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

GENETICS AND FACULTY OF MEDICINE UK, PLZEN

XXVth SYMPOSIUM OF IMMUNOLOGY AND BIOLOGY OF REPRODUCTION WITH INTERNATIONAL PARTICIPATION

in memory of Dr. Radslav and Thamar Kinsky

PROGRAM AND ABSTRACTS

Liblice Chateau, May 24 – 25, 2019

Published by: Institute of Biotechnology
The Czech Academy of Sciences, v. v. i, Vestec
Program: Pěknicová J., Ulčová-Gallová Z.
Arranged by: Kubátová A.
Number of pages: 45
Year of issue: 2019

XXVth Symposium of Immunology and Biology of Reproduction
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PROGRAM

Friday, MAY 24, 2019

9.30-10.30 **Arrival, coffee, accommodation of participants**

10.35-10.50 **OPENING CEREMONY:**
Ulčová-Gallová Z., Pěknicová J.

Chairpersons: Ulčová-Gallová Z., Jonák J.

10.50–11.20 **Chaouat G. (France):** Preeclampsia and immunology**.**
11.20-11.40 **Jankovičová J. (Slovakia):** Expression of cluster of differentiation molecules 9 and 81 in cow oocytes and embryos.
11.40-12.00 **Bartóková M. (Slovakia):** Study of CD63 tetraspanin on bull sperm.
12.00-12.20 **Nagyová E. (CZ):** Expression of inter-alpha-trypsin inhibitor molecules in ovarian follicles.
12.20-12.40 **Frolíková M. (CZ):** Capturing differences in behavior of importins during mouse spem acrosome reaction using Structural Illumination Microscopy (SIM).
12.40-13.00 **Děd L. (CZ):** New microscopic and picture analysis approaches to study the epigenetic states in sperm and testicular tissue.

13.00-14.30 **LUNCH**

Chairpersons: Hortová K., Antalíková J.

14.30-14.50 **Liška F. (CZ):** Expression profiles of testis elutriation fractions in centrobin transgenic rats.

14.50-15.10 **Postlerová P. (CZ):** Molecular mechanism involved in the formation of sperm oviductal reservoir.

15.10-15.30 **Svobodová J. (CZ):** Monitoring of protein network dynamics: The role of Fcrl proteins during sperm egg-membrane interaction.

15.30-15.50 **Tůmová L. (CZ):** Degradation of protein on boar sperm surface during capacitation regulated by ubiquitin-proteasome system.

15.50-16.30 Coffee break

Chairpersons: Pěknicová J., Madar J.

16.30-16.50 **Krejčířová R. (CZ):** Detection and localization of estrogen receptors in boar reproductive tissues and spermatozoa.

16.50-17.10 **Antalíková J. (Slovakia):** Estrogen receptors in bull spermatozoa.

17.10-17.30 **Ulčová-Gallová Z. (CZ):** NK cells in ovulatory cervical mucus are not found so often in comparison with NK cells in endometrium in patients with repeated pregnancy loss.

17.30-17.50 **Dzurillová Ž. (Slovakia):** Monitoring of peripheral T-regulatory lymphocytes dynamics in pregnant women with fertility disorders undergoing immunomodulatory therapies.

17.50-18.10 **Teplá O. (CZ):** Vitrification of oocytes: Correlation of IVF steps timing with the embryo quality.

19.00 DINNER (RAUT) – Restaurant and Terrace – Wine bar, 00.00

19.00-23.00 Band REGENT

Saturday, MAY 25, 2019

7.00-9.45 Breakfast

Chairpersons: Sedmíková M., Nagyová E.

10.00-10.20 **Cibulka J. (CZ):** Reccurent miscariages: Role of inherited trombophilia.
10.20-10.40 **Honzíková M. (CZ):** Systemic enzyme therapy as a support of postoperative healing in gynecology and obstetric.
10.40-11.00 **Malíčková K. (CZ):** Intralipids for treatment of recurrent implantation failure in women with NK cells pathologies.

Chairpersons: Jonáková V., Liška F.

11.00-11.20 **Kestlerová A. (CZ):** New methodological approaches for extension of prenatal diagnostics.
11.20-11.40 **Páleníková V. (Slovakia/CZ):** Differences in glycoconjugates of spermatozoa and seminal plasma of men with normal and pathological spermiograms.
11.40-12.00 **Krátká Z. (CZ):** Sperm selection strategy – which one is the best for semen samples with high concentration of apoptotic cells?

12.00-12.20 CLOSING OF SYMPOSIUM

Pěknicová J., Ulčová-Gallová Z.

12.30-14.30 LUNCH

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ABSTRACTS

Friday, MAY 24, 2019

PREECLAMPSIA AND IMMUNOLOGY

Chaouat G.

U976 INSERM Hopital Saint Louis Pavillon Bazin, Paris, France

In this presentation (from the last Reunion PE workshop) I discuss a few assertions on preeclampsia, then turn on a (not fully testable) model where an embryonic defect in expression of embryo/ placental regulatory proteins results in complement activation, itself responsible for a down regulation of the T regs activity, resulting in a very early lack of complete down regulation of the preimplantation decidual inflammation, causing in the post implantation stage a low grade but chronic inflammatory state.

