REACTION PATTERN OF MONOCLONAL ANTIBODY IVA-50 (CD9) DURING CAPACITATION OF BULL SPERM

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Ejaculated mammalian spermatozoa must undergo several biochemical and morphological changes in the female reproductive tract to acquire the ability to fertilize the egg. These changes, collectively called capacitation, include also the rearrangement of sperm surface proteins acquired from seminal plasma as well as epididymal secretion. One of the proteins recently detected exclusively on plasma membrane of bovine sperm is CD9 molecule, tetraspanin, whose essential role for gamete fusion was previously confirmed on oocytes, but no data are available regarding the dynamics of this molecule during capacitation in cattle.

It is well known that process of cryopreservation influences the sperm plasma membrane permeability and changes its protein composition; frozen-thawed bovine spermatozoa were referred to as capacitated or able to capacitate very easily (in 30 min). The aim of the present study was to monitor the pattern of CD9 on freshly ejaculated, frozen-thawed (capacitated-like) sperm and also on sperm during *in vitro* capacitation process using anti-CD9 antibody (mAb IVA-50). The chlortetracycline fluorescence analysis, based on the assessment of Ca-related changes during the capacitation of spermatozoa, was applied to detect the portion of capacitated sperm. When frozen-thawed or freshly ejaculated sperm (capacitated for 30 min or 4 h in TL medium for sperm cell capacitation at 39 °C with 5 % CO_2 in humidified atmosphere) were analysed, comparable results were obtained. The capacitation process did not change the pattern of CD9 molecule on freshly ejaculated and frozen-thawed sperm. IVA-50 reactivity exceeded 77 % in all tested samples and the positive immunofluorescent signal remained unchanged in form of fine grains either on the apical part or through the entire anterior region of the sperm head. When CD9 study was carried out in mice, only 10 % of capacitated or freshly recovered cauda epididymal sperm have been stained and fluorescent signal appeared mainly as a thin line in the acrosomal region. It seems that despite the fact, that CD9 is highly conserved molecule in mammals, species-dependent differences in gamete protein organization are obvious and distinct mechanisms of involvement of CD9 in the fertilization process are assumed.

Key words: spermatozoa; capacitation; CD9 molecule

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THE USE OF MUTANT *LOX66* AND *LOX71* SITES TO TARGET TRANSGENE INTEGRATION AT A PRE-CHARACTERIZED GENOMIC LOCUS

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Genetic engineering strategies usually require permanent modification of the target genome. There are many techniques available for stable integration of transgenes into mammalian cells. However, these methods mostly result in integration at random chromosomal locations of an uncontrolled number of transgene copies that express at levels which generally cannot be predicted or reproduced because of the position effect. The inability to control the site of integration, the number of integrated copies and the expression level has impeded progress in studies of both gene expression and the physiological effects of transgenes. To change this situation, several methods have been developed that enable a targeted integration into stable and consistently expressed genomic loci. Targeting transgenes into pre-characterized loci yields predictable expression patterns due to the invariable transcriptional control exerted by the given endogenous regulatory sequences. While homologous recombination can provide great specificity to the integration process, its efficiency for most biotechnologically relevant cell lines is much too low. In this situation, site-specific integration systems are of increasing relevance, as they provide targeting frequencies at least three orders of magnitude higher than those resulting from homologous recombination.

Cre-mediated site-specific recombination in mammalian cells represents a useful tool for genome engineering, allowing precise and repeated site-specific integration. The strategies are based either on heterospecific *lox* sites carrying mutation(s) in the central 8-bp spacer region (RMCE strategy) or on *lox* sites mutated in the left (L) and right (R) inverted repeat region, allowing integrative recombination. Recombination between an L mutant *lox* and R mutant *lox* sequences results in the generation of a double mutant *lox* site having mutations in both repeat regions and a wild type *lox*P site. The double mutant *lox* site is not an effective substrate for Cre recombinase, therefore the recombination reaction proceeds exclusively in one direction. Integrative recombination is useful tool for the production of transgenic cells or animals because any DNA of interest can be introduced into a chromosomally located *lox* site.

In this work we have demonstrated successful integrative recombination of DsRed gene joined with *lox*71 into pre-characterized genomic locus containing *lox66*-EGFP gene in stable transformed mouse NIH 3T3 fibroblasts. Although the integrative recombination efficiency using *lox71*/*lox66* sites without any selection of recombinants is low, the advantage of this strategy is its simplicity.

Key words: integrative recombination; mouse fibroblasts; Cre recombinase; *lox71*/*lox66*

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THE EFFECT OF ANTLEROGENIC STEM CELLS ON THE QUALITY OF CRYOPRESERVED BOAR SEMEN

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The aim of this study was to assess the effect of nutritional supplementation of a homogenate derived from the antlerogenic stem cells (HOMASC) of *Cervus elaphus* on sperm cell characteristics in frozen-thawed boar semen. The study was carried out on 49 ejaculates collected from 7 boars of Polish Line 990 breed from the experimental group and the same number of animals, ejaculates and the breed of boars in the control group. Only in the experimental group each male received in the ration homogenate formulation of the antlerogenic stem cells at the amount of 1 ml/30 kg body weight, every day for 90 days boars. After supplementation of HOMASC the semen was collected and frozen. The sperm characteristics were assessed using CASA analyzer and flow cytometry. The motility parameters obtained by CASA were: VAP, VSL, VCL, ALH, BCF, LIN, MOT, PMOT and RAPID. Sperm membranes and acrosome integrity (SYBR-14/PI and PNA/PI), assessment of mitochondria activity (JC-1), rupture of DNA (TUNEL), chromatin status (SCSA), membrane fluidity and apoptosis (YO-PRO-1/M540) and membrane lipid peroxidation (C11 BODIPY581/591) were evaluated. The addition of the HOMASC of Cervus elaphus into the boars' diet significantly decreased ($P \leq 0.01$) the percentage of dead sperm in the frozen-thawed semen but also caused an increase in the percentage of dying sperm. Furthermore, the addition the homogenate significantly increased ($P \leq 0.01$) the percentage of live sperm with intact acrosome and decreased the percentage of dead sperm with a damaged acrosome. There was no effect of boars' diet supplementation on apoptotic and capacitation changes in frozen-thawed sperm. After thawing a significant reduction ($P \leq 0.01$) in lipid peroxidation in spermatozoa of boars fed with homogenate was observed. Nutritional intake of homogenate significantly ($P \leq 0.01$) decreased the percentage of sperm with damaged chromatin in frozen-thawed sperm, while no changes were observed in the proportion of immature spermatozoa. The use of the HOMASC for boar feeding significantly ($P \leq 0.01$) increased the percentage of motile sperm, sperm with progressive and rapid movement and significantly ($P \leq 0.01$) increased speed, straightness and linearity of frozen-thawed sperm.

