methods: We used spermagglutinating tests for detection of sperm antibodies and classical ELISA methods for detection of *zona pellucida* and eight selected antiphospholipid antibodies.

Results: We found in our patients IgG positive predominance against ph-inositol, ph-ethanolamine, ph-serine, cardiolipin and beta2-glycoprotein, less of *zona pellucida* antibodies in IgG, and only in few women cervical and serum sperm antibodies.

Conclusion: 15 new pregnancies were achieved but only eleven were successful.

THE ROLE OF VITAMIN D IN THE REPRODUCTIVE SYSTEM

Tibenská E, Girovská B

Medirex a.s., Bratislava

Vitamin D is primarily known for its participation in the field of bone metabolism. Maybe a little less known but no less important is its participation in the field of reproductive system of men as well as of women. It has also a significant impact on the immune system.

In men it was observed, that it has a possitive effect on increasing the level of testosterone. Vitamin D has, simultaneously, also an important role in spermatogenesis and sperm maturation, i.e. it has a positive effect on the semen quality (observed effect on the sperm motility and also on the induction of acrosome reaction in already mature sperm).

In terms of its role in the reproductive system of women it is important to mention the fact, that vitamin D is involved in regulating the expression of a large number of genes in reproductive tissues, it helps the ovaries to increase the production of progesterone and estrogen and it also helps the placenta during pregnancy. Patients with the deficit of vitamin D were compared to a control group of healthy women and they were observed to have decreased fertility, worse results at IVF, and higher risk of complications such as preeclampsia or preterm labor during the pregnancy.

At the same time, vitamin D is considered to be a potent immunomodulator. Its receptor (called VDR) was found on the surface of most immunocompetent cells. Several studies have proven its positive impact on reducing the incidence or suppressing some autoimmune diseases symptoms.

It has also been described its immunosuppressive effect through the suppression of TNF-α indicated proinflammatory „tuning“ of the immunity and an effect to reduce the activity of NK cells, which may be important in some patients with recurrent spontaneous abortions.

This group of patients has been involved in a quite interesting study with the use of vitamin D3 therapy and its analogs. Although the mechanism of its action has not yet been explored into details, it is assumed that it has the ability to reduce the production of Th1 cytokines (IFN-ϒ, IL-2, IL-1, IL-6. IL-8) together with the ability to increase the production of so-called TH2 cytokines by T-cells.

Recent years have repeatedly confirmed that a relatively significant part of the world´s population suffers from a deficiency of vitamin D. In this regard, many experts talk about a pandemic of its deficiency, while it does not appear only in high-risk groups (the elderly, etc.) but also in people of the middle and the younger age.

In a study performed in our laboratory, with nearly 13,000 of patients examined in the period from december 2010 to august 2011, only 22% had a concentration higher than 30μg/l (the lower limit of currently accepted standard of vitamin D). The literature reffers, that the percentage of deficient patients in couples with reproductive problems can be even higher, what we have proved in a yet small group of patients.

There is still a lot of questions remaining in terms of the adequate recommended dose when supplementation, since a dietary intake is minimal and the synthesis takes place in our country only in the spring and summer months between 10 A.M. and 3 P.M. and also only without using sunscreen products with UV factors (those dramatically reduce its synhesis). There was performed a study on pregnant women in the second and third trimester carried out in the USA, resulting in the recommendation of increasing the existing recommended dose in pregnancy from 600-800 IU/day up to 4000 IU/day, as only in this group of women was achieved a sufficient level of vitamin D in the blood. In this group was also observed lower risk of complications such as preterm labor and preeclampsia, and yet a lower incidence of infections.

MICROBIOME AND MUCOSAL BARRIER FUNCTION IN PATHOGENESIS OF INFLAMMATORY DISEASES

Tlaskalová-Hogenová H, Kokešová A, **Coufal Š, Hansen C, Štěpánková R.,** Hudcovic T, Klimešová K, Kverka M, Zákostelská Z, **Hrnčíř T, Kozáková H,** Schwarzer M, Rossmann P

Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

The human fetus lives in a germ-free intrauterine environment and enters the outside world containing microorganisms. After delivery, full-term vaginally born infants are completely colonized with a diverse array of bacteria (microbiota) which is necessary for the development of appropriate innate and adaptive immune responses. Bacterial colonization is influenced by the effect of breast milk oligosaccharides, immune factors and bacteria present in maternal milk. Colonizing bacteria communicating with the gut epithelium and underlying lymphoid tissues result in a functional immune phenotype. Barrier formed by intestinal epithelium separates intestinal microbiota from underlying tissues, preventing bacterial infiltration and subsequent inflammation. Barrier integrity can be compromised, due to uncontrolled death of enterocytes. Disruption of normal colonization process can lead to aberant mucosal immune responses and to alteration in the symbiotic relationship necessary for immune homeostasis. Most of complex immune mediated diseases occur as result of disturbances of mucosal barrier function and mechanisms regulating immune responses. By exerting pathological or beneficial effects microbiota could participate in pathogenesis of various intestinal and systemic diseases.
Inadequate intestinal colonization with premature delivery, delivery by Cesarean section and excessive use of perinatal antibiotics results in the absence of adequate bacterial-epithelial crosstalk and an increased incidence of immune-mediated diseases (e.g. necrotizing enterocolitis - NEC, allergy). Necrotizing enterocolitis, an intestinal inflammatory disease of premature infants, is caused, in part by an excessive inflammatory response to initial bacterial colonization due to the immaturity of innate immune system. We studied the possibility to use biomarkers of intestinal mucosal cell

damage as diagnostic tool for distinguishing early NEC from sepsis. Our results suggest that intestinal fatty acid binding protein (i-FABP) can distinguish early stadium of NEC from sepsis. Infants with inadequate intestinal colonization can be restored to a bacterial balance with the intake of probiotics.