Keywords: pre-eclampsia - Embryo complement - Treg inflammation

EXPRESSION OF CLUSTER OF DIFFERENTIATION MOLECULES 9 AND 81 IN COW OOCYTES AND EMBRYOS

Jankovičová J., Sečová P., Horovská Ľ., Bartóková M., Antalíková J.

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

Mammalian fertilization is a very complex and challenging process comprising gamete development and their functional maturation in environment of male and female reproductive system leading to sperm-egg interaction and in case of their successful cooperation, a new individual emerges. The subjects of the current study were cluster of differentiation molecules 9 and 81 (CD9 and CD81), both members of tetraspanin superfamily. CD81 molecule shares 45% of CD9 amino acid sequence homology throughout the transmembrane domains but their involvement in tetraspanin web is probably different. In mice it was shown that both proteins are involved in fertilization process although their precise role remains to explore. The analysis was performed on bovine model to determine the localization/expression of CD9 and CD81 on cow oocytes of different maturation stages as well as on in vitro produced embryos using the indirect immunofluorescence assay. Both tetraspanins, CD9 and CD81 were detected on the plasma membrane of bovine oocytes during maturation and it was suggested that CD9 and CD81 molecules accumulate in perivitelline space of fertilized oocytes. Moreover, our observations suggested that CD9 could be involved in zona pellucida transzonal projections, communication channels between cumulus cells and oocyte. The achieved results could contribute to understanding of the processes that occur at gamete interaction in mammals.

This work was funded by grants VEGA-2/0037/16, APVV-15-0196 and bilateral projects SAS-CAS 15-05 and 18-17.

STUDY OF CD63 TETRASPANIN ON BULL SPERM

Bartóková M., Horovská Ľ., Sečová P., Antalíková J., Jankovičová J.

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

The importance of studying tetraspanins in bovine gametes results from the ability of these proteins to interact with cell receptors, change the structure of the cell membrane, affect cell motility and to mediate cell-cell interaction. All of these processes are important in the mammalian fertilization. One of the proteins of the tetraspanin family is the CD63 molecule. We detected this molecule on the bovine sperm-cell surface by indirect immunofluorescent labelling and evaluated its localization, as the sperm cell is highly structurally and functionally compartmentalized. We observed this molecule with an emphasis on dynamics during sperm capacitation and acrosome reaction and assessed the differences between frozen-thawed and freshly ejaculated bull spermatozoa. Evaluating our results, we found a definite pattern in the equatorial segment; a segment participating in the sperm-egg interaction of bull spermatozoa. The presence of this pattern was altered after inducing the acrosome reaction, as well as by sperm processing (fixation) methods. Further research will be necessary to give us a better understanding of these preliminary findings.

This work was funded by grants VEGA-2/0037/16, APVV-15-0196 and bilateral project SAS-CAS 18-17.

EXPRESSION OF INTER-ALPHA-TRYPSIN INHIBITOR MOLECULES IN OVARIAN FOLLICLES

Nagyová E., Tětková A.

Institute of Animal Physiology and Genetics CAS, v.v.i., Libechov, Czech Republic

The extracellular matrix (ECM) is an important structure that is present in all tissues. The ECM interacts with cells to regulate a wide range of functions, including adhesion, proliferation, apoptosis and differentiation. The ECM can also locally release growth factors, such as epidermal growth factor, fibroblast growth factor, and other signaling molecules such as transforming growth factor and amphiregulin (Theocharis et al. 2016). After gonadotropin stimulus, cumulus cells expand and form hyaluronan (HA) - rich cumulus ECM. In pigs, the proper structure of the cumulus ECN depends on the interaction between HA and serum – derived proteins of the inter-alpha trypsin inhibitor (IαI) protein family. We have evaluated the covalent linkage of heavy-chains of inter-alpha-trypsin inhibitor family proteins to HA, as the principal component of the expanDed HA-rich cumulus ECM in porcine oocyte-cumulus complexes (OCC; Nagyova et al. 2004). In addition; we investigated the spatiotemporal expression of IαI in porcine follicles. Porcine OCC stimulated in vivo and OCC stimulated in vitro with gonadotropins were analyzed by confocal and immunofluorescence microscopy. Both, in vivo and in vitro FSH-stimulated OCC cultured in serum-supplemented medium accumulated IαI in the expanDed cumulus ECM. In contrast, OCC cultured in medium without serum were not able to form cumulus ECM. Our results confirm that HA-rich oocyte-cumulus ECM does not form in serum- free conditions, while it does in the presence of purified IαI molecules (Chen et al. 1992).

Supported by grant MSMT (EXCELLENCECZ.02.1.01/0.0/0.0/15_003/0000460 OP RDE).