Key words: boar; spermatozoa; cryopreservation; motility; membrane

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MYCOTOXINS AS EPIGENETIC FACTOR

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Mycotoxins are secondary mould metabolites and generally ubiquitous contaminants of food and feed. Epigenetic factors modulate the structure of the chromatin, thereby affecting the transcription of genes in the genome. Changes in chromatin structure are associated with the activation and silencing of gene transcription, and reversible post-translational modifications of histones are related to chromatin structure transitions. Mycotoxins and organic chemicals can interfere in the cascade of cell machinery and thus affect cellular function. Mycotoxins with carcinogenic potency include aflatoxins, sterigmatocystin, ochratoxin, fumonisins, zearalenone, and some *Penicillium* toxins. Most of these carcinogenic mycotoxins are genotoxic agents. Mycotoxin toxicity is exerted via multiple pathways, such as DNA and RNA synthesis inhibition, inhibition of microtubule assembly and of tubulin polymerization, alteration of mitochondrial functionality with consequent increase in reactive oxygen species (ROS) production, inactivation of the heat shock protein and activation of the signal transduction pathway and the caspase-cascade system that results in apoptotic cell death. Based on this, the aim of our study was to determine the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and accumulation of Hsp70 in porcine ovarian granulosa cells after deoxynivalenol (DON) and zearalenone (ZEA) exposure *in vitro*. Porcine ovarian granulosa cells were incubated with DON/ZEA administrations for 48 h as follows: group A (10/10 ng/ml), group B (100/100 ng/ml), group C (1000/1000 ng/ml). We found that both mycotoxins induced stress reaction in porcine ovarian granulosa cells and promoted accumulation of Hsp70, what resulted in decreasing activities of SOD and GPx. These results contribute towards the understanding of cellular stress and its response to mycotoxin exposure. Mycotoxin exposure can lead to formation of reactive oxygen species in the body that could activate and deactivate various epigenetic mechanisms leading to the emergency of various diseases.

Key words: mycotoxins; capacitation; epigenetic factors

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ADIPONECTIN MAY PLAY A COMPLEMENTARY ROLE TO INSULIN IN STIMULATED GLUCOSE UPTAKE IN MOUSE BLASTOCYST

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Experimental data indicate that insulin stimulates glucose uptake in mouse blastocysts via IGF-I receptor-mediated

translocation of GLUT8 glucose transporter. In rabbit blastocyst, adiponectin, but not insulin, stimulated glucose uptake, and the increase in glucose transport was associated with translocation of GLUT4 glucose transporter to the cell membrane. The aim of this study was to find out whether adiponectin can stimulate glucose uptake in mouse blastocyst and to examine the expression of GLUT4 in mouse blastocysts. Mouse blastocysts were cultured in the medium supplemented with full-length or globular adiponectin for 2 h, afterwards the blastocysts were transferred to pulse droplet containing 0.3 mM of 3-O-methyl-D-[1-3H] glucose and the radioactivity of embryos was determined in liquid scintillation analyser. We found significantly higher uptake of 3-OMG in blastocysts treated with full-length adiponectin when compared to the control group. Dubious effects were observed after globular adiponectin treatment; therefore, additional measurements are necessary to obtain conclusive results. Using RT-PCR with specific oligonucleotide primers, we detected GLUT4 and GLUT8 transcripts in ICR mouse blastocysts. Moreover, our immunohistochemical study showed the presence of GLUT4 protein in mouse blastocysts. In summary, our results indicate that adiponectin can stimulate glucose uptake in mouse blastocysts. We also confirmed expression of GLUT4 in mouse blastocysts, what suggests possible involvement of this transporter in the adiponectin-stimulated glucose uptake.

Key words: mouse; embryo; glucose uptake; adiponectin

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EMBRYO TRANSFER AND ENDANGERED BREEDS

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Preservation of livestock breeds that may be in danger of extinction is very important. Of the 3831 breeds or breed varieties of donkeys, buffaloes, cattle, goats, horses, pigs and sheep believed to exist or to have existed in the past century, 618 (16 %) have apparently extinct. A few years ago, the Food and Agriculture Organisation (FAO)s Global Data Bank for Domestic Livestock, which carried 2047 entries, showed 221 cattle breeds to be at risk, and most of them (60 %) in the developed countries. In developing countries threats to genetic diversity usually take the form of increased use of AI and indiscriminate crossbreeding of indigenous breeds. The intensification of farming in these countries can mean that indigenous breeds are in danger of being pushed to extinction because native farmers, aiming at greater productivity, employ exotic breeds such as Holsteins and Friesians. A thousand species have been lost during the last centuries and today it is estimated that one-third of breeding animals are threatened to extinction. The disappearance of many breeds has usually been in the name of progress, driven by intensification of food production methods, which has favoured the most productive breeds. Embryo transfer technology is now regarded as a vital tool for genetic preservation of endangered species and breeds. It enables the establishment of embryo banks and embryo to be transferred into populations with decreased biodiversity. In native breeds of cattle, embryo transfer can be used to preserve genetic lines with good maternal characteristics, fertility, adaptation to extreme climatic or nutritional conditions, and natural resistance to disease.