Supported by projects of the Ministry of Health of the Czech Republic (NT13483-4/2012), the Czech Science Foundation (P303/12/0535 and P304/11/1252), and by the Institutional Research Concept of Institute of Microbiology AS CR (RVO: 61388971).

References:

Tlaskalová-Hogenová H. et al. Gut microbiota (commensal bacteria) and mucosal barrier in pathogenesis of inflammatory, autoimmune diseases and cancer: Contribution of germ-free and gnotobiotic models of human diseases. Cellular and Molecular Immunology 8, 110-120, 2011

Kverka M., Tlaskalova-Hogenova H. Two faces of microbiota in inflammatory and autoimmune diseases: triggers and drugs. APMIS 121, 403-21, 2013

Saturday, MAY 24, 2014

FLOW CYTOMETRY (FCM) SPERM ASSESSMENT IN NORMOZOOSPERMIC AND ASTHENOZOOSPERMIC MEN USING MONOCLONAL ANTIBODIES AGAINST SPERM PROTEINS

[1] **Čapková J**, [1] Kubátová A, [1] **Děd L,** [2] Teplá O, [1] **Pěknicová J**

[1]Laboratory of Reproductive Biology, Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic
[2]Clinic Center IVF ISCARE, Prague, Czech Republic

Recent studies have shown that infertility affects an estimated 15% of all couples. Male infertility is the primary or contributing cause in 60% of these cases. Consequently, application of methods of assisted reproduction is increasing. These methods would benefit from extended evaluation of the sperm quality. For this purpose, we analyzed sperm proteins in men with normal spermiograms and with asthenozoospermia. Ejaculates of both groups were tested with a set of well-characterized monoclonal antibodies (MoAbs) to human sperm.

No statistically significant differences were found between normospermics and asthenospermics in the expression of sperm surface proteins clusterin, evaluated by Hs-3 MoAb, and semenogelin, evaluated by Hs-9 MoAb.

On the other hand, flow cytometry revealed quantitative differences between normozoospermic and asthenozoospermic men in GAPDHS (glyceraldehyde phosphate dehydrogenase human sperm-specific glycolytic enzyme), evaluated by Hs-8 MoAb, VCP (valosin-containing protein), detected with Hs-14 MoAb, and PRKAR2A (cAMP-dependent protein kinase type II – alpha regulatory subunit) detected by MoAb Hs-36.

Asthenozoospermic men displayed significantly reduced expression of intra-acrosomal proteins with a likely decrease in sperm quality, and thus a negative impact on successful reproduction.

This work was supported by grant No. P503/12/1834 of the Grant Agency of the Czech Republic, and by project AVOZ 50520701 awarded by the Academy of Sciences of the Czech Republic and by BIOCEV project CZ.1.05/1.1.00/02.0109 from the ERDF.

STUDY OF IMMUNOLOGICAL PROPERTIES OF SPERM AND SEMINAL PLASMA ANTIGENS

[1] Brázdová A, [1] Zídková J, [1] **Melčová M,** [2] Senechal H, [2] Poncet P, [3] **Ulčová**-Gallová Z, [2] Peltre G

[1]Department of Biochemistry and Microbiology, Institute of Chemical Technology Prague, Czech Republic
[2]Service de Biochimie et de Biologie Moléculaire Equipe "Allergie & Environnement", Hôpital d'Enfants Armand Trousseau, Paris, France
[3]Counseling and Laboratory for Reproductive Immunology, Genetics Pilsen, Czech Republic.

Infertility affects approximately 10% of couples at reproductive age. Active immune mechanism in the female reproductive tract may produce high levels of anti-seminal/sperm antibodies. It seems that iso-immunization is also associated with infertility.

We focuse on the identification of sperm and seminal fluid proteins to illustrate in detail the IgG immune responses of infertile women. The biochemical characterization was performed by two-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis followed by immunoblotting analyses. The IgG-binding patterns of interest were identified by mass spectrometry.

The dominant sperm as well as seminal fluid antigens were detected within the range of 25 - 52 kDa and pI 4.5 - 9. The group of heat shock proteins was successfully determined as major sperm antigen. The association of the above mentioned proteins and other has been proved with female immune infertility and is under the study.

The aim of our study consisted also in the profiling of specific serum immunoglobulin classes and subclasses of infertile women. We focused on the distribution of serum seminal/sperm-specific antibodies in order to find those apparently related to iso-immunization.