CAPTURING DIFFERENCES IN BEHAVIOR OF IMPORTINS DURING MOUSE SPERM ACROSOME REACTION USING STRUCTURED ILLUMINATION MICROSCOPY (SIM)

Frolíková M. [1], **Loveland K. L.** [2, 3], **Dvořáková-Hortová K.** [1, 4]

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Importin proteins belong to karyopherin protein family, soluble molecules that mediate nucleocytoplasmic transport. They are primary responsible for active transport of cargo proteins from cytoplasm to nucleus through the nuclear pores. However, an intensive investigation of these proteins shows that importins also participate in other physiological processes such as control of transcription, embryonic stem cell pluripotency, cellular stress adaptation, nuclear envelope and lamin assembly, and spindle formation. Moreover, the essential role of some importins in germ cell maturation has been established. In mammalian testes, importins were detected in germ cells with expression levels differing between individual spermatogenic stages. These facts indicate the potential of importins to participate in regulation of key steps of sperm differentiation. Using SIM, we detected the precise localization of individual importins in acrosome-intact epididymal mouse sperm and changes in their localization that occur during the sperm acrosome reaction (AR) for the first time. Although in classical nucleoplasmic transport, importin αs serve as mediators between cargo proteins and importin β1, the stoichiometry of α-β importins is not 1:1 in spermatogenic cells. The levels of importin αs, specifically α2, α3 and α4, is significantly higher than that of importin β1 in spermatids. This indicates that, in sperm, importin αs could function independently from β1, and our current results support this theory. The SIM immunofluorescent imaging of α2, α3, α4 and β1 importins revealed differences in subcellular relocalization behavior between importins α and β1 after the AR. Although both α and β1 importins are present in apical acrosomal area in the acrosome-intact sperm head, their localization dramatically differs after AR, when importin αs are detected in the

connecting piece of sperm, the β1 importin is localized in equatorial segment, and in later AR stages it covers the whole sperm head area. These findings bring novel information about importin behavior during the AR, which represents a final step of sperm maturation that gives sperm the ability to fuse with the egg.

This study was supported by the Grant Agency of the Czech Republic No. GA-18-11275S, by the Institutional support of the Institute of Biotechnology RVO: 86652036, and by the project "BIOCEV" – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University" (CZ.1.05/1.1.00/02.0109) from the European Regional Development Fund (www.biocev.eu). K.L. was supported the National Health and Medical Research (Fellowship, ID1079646) and the Victorian State Government Operational Infrastructure Scheme. We acknowledge Operational Program Prague Competitiveness (CZ.2.16/3.1.00/21515) funDed by European Regional Development Fund.

NEW MICROSCOPIC AND PICTURE ANALYSIS APPROACHES TO STUDY THE EPIGENETIC STATES IN SPERM AND TESTICULAR TISSUE

Děd L. [1], Žatecká E. [1], Valášková E. [1], Frolíková M. [1], Dorosh A. [1], Margaryan H. [1], Elzeinová F. [1], Kubátová A. [1], Pěknicová J. [1], de la Iglesia A. [2], Castillo J. [2], Oliva R. [2], Paradowska-Dogan A. [3], Steger K. [3], Dvořáková-Hortová K. [1, 4]

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In recent years the field of microscopic analysis of biological samples experiences dramatic changes. Many new optical and data processing techniques have been developed and introduced to the scientific community. Despite this immense progress, the developed advance microscopic techniques are only limitedly use in epigenetic research as compared to methods of genetic and bioinformatic analysis. Therefore, we developed several data and sample processing techniques to study the epigenetic states in the mice and human sperm and testicular tissue. These methods include: 1) Topological analysis of the distribution of histones and histone post-translational modifications (PTMs) in mice sperm; 2) Topological analysis of the distribution of histone PTMs in mice testicular tissue sections; 3) Cluster analysis of the histone PTMs in decondensed human sperm. Using our developed image processing platforms (1) we have been able to uncover the differences in the abundances of histone H3 in the mice sperm from control group and group exposed to 17α-Ethynylestradiol (Control; EE2 -2.5 μg/L; 55.4 ± 9.7 RFU vs 69.1 ± 11.2 RFU) and the potential cause of this phenomena (histone hypoacetylation and impaired histone-to-protamine process in spermatids (2)). Additionally to our methods allowing to analyze high numbers of individual cells, we have also developed methods to analyze fine spatial patterns of the histone PTMs in human decondensed sperm nuclei (3). Using this approach

we observed interesting spatial distribution of two histone PTMs (H4K5Ac and H4K5Bu) during the imaging by high-resolution confocal microscopy. Our next steps will be to apply the newly developed image processing platform also to the data obtained by super-resolution microscopy and 3D volume imaging and thus open the new ways how to study epigenetics states in testes and sperm using advance microscopic methods.