Key words: embryo transfer; livestock breeds; preservation

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EXPRESSION OF ADRENERGIC RECEPTOR TRANSCRIPTS IN MOUSE EMBRYONIC STEM CELLS AND PREIMPLANTATION EMBRYOS

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Adrenaline and noradrenaline were detected in the female reproductive tract, and experimental results indicate that these catecholamines can influence preimplantation embryo development. Moreover, accumulating evidence indicates that catecholamines can influence cell proliferation and differentiation in mouse embryonic stem cells (derived from preimplantation embryos) as well. Using RT-PCR we examined expression of adrenergic receptor subtypes in undifferentiated and spontaneously differentiating mouse embryonic stem (ES) cells, and compared their expression with the expression profiles found in mouse preimplantation embryos. We detected eight adrenergic receptor subtypes in undifferentiated mouse ES cells, but only three subtypes were found in mouse blastocysts. In three adrenergic receptors (α1D, α2B, β1), we found higher expression in the spontaneously differentiating ES cells than in undifferentiated ES cells, and the α1B adrenoceptor was not even detectable in the undifferentiated cells. These results indicate that genes encoding all types of adrenergic receptors are transcribed in mouse embryonic stem cells, and some of them are differentially expressed during ES cell differentiation. In addition, our results showed significant differences in the expression of adrenoreceptor transcripts between embryonic stem cells and mouse blastocysts (from which ES cells are derived).

Key words: catecholamines; embryonic cells; G-protein-coupled receptors

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CHANGES IN THE REACTION PATTERN OF MONOCLONAL ANTIBODY IVA-50 (CD9) ON BULL SPERM AFTER ACROSOME REACTION

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Acrosome reaction is a necessary part of gamete interaction leading to successful fertilization of mammals. This process is initiated by the binding of sperm to *zona pellucida* proteins and helps the sperm get through the *zona pellucida* to enter oocyte plasma membrane. In this study, changes in CD9 distribution during the acrosome reaction of bull spermatozoa were evaluated using mAb IVA-50 that recognizes the CD9 molecule. Tetraspanin molecule CD9 is located in plasma membrane in the acrosomal region of ejaculated as well as capacitated bovine sperm. In our analysis, frozen-thawed spermatozoa were treated and acrosome reaction was stimulated *in vitro* either physiologically (by *zona pellucida*-intact oocytes) or artificially (by calcium ionophore). We can summarize that the ratio of sperm reactive with mAb IVA-50 decreased simultaneously with prolonging the time of induction of an acrosome reaction. Significant differences in number of IVA-50 stained sperm were observed after 40 or 60 minutes of treatment. Obtained results were independent of the way of acrosome exocytosis stimulation. Based on these results, the role of sperm CD9 molecule in the events preceding the sperm-oocyte fusion can be supposed.

Key words: CD9; tetraspanin; bull; sperm; acrosome reaction

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THE ROLE OF ADIPOSE TISSUE IN METABOLISM

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Adipose tissue plays an important role in an active body as it produces many bioactive substances. Adipose tissue is a reservoir of energy that we need. If we have a reasonable amount of fat, fat cells actually produce health-promoting substances. Abundance of fat in the cells results in the production of substances that are involved in the development of serious cardiovascular and cancer diseases or *diabetes mellitus* 2. Adipocytes play an important role in energy and glucose metabolism. In addition to serving as a site for energy storage (in the form of triglycerides), adipocytes act as endocrine cells, secreting molecules that regulate energy expenditure, food intake, and glucose metabolism. Recent findings suggest that the size of adipocytes is a major modulator of their endocrine function. For example, hypertrophic adipocytes secrete greater amounts of tumour necrosis factor α and free fatty acids than normal adipocytes, and this excess secretion has been hypothesized to cause insulin resistance. Obesity is associated with inflammation in adipose tissue, namely an infiltration and expansion of macrophages, which produce inflammatory cytokines that interfere with insulin signalling, and a loss of protective cells that promote adipose homeostasis. Thus, it is now clear that inflammation is an underlying cause or contributor to diabetes II, as well as many other obesity-induced diseases, including atherosclerosis and cancer. Inflammatory pathways contribute to impaired glucose handling by adipocytes, hepatocytes, and muscle cells and interfere with insulin production and insulin signalling. Further investigations are necessary to clarify the contribution of individual cellular components of adipose tissue in order to determine the function of these components as a cohesive unit.

Key words: adipocytes; metabolism; insulin resistance; obesity

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CHANGES IN LIPID PARAMETERS OF RABBIT´S BLOOD FOLLOWING APPLICATION OF PATULIN

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Excessive energy load, in combination with inflammation-impaired *de novo* adipogenesis, results in the hypertrophy of existing adipocytes that eventually fail to store fatty acids, resulting in their leakage into circulation and infiltration into other organs. The aim of the submitted abstract was to find out an impact of intramuscular application of patulin in concentration 10 $\mu g.kg^{-1}$ of body weight on the parameters of lipid metabolism (cholesterol, triacylglycerol - TAG, high density lipoproteins - HDL, low density lipoproteins - LDL) of rabbit's blood. Patulin was administered two times per week. Female rabbits of maternal albinotic line and paternal acromalitic line were used. The animals were divided into experimental group E1 and the control group C. The water and feed were available *ad libitum*. After 30 days of feeding rabbits were slaughtered and blood samples (n = 5 in each group) were obtained. The average value of cholesterol (2.51 ± 0.69 $mmol.l^{-1}$), serum concentration of TAG (1.08 ± 0.40 $mmol.l^{-1}$), value of HDL (0.86 ± 0.20 $mmol.l^{-1}$) and LDL (0.72 ± 0.33 $mmol.l^{-1}$) in the experimental group did not differ significantly ($P > 0.05$) compared to the control group (2.20 ± 0.62 $mmol.l^{-1}$, 0.71 ± 0.26 $mmol.l^{-1}$, 0.51 ± 0.40 $mmol.l^{-1}$ and 0.39 ± 0.28 $mmol.l^{-1}$, resp.). We assume that the particular dose of patulin used in our study was probably not enough to change all the investigated parameters.