Based on the poorly detectable levels of semen specific IgE, M, A1,2, G3, the markers of pathologic female iso-immunization appear to be the serum IgG1 and IgG4. Early determination of serum seminal/sperm-specific IgG subclasses might make patient

profiling more precise. Anti-seminal/sperm IgG1,4 could be of interest for further immunotherapy.

This work was supported by the Grant Agency of the Czech Republic (GACR 13-04580S) and by a specific university research grant (MSMT No 20/2014).

SEROLOGICAL IMMUNOBLOTTING DIAGNOSTIC OF CHLAMYDIAL INFECTION IN INFERTILE MEN AND ITS ASSOCIATION WITH SELECTED IMMUNOLOGICAL PARAMETERS (PILOT STUDY)

[1] Bobák L, [1] Váczy Z, [2] Halagovec A, [1] Rosocha J, [1] Zachar L, [3] Hulíková M

1 Laboratory for infertility diagnostic and sperm bank of Associated tissue bank UNLP a LF UPJŠ, Košice, Slovak Republic
2 Clinic of dermatovenerology UNLP, Košice, Slovak Republic
3 Clinic of heamostasis and thrombosis, Košice, Slovak Republic

Chlamydia trachomatis (C. trachomatis) is the most frequently reported sexually transmitted infection in EU/EEA countries with nearly 340,000 reported cases in 2010. Over the last 10 years reported incidence rate more than doubled, partly as a result of measures taken by Member states to improve diagnosis and reporting infections, including active case finding (Annual epidemiological report 2012 of ECDC - European Centre for Disease prevention and Control). Prevalence of *C. trachomatis* in the European population is four times more common than gonorrhea and thirty times more common than syphilis (Malik et al., 2006) and is thus the most common sexually transmitted agent, predominantly asymptomatic. There is no established surveillance of the infection in Slovakia which with unbalanced diagnostics and high contagiosity of *C. trachomatis* creates a presumption of its high prevalence in the population of people not only with fertility issues.

In this small pilot study, we examined 40 men with fertility issues, by using immunoblotting test CHLAMYCHECK IgG & IgA, for detection of *C. trachomatis*, *pneumoniae* and *psittaci* antibodies in the blood serum. These men were divided into following categories: 1. previous treatment/re-treatment for *C. trachomatis* (20 cases), 2. chronic and recurrent urogenital inflammation of undetermined pathogens (12 cases), 3. spontaneous abortions/missets of a female partner (6 cases), 4. assisted reproduction failure (5 cases), 5. lupus erythematosus + arthritis (1 case); there were also combined causes in some of the patients. From the results of the ejaculate examination, we focused on indicators for urogenital infections: PMNL >0,1.106/ml (25 cases), positive

MAR IgG/IgA (15 cases), DNA fragmentation (21 cases). The positivity for antibodies to *C. trachomatis* was determined in 5 cases in IgG class and 1 case in IgA class.

Our results confirm the manufacturer's instructions, that antibody assay is appropriate supplementary examination for the direct method. However, it is important to assess medical history, clinical and laboratory data of the patient/couple, because of potential indications for urogenital infections.

DIAGNOSTIC AND PROGNOSTIC VALUE OF PRESEPSIN IN PRETERM DELIVERIES

[1] **Malíčková K**, [2] Koucký M, [2] **Pařízek A,** [1] Pelinková K, [1] Brodská H, [2] Hájek Z, [2] Germanová A, [1] Zima T

[1]Institute of Medical Biochemistry and Laboratory Diagnostics, General University Hospital and 1st Faculty of Medicine of Charles University in Prague, Czech Republic
[2]Department of Gynecology and Obstetrics, General University Hospital and 1st Faculty of Medicine of Charles University in Prague, Czech Republic

Aim: To evaluate the association between serum presepsin (soluble CD14 antigen subtype, sCD14-ST) levels early after the signs of preterm delivery appearing and preterm delivery within 48 hours, before 34th and 37th gestational weeks; the comparison of presepsin and established markers of inflammation severity; and possible additional value of concurrently evaluated ultrasound vaginal cervicometry with serum presepsin measurement.

Patients and methods: A total amount of 60 females were included. Serum presepsin was measured by chemiluminiscent immunoassay Pathfast Presepsin (Mitsubishi Chemical, Japan). Sonographic evaluation of cervical length in all females was conducted by transvaginal ultrasound.

Results: Three quarters of examined females delivered prematurely. There were not age differences between cohorts with and without preterm delivery. Patients who have delivered until 48 hours after the analyses have shown significantly higher presepsin concentrations compared to females with later deliveries. Higher presepsin was proven also for deliveries before the week 34 compared to post-week 34 as well as for before/after the week 37. The optimal cutoff point for the preterm delivery was established at the concentration of presepsin 623.5 pg/mL. Combined finding of cervical length shortening below 18 mm and presepsin level increasing above 454 pg/mL could point out to the significantly high risk of preterm delivery.

Conclusion: Development of strategies for risk stratification and prediction of morbidity in preterm deliveries include identification of simple, rapid, and safe markers of

inflammation in women that are at increased risk for preterm birth. This study provided new information, suggesting that elevated maternal serum concentrations of sCD14-ST could be such independent and relevant risk factor for preterm delivery.