This work was supported by the project International Mobility of Researchers of Institute of Biotechnology, CAS, v.v.i. (CZ.02.2.69/0.0/0.0/16_027/0008353) from the ERDF, by the Grant Agency of the Czech Republic No. GA-18-11275S, by the Charles University in Prague No. SVV260440, by grants from the Spanish Ministry of Economy and Competitiveness (Ministerio de Economía y Competitividad; FondosFEDER 'Una manera de hacer Europa') to R.O (PI13/00699, and PI16/00346), J.C (CD17/00109), and A.I (FI17/00224), by the Institutional support of the Institute of Biotechnology RVO: 86652036, and by the project "BIOCEV" – Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University" (CZ.1.05/1.1.00/02.0109) from the European Regional Development Fund (www.biocev.eu).

EXPRESSION PROFILES OF TESTIS ELUTRIATION FRACTIONS IN CENTROBIN TRANSGENIC RATS

Liška F. [1], Valášková E. [2], Frolíková M. [2], Dostálová P. [2], Flint J. [1, 2], Janků M. [1], Maňásková-Postlerová P. [2], Chylíková B. [1], Dvořáková-Hortová K. [2, 3]

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Centrobin deficiency in the rats with hypodactyly mutation leads to acephalic spermatozoa syndrome. CMV promoter-driven transgenic expression of wild-type centrobin rescues the limb malformation and increases sperm production; however, the sperm heads detachment is persisting. We used elutriation, a technique combining centrifugation with medium flow in the opposite direction to separate cells (based on size, shape and density) from the testis of wild-type and transgenic mutants. qRT-PCR testing of the fractions for various cell specific markers showed similar profiles in wild-type and mutants. The peptidylprolylisomerase (Ppia) gene was used as the reference gene. Specific gene markers for germinal cells and somatic cells were used to determine elutriation fractions. The mRNA expression of a target genes was calculated, based on the threshold cycle difference (ΔCt) of a testicular elutriation fractions versus testis. Primers with R2 > 97% and concentration-Ct slope (efficiency) 0,9-1,1 and with one specific melting peak were used for the analysis. Negative control was prepared in the same conditions except that cDNA was replaced by nuclease free water. RT- negative control for cDNA synthesis was also analysed. Therefore, we can conclude that the cause of the acephalic spermatozoa in rats with centrobin hypodactyly and centrobin CMV-transgene is confined to the morphogenesis of elongating spermatids, which will be subjected to detailed proteomic analysis targeting a centrosome-derived head to tail coupling apparatus and a manchette formation during spermiogenesis.

MOLECULAR MECHANISM INVOLVED IN THE FORMATION OF SPERM OVIDUCTAL RESERVOIR

Postlerová P. [1, 2], **Smejkal V.** [2], **Dvořáková-Hortová K.** [1, 3], **Jonáková V.** [1]

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During formation of the sperm oviductal reservoir, it is supposed that the sperm surface protein coat interacts with glycoconjugates of the oviductal epithelium. The ability of sperm to interact with oviductal glycoconjugates changes during the course of the fertilization process, probably due to: (i) changes of the sperm membrane and protein coat; (ii) the presence of inhibiting or activating factors in the oviductal fluid. Thus, the loss/removal of certain sperm proteins in the course of capacitation is required for the sperm membrane remodeling resulting in the maturation of fertilization-competent spermatozoa and their detachment from oviductal reservoir. However, the binding molecules on sperm and corresponding binding partners on oviductal cells are still not fully described in almost mammals. Moreover, nothing is known about changes on the surface of oviductal epithelial cells during the hormonal cycle of the female. The aim of our study was to monitor the binding of oviductal and sperm surface proteins depending on the female hormonal cycle and sperm capacitation state in the pig. The binding of surface proteins and oviducts in preovulatory and luteal phase to surface proteins of ejaculated and capacitated sperm showed that proteins of ejaculated spermatozoa bind to oviduct proteins within a range of molecular masses with higher intensity than proteins of capacitated sperm. Additionally, we detected different carbohydrate structures of the oviductal glycoproteins using selected biotin-labelled lectins. Our results showed that the binding of sperm and oviductal proteins varies depending on the stage of the female hormonal cycle. These changes may also be related to modifications in carbohydrate structures on the surface of spermatozoa and oviductal cells. The identification of molecules mediating the sperm-oviduct interaction leads to the understanding of molecular mechanism of the sperm oviductal reservoir formation and sperm capacitation process.

This work was supported by CAS (RVO: 86652036), GA-18-11275S, SGS projects 21230/1312/213157 and 21230/1312/213181, and project BIOCEV (CZ.1.05/1.1.00/02.0109) from the ERDF. CellFit COST Action CA16119, MSMT INTER-COST LTC 18059.

MONITORING OF PROTEIN NETWORK DYNAMICS: THE ROLE OF Fcrl PROTEINS DURING SPERM – EGG MEMBRANE INTERACTION.

Svobodová J.