Key words: patulin; cholesterol; TAG; HDL; LDL; rabbit

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POLYMORPHISM OF BOLA-DRB3 GENE AND ITS ASSOCIATION WITH SPONTANEOUS EMBRYONIC LOSS AFTER *IN VITRO* FERTILIZATION IN HOLSTEIN-FRESIAN CATTLE

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The primary function of the major histocompatibility complex (MHC) is the induction of the immune response. It has been also suggested that the MHC genes may play a role in mate selection. The main hypothesis about their role assumes that animal sexual preferences stem from and are driven by the pursuit of the greatest genetic diversity at MHC loci among mates which should result in heterozygous offspring. It has been confirmed by studies on rodents, lemurs, human etc. BoLA- DRB3 is the most polymorphic gene of the major histocompatibility complex in cattle. Many studies showed that polymorphism at BoLA- DRB3 locus was associated with susceptibility or resistance to some infectious diseases in cattle (mainly with susceptibility to clinical and subclinical mastitis in dairy cattle). Furthermore, in the study of Kovalyuk *et al*. (2012) it was observed that in case of using semen of bulls with the rare BoLA- DRB3 alleles, the number of semen doses per effective insemination was at least 15 % lower than in case of using semen of bulls carrying common alleles. The aim of our study was to analyse the association between polymorphism at the BoLA-DRB3 locus and embryonic loss after *in vitro* fertilization. Bovine oocytes recovered from bovine ovaries at slaughtering were matured in E199 medium with 10 % FCS and 1IU FSH and LH and fertilized *in vitro* with sperm of Holstein-Fresian bull during 20 h. Presumptive zygotes were cultured in ISM1 culture medium for three days, afterwards the embryos were evaluated and selected into two groups: 1) cleavage (2-, 4-, 6- or 8- cell stage) and 2) undeveloped embryos (no cell cleavage). The genotypes at BoLA-DRB3 locus of 72 developed and 119 undeveloped embryos were identified by PCR-RFLP method using RsaI, HaeIII, BstUI and PsuI endonucleases. From among 24 identified alleles the most frequent was DRB3.2*24 which is typical for Holstein- Fresian cattle. It was found that embryonic mortality was significantly associated with homozygosity at BoLA-DRB3 locus.

Key words: cattle; embryo; *in vitro*; MHC; BoLA gene

MORPHOLOGICAL CHANGES OF MITOCHONDRIAL-ENDOPLASMIC RETICULUM ASSOCIATION DURING *IN VITRO* FERTILIZATION OF BOVINE OOCYTES

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Bovine oocytes used for *in vitro* fertilization (IVF) are characterized by the level of both nuclear and cytoplasmic maturation. One of the most important parameters of cytoplasmic maturation is mitochondrial status of oocytes. Mitochondrial clusters are formed as large circular agglomerates around the peripheral network of endoplasmic reticulum (ER) during oocyte *in vitro* maturation (IVM). The association of mitochondrial clusters with ATP reserves has been verified. The aim of this study was to characterize the morphological changes of mitochondrial-ER association in bovine oocytes during maturation and fertilization. The oocytes were collected from the ovaries of slaughtered cows and selected according to the ooplasm and cumulus morphology. Only the oocytes suitable for IVM-IVF procedures were matured for 24 hours using a standard protocol. Subsequently, the oocytes were transferred into IVF-Talp medium and either inseminated with spermatozoa of a proven bull or cultured without spermatozoa in the same medium. Adequate number of oocytes was collected at 24 h after maturation and at 6, 12 and 18 h after insemination or culture. They were stained with Sytox-Green for both chromatin and mitochondria visualization and with Calnexin antibody for ER network morphology, and assessed by confocal microscopy. About one half of mature oocytes (49.2 %) showed mitochondrial clusters after maturation. The proportion of oocytes with mitochondrial clusters decreased significantly ($P \leq 0.01$) in the fertilized oocytes (12.5 %) but, on the other hand, it was not changed in the unfertilized oocytes (47.5 %) or oocytes cultured without spermatozoa (47.1 %) at the 6h interval. The proportion of the oocytes with clusters even decreased ($P \leq 0.05$) in the fertilized oocytes (1.1 %) or in the oocytes cultured without spermatozoa (16.7 %), but it increased to 62.1 % in the unfertilized oocytes at the 12h interval. The proportion of oocytes with clusters was not changed in the fertilized oocytes and decreased in the unfertilized oocytes (35.1) or the oocytes cultured without spermatozoa (21.1 %) at the 18h interval. It can be concluded that mitochondrial status of bovine oocytes changes during IVM-IVF. While in the fertilized oocytes the mitochondrial-ER association is fast disintegrating due to the fertilization process, in the unfertilized oocytes it is maintained for 12 hours following the oocyte contact with spermatozoa.