Supported by projects PRVOUK P25/LF1/2 (2012–2016) of Charles University in Prague and RVO VFN 64165 of Ministry of Health.

THE IMPACT OF THYROID DISORDERS ON FERTILITY AND IMPACT OF ASSISTED REPRODUCTION ON THE THYROID GLAND

[1] Nováková D, [1] **Křenek M,** [1] Vošmiková K, [1] Sýkorová P, [2] Límanová Z, [1] **Vlček P**

[1]Department of Nuclear Medicine and Endocrinology, 2nd Faculty of Medicine Charles University Prague and University Hospital Motol, Prague, Czech Republic
[2]Third Department of Medicine, General University Hospital and First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

Thyroid disease is one of the most common endocrine pathological conditions affecting women during reproductive age. Hypothyroidism is ten times more common then hyperthyroidism. Overt hypothyroidism has been proposed as a possible cause for several etiologies of infertility, including: impaired ovulation, fertilization, and implantation and also risk factor for miscarriage and late pregnancy complications. Abnormal isolated elevation of serum TSH, subclinical hypothyroidism, is also known to be a risk factor for ovulatory disturbances. According to literature data upper limit of serum TSH level reference range for healthy fertile women is 2.5 mIU/L. The pregnancy rate in women undergoing assisted reproduction (ART), including *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) appears to be significantly lower in those with positive organ-specific antithyroid autoantibodies.

Women undergoing ART with gonadotropins may have increased Estrogen levels witch may result in alteration of TSH (Thyrotropin), T3 (triodthyronine) and T4 (tetraiodthyronine).

Thyroid cancer is the most common endocrine malignancy. Papillary thyroid carcinoma is the common form of thyroid carcinoma. In Czech Republic thyroid cancer was found to affect women more often than men by a ratio of 4 to 1. At the Department of Nuclear Medicine we noticed in recent years 5556 microcarcinomas cases of thyroid gland. Of this number 79,4 % were women. Some of our patients undergoing ART.

Greater exposure of estrogen may be a risk factor for thyroid cancer. Links between estrogen and thyroid cancer have been reported. Estrogen receptors are present in

thyroid thyroid tissue and treatment with 17ß-estradiol can result in increase of both benign and malignant thyroid cells.

Supported by grant: IGA MZČR, NT 11455-5/2010.

PREVALENCE OF HPV INFECTION IN INFERTILE WOMEN AND OOCYTE DONORS

[1] Ondryášová H, [2,3,7] Oborná I, [1] Koudeláková V, [1] Vrbková J, [3] Sobek A, [2,4] **Březinová J,** [2,4] **Filipčíková R,** [5] Doležal J, [6] Novotný J, [1,2] Hajdúch M

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

Backround: Human Papilloma virus (HPV) infection is a cause of many cancers, especially the cervical cancer, but also a potential factor affecting human fertility. HPV infection could be transmitted from mother to fetus before or during parturition. It may also be associated with a higher risk of spontaneous abortion, though this has not yet been clearly established. In contrast to other sexual transmitted diseases, HPV test is not used in infertility because the association of HPV infection have not been published yet. HPV testing is also not used in oocyte donors in whom undiagnosed HPV infection may be a risk factor, too.

Material and methods: Cervical smears were obtained from 417 women (339 infertile women and 78 oocyte donors). These samples were analysed for presence of HPV16/18 and other hrHPV genotypes on Cobas 4800 system (Roche). All samples were then tested on PapilloCheck HPV- Screening system (Greiner bio-one). Results of these methods were compared. All study participants fulfilled a questionnaire focused especially on sexual life (number and age of sexual partners, STDs) and fertility (its duration, infertility in family, abortions, genital surgery, IVF treatments). Obtained data were statistically processed. All study participants had signed informed content to be

included in the study. The research was approved by the ethical committee of Faculty of Medicine and Dentistry, Palacky University Olomouc.

Results: Twenty eight of 78 (36%) oocyte donors were HPV positive. Of the HPV positive donors 25 (32%) were hrHPV positive and 6 (24%) had multiple HPV infections. The most frequent HPV genotypes found in oocyte donors were HPV16 (18%) and HPV39 (18%). HPV infection was detected in 73 of 339 (22%) women from infertile couples. In 7 (10%) cases only hrHPV infection was found. The most frequently detected hrHPV type was HPV42 (60%). Sixty six (19%) infertile women were hrHPV positive, in 11 (17%) cases multiple hrHPV infection was detected. The co-infection of HPV16 (55%) and/or HPV31 (45%) was the most frequently present. In infertile women HPV16 (26%), 51 (12%) and 31 (12%) were most frequently detected.

Conclusion: In a group of 417 asymptomatic women of whom 15 had a history of HPV associated disease, 24% were HPV positive. Oocyte donors were more frequently HPV positive than women from infertile couples. For the definitive confirmation or exclusion of the HPV infection impact on fertility a much larger group of women should be analyzed.

This work was supported by the grants IGA UP LF_2013_015 and Biomedreg CZ.1.05/2.1.00/01.0030.