DEGRADATION OF PROTEINS ON BOAR SPERM SURFACE DURING CAPACITATION REGULATED BY UBIQUITIN-PROTEASOME SYSTEM

Tůmová L. [1], **Zigo M.** [2, 3], **Sutovsky P.** [3], **Jonáková V.** [2], **Sedmíková M.** [1], **Postlerová P.** [1, 2]

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Capacitation is an important step in post-testicular maturation of mammalian spermatozoa that is essential for the process of fertilization. It is associated with biochemical and physiological changes both on the surface and inside the sperm cells. For successful capacitation, specific proteins must be released from the sperm surface. An important regulatory mechanism is proteins degradation through the ubiquitin-proteasome system (UPS) that is significantly involved in the regulation of signalling pathways via ubiquitination, a post-translational modification of proteins. It has been described a several sperm surface proteins that are degraded by UPS during capacitation; however there are a number of other proteins where is not sure, whether are degraded by this system. We monitored the degradation of boar sperm surface proteins during in vitro capacitation by UPS with addition of proteasomal and ubiquitination activity inhibitors. For protein detection after UPS inhibition, we used the Image Stream analysis, fluorescence microscopy and Western blot. Elucidation of the molecular mechanism of mammalian sperm capacitation including UPS is important for understanding the processes that control gamete development and fertilization or for improving IVF.

Project was supported by SGS projects 21230/1312/213157 and 21230/1312/213181, CAS (RVO: 86652036), and project BIOCEV (CZ.1.05/1.1.00/02.0109) from the ERDF.

DETECTION AND LOCALIZATION OF ESTROGEN RECEPTORS IN BOAR REPRODUCTIVE TISSUES AND SPERMATOZOA

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Estrogens effect many physiological processes in the female and male organism and their effect is conditioned by the presence of specific estrogen receptors (ERs) in target tissues. Three ERs are currently known, the classical nuclear receptors ERα and ERβ, and the transmembrane receptor GPER. ERs were found in the male reproductive tract and spermatozoa of several animal species. The absence of ERs can result in the failure of spermatogenesis and sperm maturation. However, in adult boars their presence has not been described completely. The aim of our work was to investigate the expression and localization of membrane GPER and classical ERα and ERβ in boar reproductive tissues, epididymal and ejaculated spermatozoa, and to monitor the changes in distribution of individual types of ERs in germ cells and in spermatozoa after capacitation and acrosome reaction. All types of ERs was localized in seminiferous tubules of boar testes, and in the secretory epithelium and spermatozoa of the epididymal tubule. In epididymal as well as in ejaculated and capacitated spermatozoa, GPER was detected especially in the acrosome and weakly in flagellum. For the first time, we localized ERα in the post-acrosomal part and equatorial segment, and in the midpiece of spermatozoa isolated from boar epididymis. Additionally, we found truncated forms of ERs in testicular and epididymal tissue extracts and epididymal sperm. Our results on the localization of ERs in the testicular and epididymal tissues of adult boars and spermatozoa during their development may indicate the evidence of estrogens involvement via their receptors in the rapid non-genomic signalling in the sperm development and their post-testicular maturation.

Project was supported by SGS projects 21230/1312/213157 and 21230/1312/213181, CAS (RVO: 86652036), and project BIOCEV (CZ.1.05/1.1.00/02.0109) from the ERDF.

ESTROGEN RECEPTORS IN BULL SPERMATOZOA

Antalíková J. [1], Horovská Ľ. [1], Sečová P [1], Jankovičová J. [1], Bartóková M. [1], Krejčířová R. [2], Postlerová P. [2, 3]

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Estrogens play an important role in reproductive processes not only in females, but also in males. Their effect is dependent on the presence of estrogen receptors (ERs) to which they bind and initiate a cellular response. Besides the ERα and ERβ nuclear receptors, which act as transcription factors in the nucleus and initiate a genomic pathway after the estrogen stimulus, the GPER membrane receptor (G-protein coupled estrogen receptor), responsible for mediating a fast non-genomic response, has also been described. Additionally, nuclear receptors are also able to translocate to the plasma membrane and thus mediate non-genomic signalling. The GPER signalling cascade includes the release of intracellular calcium ions, a process closely associated with the sperm capacitation and acrosome reaction. Therefore, it is obvious that in order to understand the biological function of both types of receptors it is necessary to know their localization. Even though that ERs expression in reproductive tissues and/or spermatozoa has been described in several animal species; there is no information on bull spermatozoa. For the first time we localized a GPER receptor in the post-acrosomal segment of bull epididymal spermatozoa and after ejaculation in addition also in the apical part of the sperm head. Additionally, we detected the presence of ERα and ERβ receptors in cryopreserved bull sperm after thawing as well as in freshly ejaculated bull spermatozoa using polyclonal antibodies. Detection of both types of receptors is a prerequisite for further study of their role in the events such as maturation, capacitation and acrosome reaction of bull sperm, and the effect of estrogens, but also the negative effect of endocrine disruptors on reproduction process.

This work was funded by grants VEGA-2/0037/16, APVV-15-0196, SGS projects 21230/1312/213157 and 21230/1312/213181, CAS (RVO: 86652036), project BIOCEV (CZ.1.05/1.1.00/02.0109) from the ERDF, and bilateral project SAS-CAS (18-17).