Key words: bovine; oocyte; *in vitro* fertilization; mitochondrial morphology

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CAPACITATION – INDUCED BULL SPERM PROTEIN CHANGES DETECTED BY A SET OF MONOCLONAL ANTIBODIES

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The process of fertilization is characterized by complex set of events. Before spermatozoa can fertilize an oocyte, it must undergo a cascade of biochemical and physiological changes that facilitate its binding and penetration into the oocyte. The sperm acquire ability to fertilize oocyte in a female

genital tract in a time-dependent process called capacitation. This process involves reorganization of membrane proteins, an increase in membrane fluidity, cholesterol efflux, ion fluxes resulting in alteration of sperm membrane potential, increased tyrosine phosphorylation of proteins and induction of hyperactivation.

In our study a set of 34 anti-sperm monoclonal antibodies (mAbs) was used to detect changes of sperm surface protein reaction patterns after capacitation. MAbs were produced using hybridoma cell lines obtained after intrasplenic immunization of BALB/c mice with intact bull sperm. Capacitation was induced by TL Sperm capacitation medium. The changes in the reaction patterns were evaluated by indirect immunofluorescence, PAGE-SDS and two-dimensional gel electrophoresis followed by detection with anti-sperm mAbs and anti-phosphotyrosine α-PY antibody to detect changes in phosphorylated protein spectra. The changes were observed in the reaction patterns of mAbs and in the percentage of reactive sperm. In the indirect immunofluorescence the percentage of reactive sperm after treating with mAbs IVA 508-1, IVA 513-16, IVA 519-19, IVA 520-4, IVA 526-7, IVA 527-10 and IVA 582 increased in comparison with control. The percentage of reactive sperm after treating with mAbs IVA 517-1, IVA 520-41 and IVA 520-42 decreased in comparison with control. Western blot analysis showed changes in molecular weight of proteins detected by mAbs IVA 519-3, IVA 520-4, IVA 520-41, IVA 520-42 and IVA 527-10. Two-dimensional electrophoresis of proteins detected by mAbs IVA 520-4, IVA 520-41, IVA 520-42 and IVA 527-10 showed that reactive region changed to less acidic region after capacitation.

Key words: bull; sperm; capacitation; mAb

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EFFECTS OF THE HPA LECTIN ON RABBIT SPERM MOTILITY AND FERTILITY

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The aim of this study was to test the effect of the HPA-lectin (the membrane biomarker with affinity to the epitope N-acetyl-α-D-galactosamine) added to the insemination dose on motility and conception rate of broiler rabbits.

The experiments were performed on males (18) and females (403) of rabbit broiler lines M91 and P91 bred at VÚŽV-NPPC Nitra and kept in a partly air-conditioned experimental hall. The CASA (Computer Assisted Sperm Analysis) system SpermVision (Minitüb, Tiefenbach, SRN) was used for the evaluation of sperm motility. To evaluate the conception rate, the control and experimental (with HPA-lectin) females were artificially inseminated (AI) with fresh heterospermic doses (0.5 ml per female). 48 hours before A.I. each female was treated with 25 I.U. PMSG (Sergon, Bioveta, Czech Republic). Immediately after the A.I. all females were intramuscularly injected with 2.5 ug of synthetic GnRH (Supergestran, Ferring Pharmaceuticals, Czech Republic). All insemination doses (ID) were diluted with a commercial diluent Minitüb (Verdünnungsmischung for Kaninchensperma with antibiotics, Germany). The sperm concentration was not less than 15.0 x 10^6 cells per insemination dose. The insemination doses were supplemented with HPA - *Helix pomatia* (garden snail, HPA) lectin (20 µl per 0.5 ml ID) and then incubated at room temperature for 0, 30 and 60 min. The differences between the experimental (with HPA lectin) and control group in the parameter VSL – straight-line velocity (µm/s) - were highly significant at 0 min ($p = 0.0015$), and a significant at 30 min ($p = 0.0497$), whilst non-significant at 60 minutes (0.4914). The motility of spermatozoa was increasing at 0 and 30 min and decreasing at 60 min. Therefore, for female insemination sperm samples at 30 min incubation with HPA lectin were chosen. Average number of live-born kits (8.93 pcs) and conception rate (56.35 %) in the group with HPA lectin were not significantly different compared to the control group (8.21 pcs; 53.79 %, resp.). The obtained results suggest a positive trend of HPA-lectin effect on sperm motility. However, HPA-lectin did not improve female fertility compared to control. In conclusion, although HPA-lectin seems to be suitable additive to improve rabbit sperm motility, however motility parameters may not be in direct association with rabbit female fertility.

Key words: HPA-lectine; rabbit; sperm; motility; conception rate; fertility

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PRESENCE OF INTEGRIN MOLECULE CD18 IN THE CATTLE REPRODUCTIVE SYSTEM

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Integrins are transmembrane receptors that are the bridges for cell-cell and cell-extracellular matrix (ECM) interactions. Integrins have two different chains, α (alpha) and β (beta) subunits. In mammals, there are eighteen α and eight β subunits. A given chain may combine with multiple partners resulting in different integrins. In molecule CD18 the β chain may be paired with four different α subunit (11a, 11b, 11c, 11d). CD18 is important in adhesive interactions of leukocytes and endothelial cells. In cattle the mutation in CD18 gene is leading to BLAD (Bovine Leukocyte Adhesion Deficiency) which causes extreme susceptibility to infection of diseased animals. Participation of CD18 in cell-cell adhesion suggested the possible role of this molecule in spermatozoa – egg fusion or in other steps of reproduction processes. The possible role of CD18 (integrins) in reproduction was studied in man and some animal species. Several experimental results support the involvement of egg integrins during sperm - egg interaction.