DEVELOPMENTAL POTENTIAL OF BOVINE OOCYTES CULTIVATED *IN VITRO* IS AFFECTED BY MINERAL OIL EXPOSED TO UV LIGHT

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

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

Optimalization of conditions for IVF culture is an essential step required for physiological studies of gametes in all species. Mineral oil (MO) cover is used to prevent the culture medium from evaporating and maintains the appropriate pH and osmotic pressure during *in vitro* fertilization (IVF). On the other hand, the use of MO can damage oocytes and embryos due to possible toxic contamination. Peroxide contamination of laboratory grade MO was reported when exposed to heat, sunlight, UV light, extended storage. The sensitivity of embryos to *in vitro* culture stress differs in various species. Decontamination of an area for IVF manipulation is made by UV light, therefore the aim of this study was to analyse the effect of MO (USB-ultrapure, Cleveland) shortly exposed to UV light on fertilization ability and developmental potential of bovine oocytes cultivated *in vitro*. Mature bovine oocytes were divided into three experimental groups. Mineral oil has been submitted to UV light for 24 hours and used to overlay the fertilization medium droplet. As a control sample, not exposed MO (with the same lot number) has been applied and as a second control sample, IVF in a "big" (500µl) droplet without MO was tested. After 24 hours, we observed a significant decrease in the number of fertilized oocytes (12,5%) in comparison with control samples (96,3% or 91,5%, respectively) (Mann-Whitney, $P \leq 0{,}001$). Consistently, when the developmental status of fertilized bovine oocytes (48 hours after IVF) was evaluated, following results were obtained. In the samples where irradiated MO has been applied, only 10% of oocytes acquired the stage of pronucleus and 5% of oocytes were divided to two and more cells. On the contrary, in the group of oocytes fertilized in droplets overlaid with non-affected MO, 46,5% embryos with at least one pronucleus were detected and moreover, almost all were divided to two and more cells. Interestingly, in a sample of oocytes fertilized in droplets where MO was completely

omitted, all 36,6% embryos were arrested in pronucleus stage and further development did not proceed. Confirming previous results, we found out that the droplets overlay with mineral oil is essential for further development of the bovine embryos. Finally, we can conclude, 24h exposure of the MO to UV light has toxic influence on bovine gametes fertilization and following development.

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

EFFECT OF CARBON MONOXIDE ON PORCINE OOCYTES MEIOTIC MATURATION

[1] **Krejčová T**, [2] Petr J, [1] Weingartová I, [1] **Dvořáková M,** [1] Nevoral J, [1] **Vyskočilová A,** [1] **Němeček D,** [1] Sedmíková M

[1]Department of Veterinary Sciences, University of Life Sciences in Prague, Prague, Czech Republic
[2]Research Institute of Animal Production, Prague, Czech Republic

Fast development of biotechnologies in reproduction requires to increase the number of high - quality oocytes matured in *in vitro* conditions. Germinal vesicle breakdown happens during meiotic maturation and then oocytes enter into metaphase of the first meiotic division followed by anaphase and telophase of the first meiotic division, when the first polar body is formed and extruded. Then oocytes enter into metaphase of the second meiotic division, where the meiosis is blocked. Many signaling molecules participate in the regulation of the proccess of porcine oocytes maturation, and carbon monoxide (CO) appears to be one of them. Endogenous production of CO is mediated by the heme oxygenase (HO). There are two known functional isoenzymes of heme oxygenase HO – 1 and HO – 2. Both isoenzymes are localized in different parts of the female reproductive system.

The first aim of this study was to localize HO – 1 and HO – 2 in porcine oocyte and to evaluate their subcellular distribution during *in vitro* meiotic maturation. The second aim was to investigate the influence of carbon monoxide on porcine meiotic maturation. For this purpose, oocytes were exposed to heme oxygenase inhibitor Zn-protoporphyrin IX (Zn-PP IX) or carbon monoxide donor tricarbonyl-RuCl (TRICK-RuCl).

During meiotic maturation, both isoenzymes (HO – 1 and HO – 2) were localized in porcine oocytes in all evaluated areas, i. e. in the nucleus, cytoplasm and cortical area. By the evaluation of their redistribution, it was found that the HO – 1 does not change significantly its localization, however, for HO – 2, statistically significant decline of signal intensity was evaluated during meiotic maturation of porcine oocytes.

Inhibition of heme oxygenase had no effect on the meiotic maturation proccess, however elevated levels of carbon monoxide led to inhibition of the porcine oocytes meiotic maturation.

Preliminary results indicate that carbon monoxide is involved in the regulation of porcine oocytes meiotic maturation.

The project was supported by grants NAZV QI101A166, CIGA CZU20142049.

S-ALLYL CYSTEINE BUT NOT ALLIIN INFLUENCES MEIOTIC MATURATION OF PORCINE OOCYTES

[1] **Dvořáková M**, [1] Weingartová I, [1] Nevoral J, [1] **Vyskočilová A,** [1] **Krejčová T,** [1] Sedmíková M, [2] Petr J

[1]Department of Veterinary Sciences, Czech University of Life Sciences in Prague, Prague, Czech Republic
[2]Research Institute of Animal Production, Prague, Czech Republic

Garlic has long been considered a food with many health benefits. Sulfur compounds are responsible for the positive effects of garlic on organisms (Gorinstein et al., 2007). Alliin and S-allyl cysteine (SAC) are among the most important garlic sulfur compounds. These two substances are able to decrease oxidative stress in the cells, with SAC being a more efficient antioxidant (Borek, 2001; Banerjee et al., 2003).