NK CELLS IN OVULATORY CERVICAL MUCUS ARE NOT FOUND SO OFTEN IN COMPARISON WITH NK CELLS IN ENDOMETRIUM IN PATIENTS WITH REPEATED PREGNANCY LOSS

Ulčová-Gallová Z. [1, 2], **Mukenšnabl P.** [3], **Haschova M.** [4], **Pešek M.** [5], **Chaloupka P.** [5], **Lošan P.** [1], **Bibková K.** [1], **Mičanová Z.** [1], **Cibulka J.** [1], **Švecová M.** [6]

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Problem: An increased number of NK cells can be associated with infertility. The aim of our research was to concentrate on the density of NK cells in ovulatory cervical mucus (OCM) and in endometrium in infertile women with repeated pregnancy loss (RPL).
Method of study: Seventy-two randomly selected healthy women resulted in fifty-seven patients with repeated unexplained miscarriages, and fifteen fertile healthy women. The hormonal status was studied including the humoral, autoimmune responses, hysteroscopy, and endometrium immunohistology. NK cells CD56+, CD16+ in OCM and uterine NK cells were identified by immunocytochemistry, aPls by ELISA, indirect immunofluorescent method for detection of serum and OCM IgM, IgG antibodies against HHV-6 levels.
Results: We found high density of NK cells CD56+ and CD16+ in OCM and in endometrium in only two infertile women. NK cells in OCM were missing in other samples of patients. The prevalence of positive density of NK cells CD56+ in the endometrium was seen in twenty three (40%), NK cells CD16+ in eleven (19%), NK cells 56+ and NK cells 16+ together in eight (14%). The rest of examined samples were without NK cells. Levels of serum IgG against HHV-6 in all examined patients were not elevated.
Conclusion: Mucosal activity in the cervical area at the time of ovulation in two infertile patients was evident. We excluded the abnormal number of NK cells owing to local and general viral infection (HHV-6). But our question still remains - are cervical NK cells fixed or still migrating from endometrium into OCM?

MONITORING OF PERIPHERAL T-REGULATORY LYMPHOCYTES DYNAMICS IN PREGNANT WOMEN WITH FERTILITY DISORDERS UNDERGOING IMMUNOMODULATORY THERAPIES

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Embryo implantation process is linked to maternal immune system activation with simultaneous induction of immune tolerance towards fetus on feto-maternal interface. Tolerance of paternal antigens of semiallogeneic fetus by maternal immune system is provided among other mechanisms by both natural and induced T-regulatory lymphocytes (nTreg and iTreg). Aim of our work was to monitor dynamics of absolute counts as well as percentages of peripheral T-regulatory lymphocytes in our patients from 1st to 3rd trimester. All these women had medical history of sterility and/or infertility disorders and underwent immunomodulatory therapy in our centre. We divided subjects into subgroups based on type of received therapy.

We entered 64 subjects into our study, 25 of them conceived spontaneously and 39 subjects became pregnant after embryotransfer. We observed continuous decrease of Treg cells from pre-pregnancy baseline (percentages as well as absolute counts) in group with spontaneous conception. In embryotransfer group we observed increase of counts and percentage of Treg lymphocytes during first trimester followed by decrease until the end of pregnancy.

One of plausible reasons to explain different dynamics of Treg lymphocytes between spontaneous conception and embryotransfer groups is probably due to various times, doses and routes of administration of progesterone. Progesterone has positive immunomodulatory properties during pregnancy it induces Treg cells proliferation. In subjects undergoing embryotransfer, progesterone is usually being administered in more potent local vaginal form immediately following transfer with usual minimum dose of 400 mg daily. In spontaneous conception group progesterone is typically administered after detection of pregnancy i.e. after positive blood test and usually in doses below 400mg/day. However, we have to point out that despite observed differencies in peripheral Treg lymphocytes dynamics, all women in both groups had no signs of pathologies associated with pre/eclampsia and they delivered their babies in term.

VITRIFICATION OF OOCYTES: CORRELATION OF IVF STEPS TIMING WITH THE EMBRYO QUALITY

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Oocytes vitrification is an essential aspect of the in-vitro fertilization process. For artificial fertilization the maturity of the oocytes, resp. timing of fertilization related to maturated oocyte collection plays essential role. Currently applied cryoprotection of oocytes by vitrification modifies the process of standard oocyte maturation. After thawing, the cells damaged by vitrification are gradually regenerated, and this regeneration is combined with continued oocyte maturation gradually making conditions suitable for fertilization. Time parameters play a crucial role throughout the whole complicated process. In our work, we correlate the main time parameters of the individual steps after the collection of the maturated oocyte with its fertilization success, the quality of embryos and pregnancies. We work with the time of vitrification after oocyte collection and the time interval between thawing oocyte and fertilization. We identify time attributes that are important to successful of cryopreserved oocytes application in IVF. A detailed analysis of the correlation functions will allow optimizing the IVF of cryopreserved oocytes.