It was found by immunofluorescence studies that both hamster and human oocytes expressed CD11b/CD18 (MAC/1) a β2 class antigen, which could mediate the sperm egg binding (Fénichel and Durand-Clément, 1998). The aim of this study was to identify the distribution of the CD18 molecule on bovine gametes and reproductive tissues.
Tissues for the histochemical staining: bovine testis, epididymis, ovary and accessory glands have been obtained at local slaughterhouses. Ejaculated spermatozoa were received from Slovak Breeding Services Inc. The oocytes at different developmental stages were obtained after the culture in *in vitro* maturation medium. The CD18 molecule was detected by indirect immunoperoxidase test (tissue sections and oocytes) and immunofluorescence test (spermatozoa) using IVA-35 the anti-CD18 monoclonal antibody. The presence of CD18 on spermatozoa and male reproductive tissues was analysed during the spermatocytogenesis in the seminiferous tubules of the bull testis and different parts of epididymis. In the cross-sections of the testes and epididymis tested in immunoperoxidase assay no positive reaction was found either in the tested tissue or in the developmental forms of spermatozoa. Positive reaction was found in luminal secrets of the epididymis, probably with soluble form of CD18. In the immunofluorescence assay only the minor population of ejaculated spermatozoa was slightly reactive. In the cow reproductive tract the reaction with CD18 molecule was found in the horn of uterus; the follicular and *in vitro* cultured oocytes were also positive.
The study demonstrates the presence of CD18 in some organs of the reproductive tract of cattle, but the expression of CD18 molecules on gametes was not detected unambiguously, although weak reaction of cow oocytes with CD18 antibody was noted.

Key words: cattle; reproductive tract; CD18; integrins

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NUCLEOLOGENESIS IN EARLY PORCINE EMBRYOS

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The initial development of mammalian pre-implantation embryos is governed by gene transcripts and polypeptides produced by and stored in the oocyte during its development. The transition from maternal to embryonic control is accompanied by a series of morphological and physiological changes. The nucleolus is the organelle that morphologically reflects changes in the metabolism and physiology of the cell. Our study investigated nucleolar dynamics (as one viability marker) in porcine embryos developed *in vivo* (IVD) and compared this physiological standard to that of embryos produced by *in vitro* fertilization (IVF), parthenogenetic activation (PA), or somatic cell nuclear transfer (SCNT). At the ultrastructural level porcine IVP zygotes and embryos display a well-synchronized pattern of chromatin dynamics compatible with genome activation and regular nucleolar formation at the four-cell stage. Production of porcine embryos under *in vitro* conditions (IVP) by IVF, PA, or SCNT is associated with altered chromatin remodeling, delayed nucleolar formation, and poorly defined lineage segregation at the blastocyst stage. The intranuclear localization of nuclear proteins is observed towards the end of the third cell cycle in IVD. IVP embryos lack labelling for topoisomerase I, and the allocation of remaining nuclear proteins is delayed by one cell cycle. Nucleologenesis in porcine IVP embryos is markedly disturbed and this may be one reason for the high rate of embryonic and fetal mortality.

Key words: nucleolus; porcine pre-implantation embryos; viability

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EFFECT OF SELECTED PLANTS ON RABBIT OVARIAN FUNCTIONS

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Rooibos, chia and yucca are plants widely used in folk medicine, food and drink production, but their influence on reproductive processes has been studied insufficiently. The aim of this study was to examine possible action of rooibos, chia and yucca on the release of hormonal regulators of reproduction (progesterone, P4, testosterone, T, estradiol, E and insulin-like growth factor I, IGF-I) and on fecundity in rabbits. Fragments of rabbit´s ovaries were incubated with rooibos, chia and yucca for 48 hours. Hormones were determinated by RIA. It was observed that rooibos addition to the culture medium inhibited P4 and T release. IGF-I release was significantly stimulated by rooibos at all doses added. Moreover, chia addition had inhibitory effect on P4 and T release, but not on IGF-I release. P4 release was stimulated after yucca treatment at all doses added. Yucca addition did not affect testosterone or estradiol release. Preliminary *in vivo* experiment showed that feeding of rabbits with yucca significantly increased their conception and kindling rate. The differences between control and yucca-treated groups in the number of liveborn, stillborn and weaned pups per doe

were not statistically significant. Results of this study suggest the multiple sites of action (release of hormones, conception and kindling rate) of these medical plants on rabbit reproductive functions and their potential applicability for improvement of reproductive efficiency.

Key words: rabbit; ovary; plant extract; reproduction

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THE EFFECT OF CROSSING TSIGAI EWES AND IMPROVED VALACHIAN WITH LACAUNE EWES ON MILKABILITY

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Sheep farming has a long tradition in Slovakia. In the 11th-12th centuries, sheep constituted 5 % of the total number of bred animals. Their original use was in the production of milk, meat, wool and fur. However, besides the production of lamb meat, only the production of milk is economically interesting today. Dual-purpose breeds Tsigai (TS) and Improved Valachian (IV) are the most-common sheep breeds in Slovakia. They have lower milk production than typical milk breeds (e.g. Lacaune-LC), but they are more robust what is important for our breeding conditions. At present, in order to improve their milk yield and milkability TS and IV breeds are crossed with Lacaune. The aim of this work was to summarise knowledge from our laboratory related to the evaluation of milkability of main sheep crossbreds used in Slovakia (project MLIEKO No. 26220220196, KEGA 006SPU-4/2014). It was demonstrated that crossbreds have higher machine milk yield and total milk yield than purebred TS and IV, but there is a tendency of higher machine stripping yield compared to the purebred animals. The effect of breed is also evident in different occurrence of milk flow types indicating different physiological response of ewes to milking stimulation. From three types of milk flow curves (1 peak - no response, 2 peak - positive response, and plateau - indicating high milk production and possible positive response) classified in sheep during machine milking, the lowest occurrence of 1 peak milk flow was noted in crossbred animals as compared to purebred animals. This can indicate a possibly higher sensitivity to udder stimulation by machine and better adaptation of both crossbred ewes to machine milking than purebred TS and IV. However, the crossing with LC negatively influenced the teat position in TS × LC and IV × LC crossbreds causing above mentioned higher stripping yield of milk. This should be taken into consideration for future breeding programmes. With the crossing, some parameters of milkability had been improved, but some parameters of udder morphology had been worsted, what could negatively affect milk out of the udder without stripping. When machine stripping yield is high, the intervention of the milker during machine milking is necessary. This may lengthen the time of milking and reduce labour efficiency during milking.