Meiotic maturation is the development from an oocyte in the germinal vesicle stage to an impregnable egg. Changes in chromatin configuration and hyaluronic acid (HA) production by cumulus cells (immediately surrounding oocytes) occur during meiotic maturation (Nakayama et al., 1996). Cumulus expansion intensity is positively correlated to successful *in vitro* meiotic maturation (Qian et al., 2003). Meiotic maturation is impaired by enhanced concentrations of reactive oxygen species *in vitro* (Combelles et al., 2009). The results of this study may be useful in the field of human medicine due to the similarities between human and porcine oocytes.

The aim of this study was to reveal the effects of garlic sulfur compounds on porcine oocyte meiotic maturation.

Porcine cumulus-oocyte complexes (COCs) were aspirated from 3 - 5 mm follicles of prepubertal gilt ovaries. Oocytes were cultured for 24 hours in modified M199 at 39 °C in an air mixture of 5 % CO_2. One experimental group was cultured with alliin at concentrations of 0,05 mM and 0,1 mM. The other experimental group was cultured with SAC at concentrations of 0,1 mM and 1 mM. Oocyte meiotic maturation was evaluated under a phase contrast microscope after orcein staining. Samples for the evaluation of HA production were prepared from COCs and a cultivation medium. Samples were

subjected to digestion by lyase and evaluated using spectrofotometry (216 nm). The data were analyzed by ANOVA (t-test) at a p level of <0.05 (SAS 9.0).

The cultivation of oocytes with alliin had no effect on nuclear maturation; however, SAC (1mM) accelerated nuclear maturation. After 24 hours of cultivation, higher number of oocytes cultured with SAC reached metaphase II than did oocytes in the control group (3,25 ± 5,83 % for control group vs. 20,61 ± 15,43 % for SAC).

After 24 hours of cultivation, SAC (1 mM) enhanced production of HA (100 % for control group vs. 211 ± 48 % for SAC). SAC enhanced production of HA in COCs (25 ± 26 % for control group vs. 145,7 ± 59,9 % for SAC); however, this was not the case with the cultivation medium (75 ± 26 % for control group vs. 65,5 ± 50,2 % for SAC).

Alliin, which has no effect on nuclear maturation of porcine oocytes, has an antioxidative effect. Nevertheless, this effect is less than that of SAC. SAC accelerated nuclear maturation and enhanced HA production in cumulus-oocyte complexes. Whether the effects of SAC are mediated by its antioxidative action has yet to be revealed.

This work was supported by the National Agency of Agriculture Sciences (NAZV QI 101A166) and the Czech University of Life Sciences, Prague (CIGA 20142049).

References:

Banerjee, S. K., Mukherjee, P. K. and Maulik, S. K. (2003) Garlic as an antioxidant: The good, the bad and the ugly. Phytotherapy Research, 17, 97-106.

Borek, C. (2001) Antioxidant health effects of aged garlic extract. Journal of Nutrition, 131, 1010-1015.

Combelles, C. M. H., Gupta, S. and Agarwal, A. (2009) Could oxidative stress influence the in-vitro maturation of oocytes? Reproductive Biomedicine Online, 18, 864-880.

Gorinstein, S., Jastrzebski, Z., Namiesnik, J., Leontowicz, H., Leontowicz, M. and Trakhtenberg, S. (2007) The atherosclerotic heart disease and protecting properties of garlic: contemporary data. Molecular Nutrition & Food Research, 51, 1365-1381.

Nakayama, T., Inoue, M. and Sato, E. (1996) Effect of oocytectomy on glycosaminoglycan composition during cumulus expansion of porcine cumulus-oocyte complexes cultured in vitro. Biology of Reproduction, 55, 1299-1304.

Qian, Y., Shi, W. Q., Ding, J. T., Sha, J. H. and Fan, B. Q. (2003) Predictive value of the area of expanded cumulus mass on development of porcine oocytes matured and fertilized in vitro. Journal of Reproduction and Development, 49, 167-174.

HYDROGEN SULFIDE: A SIGNAL MOLECULE IN OOCYTE MATURATION AND CUMULUS EXPANSION

[1] Weingartová I, [1] **Dvořáková M,** [1] **Vyskočilová A,** [1] Nevoral J, [1] Sedmíková M, [1] **Krejčová T,** [1,2] Petr J

[1]Department of Veterinary Sciences, Czech University of Life Sciences Prague, Czech Republic;
[2] Research Institute of Animal Production, Prague, Czech Republic

Progress of reproductive biotechnologies depends on improved methodologies for successful meiotic maturation of oocytes *in vitro*. Oocyte maturation and acquisition of developmental competence are supported by surrounding cumulus cells and their expansion. Thus, the extent and quality of cumulus expansion may be a possible biomarker of oocyte quality. The cumulus expansion encompasses synthesis and accumulation of extracellular matrix rich in hyaluronic acid. These processes are regulated by a wide range of upstream signalling molecules. Hydrogen sulfide has been revealed as an essential signalling molecule in reproductive tract. We hypothesized that exogenous hydrogen sulfide influences meiotic maturation and cumulus expansion of porcine cumulus-oocyte complexes (COCs) and oocytectomized complexes (OOXs) after oocyte removal.