Saturday, MAY 25, 2019

RECURRENT MISCARRIAGES: ROLE OF INHERITED TROMBOPHILIA

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Recurrent miscarriages / early (within 20th gestation week) recurrent pregnancy losses (RM/eRPL) is a common health problem, with three or more loses affecting 1-2% and two or more losses affecting up to 5% of women at the reproductive age. We know that there are mainly chromosomal abnormalities playing a dominant role in RM with a proportion of up to 80% depending on age, but their issue is not the subject of this report.

Pregnancy is a hypercoagulating state and if affected by thrombophilia, the tendence is amplified and may generate clots in the placental blood vessels impairing placentation with all known consequences. Among the prothrombotic states, antiphospholipid syndrome (APS) is an established (and treatable) cause of RM. However, for reasons to be discussed, the same cannot be said for inherited thrombophilia (IT).

The evidence for IT being the underlying cause of RM was examined on three basic moments: (a) whether there is increased prevalence of IT in women experiencing RM, (b) whether there is higher incidence of RM among women with IT and (c) whether there is benefit of using thromboprophylaxis to prevent RM among women with IT. Two relevant candidates were examined: factor V G1691A (FVL) and prothrombin G20210 (FII).

The conclusions of numerous meta-analyzes suggest that association between recurrent miscarriages and FVL or FII is slightly increased, but not significant. LMWH is only relevant to prevent thromboembolism in late pregnancy, but not abortion.

SYSTEMIC ENZYME THERAPY AS A SUPPORT OF POSTOPERATIVE HEALING IN GYNECOLOGY AND OBSTETRICS

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Systemic enzyme therapy (SET) is a treatment method which uses oral application of exogenous hydrolytic proteases of animal (trypsin, chymotrypsin) and plant origin (bromelain, papain) with rutin in the form of acido-resistant tablets for treating inflammatory conditions. SET medicinal preparations are known mainly for their anti-inflammatory, anti-edematous effects. These preparations support and accelerate healing of wounds, so they are used in surgery fields including gynecology and obstetrics (e.g. laparoscopically assisted vaginal hysterectomy, plastic surgery of female genitals).

With increasing frequency of Caesarean sections (CS) there are more cases with abnormal healing of uterotomy wound which can be cause of risks for subsequent gravidities. This situation prompted the project to verify positive influence of SET on uterine scar healing. SET medicinal preparation was given to women after CS for 21 days. The main indicator of treatment efficiency was ultrasound examination of a scar and comparison to the control group. The quality of scars in women with SET treatment was significantly better ($p=0,01$) compared with control group, as well as other monitored parameters (uterine cavity dilatation, post-operative pain, CRP).

INTRALIPIDS FOR TREATMENT OF RECURRENT IMPLANTATION FAILURE IN WOMEN WITH NK CELLS PATHOLOGIES

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Introduction: Intralipid is a fat emulsion containing soybean oil, glycerin and egg yolk derived phospholipids. Fatty acids in intralipid have immunomodulatory effects. In reproductive immunology, their influence on peroxisome proliferator activated receptors (PPAR) leading to the suppression of proinflammatory cytokines production in NK cells is used.
Objectives: (1) Analysis of indications for intralipid usage, and (2) a retrospective analysis of implantation and pregnancy rate in intralipid-treated cohort at the GENNET clinic in 2018.
Patients and Methods: A retrospective analysis of our own cohort of patients indicated for therapeutic immune intervention in 2018 was performed. Statistical analysis was executed on data about 32 women, in whom the clinical immunologist deciDed about intralipid immune - intervention treatment.
Results: Only in 6 % out of the 915 patients with laboratory confirmed immunopathology affecting fertility, clinicians deciDed to use intralipid treatment. The main clinical criteria for this therapy were (1) repeatedly unsuccessful embryo transfer of high-quality embryos and/or repeated early miscarriages; and (2) failure of prior immune intervention by other immunomodulators. The main laboratory criteria for this therapy were (1) high numbers of circulating NK cells in peripheral blood and/or (2) pathological cytotoxic activity of NK or NKT cells after stimulation by trophoblast antigens. From a total of 32 females, embryo transfer was performed and therefore at least 2 immunomodulation doses of intralipid were given to 25 subjects. The implantation rate in intralipid-treated cohort was 60%, and the pregnancy rate was 52%.
Conclusion: Intralipids are useful immunomodulators in the treatment of immunological causes of repeated embryo transfer failure, particularly in laboratory-verified pathologies in the number or functions of NK cells.