Key words: dairy ewes; crossing; milkability; udder morphology

IMMUNOPHENOTYPING OF THE RABBIT ADULT STEM CELLS

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There are three criteria that cells require to meet in order to be qualified as a stem cell: 1) they need to be capable of self-renewal, i.e. undergoing symmetric or asymmetric divisions; 2) a single cell must be capable of multilineage differentiation and 3) capacity for *in vivo* re-population and functional reconstitution of a given tissue. Stem cells are able to differentiate into cell types beyond the tissues in which they normally reside. This is often referred to as stem cell plasticity. Stem cells are also believed to be slow cycling but highly clonogenic and generally represent a small percentage of the total cellular make-up of a particular organ. Adult stem cells (ASCs) are multipotent stem cells that can differentiate into a range of cell types related to the tissue of origin of these cells, so that their differentiation potential is limited compared to pluripotent stem cells. They constitute a pool of cells that proliferate and differentiate into cells required to maintain the integrity of the tissue, especially in case of injury. Most common ASCs used in biomedicine are hematopoietic (HSCs), mesenchymal (MSCs) and amniotic fluid stem cells (AFSCs). HSCs generate all the blood cells and can thus be considered as being multipotent and capable of regenerating the complex hematopoietic system. Although the phenotype of human and mouse HSCs is well-known, the phenotypic expression of the rabbit HSCs is still unclear. Regarding the MSCs, according to the International Society for Cellular Therapy, the human MSCs are defined as cells that are (i) plastic adherent; (ii) express typical surface molecules e.g. CD105, CD73 and CD90 etc.; and (iii) can differentiate into osteogenic, chondrogenic and adipogenic lineages. Rabbit MSCs share some markers with human MSCs. AFSCs clearly display a unique phenotype that is mostly multipotent but borders on pluripotency. Their phenotype is very similar to that of MSCs. Adult stem cells can be isolated using different methods, e.g. magnetic-activated cells sorting (HSCs, MSCs) or plastic adherence (MSCs, AFSCs). These cells could be then identified and recognized through:

(i) their morphology observed by phase contrast microscopy; (ii) ultrastructural analysis using transmission electron microscopy (TEM); (iii) CD marker expression, detected using immunostaining, reverse transcription-polymerase chain reaction (RT-PCR) and flow cytometry analysis; and (iv) differentiation ability using a commercial assay (e.g. osteogenic, adipogenic and chondrogenic differentiation assay; Tan *et al*., 2013). The importance of the animal ASCs for the agricultural applications lies in the preservation of the animal gene resources via these cells.

Key words: rabbit; adult stem cells; FACS; PCR; TEM

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DIETARY EFFECT OF *LIPPIA CITRIODORA* EXTRACT ON SEMEN QUALITY CHARACTERISTICS IN MALE HARES (*Lepus europaeus Pallas*, 1778)

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Natural antioxidants have been widely reported to have potent antioxidant, anti-inflammatory, and antimicrobic activities related especially to their phenolic content.

The aim of the present study was to evaluate the effect of dietary supplementation with a natural extract of *Lippia citriodora*, titrated in verbascoside, on some quality traits of semen in male hare. Welfare status of animals was also monitored.

Hares were randomly divided into four groups of 3 animals each, homogeneous by age and body weight, and fed *ad libitum* and free access to water until the end of the trial. Animals were fed for 240 days a commercial diet assigned to four dietary treatments: control diet (CON) and diet supplemented with 1 $g.kg^{-1}$ of natural extract (low natural extract - LNE) or 1.5 $g.kg^{-1}$ of natural extract (medium natural extract - MNE) or 2 $g.kg^{-1}$ of natural extract (high natural extract - HNE). All hares were subjected to the following experimental measurements: weekly pattern of feed intake, body weight and blood samples at 0 days and a 240 days of trial, and semen collection at 180 days, 210 days and 240 days of trial.

The body weight and feed intake of the hares were not affected by the experimental treatment. At the end of the trial, sperm volume, pH and sperm concentration values were not affected by *Lippia citriodora* extract treatment, and the mean values recorded were 0.543 ml, 7.4 and $263.25x10^6$ per ejaculate, respectively. The dietary treatment negatively affected ($P < 0.05$) the sperm motility values in LNE, MNE and HNE groups.

In conclusion, the results of the present work underline a possible negative effect of the *Lippia citriodora* extract on the semen quality characteristics, besides the improvement in welfare status of the treated hares, expressed by a better lipid profile and improved plasma oxidative markers.

Key words: antioxidant supplement; biochemical parameters; hare spermatozoa

CONSERVATION OF ANIMAL GENETIC RESOURCES IN THE REPUBLIC OF SERBIA

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Organized work on the conservation of animal genetic resources in Serbia started in late 1994 by identification and creation of inventory of existing domestic animal breeds and strains. There were a total of 35 identified and described breeds of cattle during this period, and data on them are forwarded to the central database of FAO, Rome. Conservation of indigenous breeds and strains started at the same time. For each breed included in the program of conservation it is anticipated to form three herds in accordance with the FAO program.