Porcine COCs was matured *in vitro* in modified M199 medium with 150-900μM $Na_2S.9H_2O$, the hydrogen sulfide donor. Concomitantly, OOXs were cultured under the same conditions. Proteolytic digestion of expanded cumuli and measurement of hyaluronic acid by Enzyme-linked ImmunoSorbent Assay (ELISA) were used for evaluation of cumulus expansion. Stage of oocyte maturation was evaluated after fixation inacetic-alcohol and orcein staining under a phase contrast microscope.

The hydrogen sulfide donor accelerated oocyte maturation. Concurrently, the hyaluronic acid production by expanded cumulus was inhibited. These differences were statistical significant ($P \leq 0.05$). The exogenous hydrogen sulfide donor had no effect on oocytectomized complexes. Our results suggest that hydrogen sulfide signalling is involved in regulation of meiotic maturation and cumulus expansion. Presumably, its

inhibitory effect on cumulus expansion is dependent on signalling cross-talk between oocyte and cumulus cells.

This work was supported by the National Agency of Agriculture Sciences (NAZV QI 101A166) and the Czech University of Life Sciences, Prague (CIGA 20142049).

DOCTOR, DO EVERYTHING.
ALTHOUGH I DO NOT KNOW WHAT I WANT.

[1,2] Malá E, [1] Urbanová I, [3] Jindra M

[1]Department of Clinical Immunology and Allergology, University Teaching Hospital Hradec Kralove, Czech Republic
[2]Department of Metabolism and Clinical Nutrition, University Teaching Hospital Hradec Kralove, Czech Republic
[3]Department of Rehabilitation, University Teaching Hospital Hradec Kralove, Czech Republic

Our case report of what we do know, what we can do, what we tried to improve, what we successfully treated, what methods we have and how we use it within multidomain knowledge and interdisciplinary access to the patient due to our medical skills.

And how everything turned out quite differently.

Although the fact we were able to almost a miracle ...

SOME ASPECTS OF FRUSTRATION

Landová V

Maternal Hope Foundation, Prague, Czech Republic

Frustration can occur in a number of different ways. It can occur when some person or object or state of affairs blocks the individual´s path to his goal. A man may be deprived of deserved promotion in his job by a superior who has a personal grudge against him. He may miss his favourite television show because the receiver has broken down. His holiday may be spoilt by bad weather. All these are examples of frustration arising from the individual´s environment.

Another very potent source of frustration is motivational conflict. The individual fails to satisfy a need because he is unable to choose between two or more courses of action which are open to him.

One kind of conflict occurs when one has to choose between two equally attractive goals such as desire to save money and the equally strong desire to buy new clothes.

Another type of conflict occurs in the presence of two equally repellent threats, when again we are caught in between. One may be afraid to ask one´s employer for an increase in salary, but also be equally afraid to go home and face the wrath of one´s wife for not doing so. This state of affairs also makes life difficult.

Not all people behave aggressively when they are frustrated. Some may become apathetic and inattentive and withdraw from the situation. The world of imagination is a comforting retreat from reality: it can be made as accommodating as we choose.

We like to believe we are always rational and under complete control of our actions, but this is obviously far from true.

These habitual, common reactions to frustration are called defence mechanisms.

The common deffence mechanisms are rationalization, projection, identification, reaction-formation, repression, and substitution.

Rationalization is the psychological process of explaining away situations in which we find ourselves in such a way as to justify our actions.

Projection is the response pattern by which we ascribe to others the undesirable qualities we ourselves have. A man who is disagreeable and unsociable may complain that other people are unfriendly and spiteful.

In *identification* we take the good qualities of others as our own and enjoy a vicarious feeling of pride and achievement. Adolescents resort to identifiaction in their effort to assert their independence from their parents.

Repression is a type of forgetting which is motivated by the unconscious need to deny the memory of any event, and the person who is repressing the past is not aware of his motives. Our tendency to forget our failures and bad times is an illustration of repression. Some kinds of amnesia, or the loss of memory and identity, are an extreme form of repression.

Substitution or sublimation is the means by which we express our anti-social impulses in an acceptable manner. Our desire to commit aggression will get us into trouble if we express it against the man in street.

Summary: Conflict and frustration are part of the normal course of life. The habitual reaction patters to these situations inlcude aggression, rationalization, projection, identification, dissociation, repression, and substitution. These defence mechanisms allow us to maintain our self-esteem.

MATERNAL HOPE FOUNDATION

Domorázková E

Maternal Hope Foundation, Prague, Czech Republic

Infertile couples and women who repeatedly miscarry are committed to the examination to immunobiological laboratory of the Institute of Care for Mother and Child in Prague-Podoli, it is 19 years now. The Maternal Hope Foundation cooperates with the laboratory to disseminate information on the need for precise, comprehensive diagnosis and modern methods of infertility treatment offered by reproductive immunology.