NEW METHODOLOGICAL APPROACHES FOR EXTENSION OF PRENATAL DIAGNOSTICS

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Introduction: Since 2011, Professor Nicolaides has been publishing a new approach in prenatal diagnosis called "Inverted Pyramid of Care", in which physical methods are the basis for the system. Therefore, in this work we deal with complex prenatal care from conception to childbirth and results in an effort to introduce a new European trend.
Materials and methods: The combination of anamnesis, results of laboratory examination (free beta-hCG, PAPP-A and alpha-fetoprotein) and physical methods (ultrasonographic measurement of nuchal translucence, venous duct, tricuspid regurgitation, arterial flow) has been established. Over four years, our team has created a set of 600 patients in 9th - 13th week of pregnancy from two clinics - Institute for the Care of Mother and Child and University Hospital in Motol. In all patients, the concentration of Placental Growth Factor (PlGF) and a Doppler ultrasonographic examination were done. Our goal was to compare the results with the published values of Professor Nicolaides in order to assess the possibility of introducing a method into clinical practice in the Czech Republic.
Results: The levels of PlGF for 3rd, 5th, 25th, 50th, 75th, and 95th percentile in 9th and 13th week of pregnancy in our patients matched the levels in physiological pregnancies published by Perkin-Elmer and by Professor Nicolaides. The linear increase of PlGF in 9th and 13th gestational week was also identical in women with consequential development of pre-eclampsia (PE). On the contrary, the levels of PlGF in women from pre-eclampsia group were significantly ($P<0.0001$) lower in comparison with the group of physiological pregnancy. These results enabled the introduction of PlGF among prenatal examinations at the Institute for the Care of Mother and Child.
Conclusion: The PlGF assay seems to be a suitable combination of ultrasound and biochemical examination searching out not only aneuploidy, but also the possible risk of pregnancy pathologies. In the future, examinations may be extended to include other methods, such as examining microparticles from the blood of the upcoming mothers.

Acknowledgement:
Professor Kypros Nicolaides, MD, BSc, MBBS, MRCO for agreement to present his results.
Professor MUDr. Tomáš Zima, DrSc., MBA for cooperation and supporting the study. Professor RNDr. Ilona Hromadníková, Ph.D., Associate profesor MUDr. Milan Macek, CSc. and Professor Dr. med. Udo R. Markert for cooperation with their working groups.
The study was supported by: PRVOUK–P25/LF1/2, MZČR–RVO–VFN 64165, FNM 64203,CZ.2.16/3.1.00/24022, IGA NT13770

DIFFERENCES IN GLYCOCONJUGATES OF SPERMATOZOA AND SEMINAL PLASMA OF MEN WITH NORMAL AND PATHOLOGICAL SPERMIOGRAMS

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Glycoconjugates are essential components of sperm cells and reproductive fluids and include the complex of different glycoproteins and glycolipids important for the sperm development and function. Expression and modification of glycoproteins occur in the entire male reproductive tract and therefore they play role as the sperm-coating antigens and immunomodulators that are important for survival of spermatozoa in the female reproductive tract. Disorders and differences in glycation can lead to the changes in the sperm development or function reducing their fertility and indicate health problems. The aim of this study was to characterize the glycoprotein differences in sperm and seminal plasma in ejaculates of men with normal and pathological spermiograms using lectins recognizing different saccharide moieties. Protein contents of spermatozoa and seminal plasma were separated by SDS electrophoresis and transferred onto the nitrocellulose membrane. Differences in glycans were tested by selected biotin-labelled lectins (MAA and SNA recognizing sialic acids; AAL and UEA recognizing fucose). Additionally, the glycoprotein profiles of seminal plasma were compared after lectin affinity chromatography. The highest variability of glycoproteins was detected mainly in the high-molecular-weight proteins of pathological ejaculates with azoospermia and oligozoospermia. Variability of sperm and seminal plasma glycoproteins can help to better understand the fertilization, reproductive disorders and improve the quality of diagnostic methods.

This work was supported by CAS (RVO: 86652036), GA-18-11275S and project BIOCEV (CZ.1.05/1.1.00/02.0109) from the ERDF.

SPERM SELECTION STRATEGY – WHICH ONE IS THE BEST FOR SEMEN SAMPLES WITH HIGH CONCENTRATION OF APOPTOTIC SPERM?

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The worldwide decline in sperm quality is a serious issue. It is necessary to implement techniques that will enhance the quality of embryos.
The combination of semen analysis and flow cytometric analysis of the viable sperm were used for the diagnosis of sperm quality. Sperm selection was performed by density gradient centrifugation (GC), swim-up (SU), magnetic sperm separation (MACS) or microfluidic separation (MFSS). The decrease of viable sperm after GC was determined in samples with <50 % of viable sperm in semen. More gentle separation techniques were tested in such samples. A significant increase of % viable sperm (38.6 % in semen) was found after MFSS (64.6 %) but not after MACS (38.2 %).
GC, MFSS or MACS are used as routine practice in GENNET. Retrospective analysis of 112 cycles was done. We compared data from 40 GC, 36 MFSS and 36 MACS cycles in which semen with lower rate of viable sperm were used. Fertilization rates were 88.0 % (GC), 82.4 % (MFSS) or 80.0 % (MACS). Utilization rates were 29.2 %, 26.0 % or 36.0 %, respectively. There were only 20.0 % pregnant women after GC, but 52.8 % after MFSS and 38.9 % after MACS.
The optimization of sperm separation methods can be of great importance. MFSS was recommenDed for samples with lower percentage of viable sperm.

Study was supported by GENNET, Prague.

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