EFFECT OF DIABETES MELLITUS ON REPRODUCTIVE PARAMETERS IN MICE

Margaryan H, **Elzeinová F, Kubátová A, Strolená E, Pěknicová J**

CHANGES IN THE EXPRESSION OF SELECTED TESTICULAR GENES IN MICE

Valášková E, **Margaryan H, Žatecká E, Pěknicová J**

Laboratory of Reproductive Biology, Institute of Biotechnology, Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic

The decrease in population fertility has become a major concern in many developed countries. Recent studies show that infertility is affecting an estimated 15% of all couples (World Health Organization, WHO, 2010). Male infertility is the primary or contributing cause in 60% of cases. Male infertility is caused by a number of factors, such as genetic background, various environmental factors and disease. Diabetes mellitus (DM), a serious health problem on its own, is also suspected to be a contributing factor to male infertility.

The aim of this project was to analyze the cellular, molecular and genetic effects of diabetic environment on spermatogenesis and sperm quality and to determine the impact of DM on the *in vivo* reproduction, using the mouse model (*Mus musculus*) inbred FVB. Diabetes was induced using streptozotocin.

We used our knowledge and tools (unique monoclonal antibodies developed by our group) to determine the status of reproductive organs, anogenital distance, and the quality of sperms. Genetic analysis was performed by a quantitative Reverse Transcription Polymerase Chain Reaction (qPCR). We tested selected genes which are expressed in testicular tissue and thus can influence process of spermatogenesis and consequently the sperm quality.

Our preliminary data strongly suggest that DM impairs male fertility. We have found significant changes in the body and reproductive organ weight of mice with DM. We have identified qualitative and quantitative changes in the expression of proteins in epididymal

fluid and sperms. We have also detected an increased number of apoptotic cells in sperm of diabetic mice compared to the control group.

To our knowledge, there is no study assessing the correlation between DM and "unexplained infertility". In view of this, it is essential to analyze the effects of DM on male fertility, sperm quality, and reproduction parameters.

This work was supported by grant No. P503/12/1834 of the Grant Agency of the Czech Republic, and by project AVOZ 50520701 awarded by the Academy of Sciences of the Czech Republic and by BIOCEV project CZ.1.05/1.1.00/02.0109 from the ERDF.

MONITORING OF PERIPHERAL NK AND REGULATORY T CELLS CD MARKERS IN WOMEN WITH REPRODUCTIVE FAILURE

Dzurillová Ž, Dzurilla M

Imunoalergológia Dzurilla s.r.o. Nitra, Slovak Republic

Natural killer (NK) cells present in peripheral blood respond to pathogens or pathogen-infected cells including cytotoxicity and production of cytokines. Most peripheral NK cells express surface marker CD16 (immunoglobulin receptor) and have low expression of CD56 (adhesion molecule) as CD56dim. In contrast, around 1% of peripheral lymfocytes are CD16-CD56bright NKcells known as decidual NK cells. Elevated NK cells numbers in peripheral blood and/or their increased activity indicates generally upregulated immune reactivity. The number of peripheral NK cells is decreased in pregnant women compared with nonpregnant women.

Decidual NK (dNK) cells are considered to be poorly cytotoxic and possess functional lytic machinery negatively regulated in healthy pregnancy and reaching a peak during the first trimester. In humans most uNK cells are CD56bright, but lack CD16-.

Increased expression of iNKT CD8+ lymphocytes (> 60%) is predictive of IVF failure, while decreased expression (40%) is significantly predictive of pregnancy failure. Conditionally normal iNKT CD8+ expression is significantly predictive of complete reproductive success after IVF. Lower iNKT CD8+ expression is associated with elevated HLA-DR expression on peripheral NK cells.

CD4+CD25+FoxP3+ Treg lymphocytes in peripheral blood show to be another marker for reproductive failure. Regulatory T lymfocytes play essential roles in implantation and allogeneic pregnancy maintenance in humans. Seminal plasma plays an important role in the induction of paternal antigen-specific Treg cells. These cells reduce the expression of class II-MHC and CD86 on uterine dendritic uDC cells, thus inducing tolerogenic DCs in pregnant uterus.

Current laboratory testing algorithm in women with reproductive failure comprises sole CD16 and CD56 detection in peripheral NK cells. New data on NK cells phenotypes and

the role of Treg lymphocytes in the induction of paternal antigens tolerance casts a new light on potential of detecting further NK cells CD markers and peripheral Treg cells population. These tests might help in diagnosing reproductive immunological disorders in the near future.

XXth Symposium of Biology and Immunology of Reproduction
with International Participation
The Castle, Třešť, May 22 – 24, 2014

AUTHOR INDEX

SEZNAM PŘEDNÁŠEJÍCÍCH / INDEX strana / page

SPONSORS

Asco-med

Beckman Coulter

Bio-Rad

Exbio

Laboserv

Merck Sharp & Dohme

Mucos

Olympus

Schoeller Pharma

Sigma - Aldrich