Agricultural Strategy of the Republic of Serbia (2005) contains elements related to the conservation and sustainable use of biodiversity, i.e. animal genetic resources. The main elements of this strategy are related to: identification and monitoring of biodiversity and processes, i.e. activities that have a significant negative impact on the state of biodiversity *in situ* and *ex situ* conservation of biodiversity, access to genetic resources, access to biotechnologies and their transfer, exchange of information, technical and scientific cooperation in the field of conservation and sustainable use of biodiversity, distribution of benefits and others.

Criteria of breed selection for conservation must be multiple and well and reasonably chosen. Criteria must respect the potential value of the breed, that is, the genetic constitution and eventually useful genes for future research at breeder discretion. The possibility of losing a breed is also one of the important criteria, because once lost genes or gene combinations can never be brought back in any way. In addition to these criteria, we should take into account the economical, social, cultural and other aspects of conservation. When it comes to population size, analysis of domestic animals species and breeds encountered in the Republic of Serbia shows that many of them are endangered and disappearing: Podolian cow, Busha cattle, Domestic buffalo, Mountain horse, Nonius, Balkan donkey, Mangalitsa, Morava pig, Resava pig, Pirot sheep, Bardoka sheep, Krivi Vir sheep, Karakachan sheep, Lipa sheep, Valachian sheep, Choka Tsigai sheep, Balkan goat, Svrlig chicken, Banat naked-neck chicken and Sombor capor chicken.

Key words: animal biodiversity; genetic resources; conservation

YUCCA SCHIDIGERA PLANT EXTRACT NEGATIVELY AFFECTS RABBIT EMBRYO DEVELOPMENT *IN VITRO*

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The goal of this study was to examine the effect of *Yucca*

schidigera plant extract (YSE) on the rabbit embryo development *in vitro*. Zygotes at the pronuclear stage were flushed from the oviducts of hormonally stimulated rabbit females and subsequently cultured *in vitro* in k-DMEM medium supplemented with 10 % of fetal bovine serum and different concentrations of YSE (E1 - 0,1 $mg.ml^{-1}$; E2 - 0,01 $mg.ml^{-1}$; E3 - 0,001 $mg.ml^{-1}$) up to the blastocyst stage (120h). At the end of culture period the blastocysts were stained with DAPI fluorochrome for the total cell number determination.

Although there were no significant differences between the experimental (E1 - 38.7 ± 23.6; E2 - 56.7 ± 36.6; E3 - 44.6 ± 32.1) and the control (C - 59.4 ± 30.0) groups in the blastocyst rate, our results suggest that increasing of YSE concentration by 0.1 $mg.ml^{-1}$ negativelly affected developmental potency of embryos. Negative effect of the highest concentration of YSE (0.1 $mg.ml^{-1}$) in the culture medium was manifested also in the decrease ($P < 0.05$) of blastocyst total cells number (E1 - 68.4±14.2), when compared to the control (C - 90.6 ± 21.0). Lower concentrations of YSE in culture medium had no effect on the blastocyst total cell number (E2 - 80.2 ± 25.6; E3 - 81.8 ± 16.1) compared to the control (C - 90.6 ± 21.0). According to our results we can conclude, that higher concentration of *Yucca schidigera* plant extract added to culture media negatively affected embryo total cell number and blastocyst rate.

Key words: rabbit; embryo; *Yucca schidigera* extract; *in vitro* development

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THE REPRODUCTION AND GROWTH CHARACTERISTIC OF ORAVKA CHICKEN

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The reproduction and growth characteristics of two inbred (OR 1 and OR 2) and one outbred (OR 3) lines of Oravka chicken were compared. Oravka is a dual-purpose chicken breed. There are important not only egg production but also the meat production. The *in situ* conserved flock kept at the National Agriculture and Food Centre - Research Institute for Animal Production (RIAP) Nitra was involved in the experiment. All three lines were of the same origin. The males and females were progeny of the same cock.

The females were randomly divided into three lines. In two lines the males from the same lines – half-sibling were used as father. In the third line the non-relative cock from the private breeder was used as a father. The reproduction (fecundity, embryonic mortality, hatchability) and growth characteristics were examined.

We observed the highest hatchability and lowest embryonic and postembryonic mortality in the outbred line. In inbred lines we also observed more chicken that were culled due to degenerative changes of limbs, substandard color etc. In the outbred line this fact was observed rarely.

The fecundity in lines OR 1, OR 2 and OR 3 was 89.62 %, 84.21 % and 85.71 % respectively. The hatchability from fertilized egg was 80.50 %, 80.26 % and 91.67 % respectively. The chicken of outbred line had higher live weight in all categories when compared to inbred lines. Significant difference was recorded between outbred line OR 3 (450.47 ± 64,45 g) and inbred line OR 1 (491.43 ± 100.30 g) at 5 weeks age ($P = 0.0027$).

According to our results we can conclude that outbred line had better reproduction characteristics than inbred lines. The outbred line chickens had higher live weight than inbred ones.

Key words: Oravka breed; reproduction; growth

MOLECULAR SCREENING FOR SINE INSERTION IN THE MITF GENE IN DOGS

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SINEs (Short Interspersed Elements) are mobile transposons of approximately 100-400 bp. SINEC_Cf is the most abundant canine -specific SINE in dog genome and it is estimated to be present in half of all genes.

Most SINE insertions induce no damage to the host genome, because only a small part of canine genome is functional. The results of many genetic studies suggest that SINE insertions play a significant and major role in mammalian evolution and the phenotypic diversity of dog breeds. Occasionally, SINEs insertion in coding regions can disrupt ORFs, modulate gene expression, alter splicing, create genomic deletions or have a post-insertional impact through unequal homologous recombination.

A SINEC_Cf insertion located 3.5 kb upstream of the MITF 1M promoter of the MITF gene causes piebald spotting in numerous canine breeds due to the predicted altered transcription of the MITF-gene (Schmutz *et al*., 2009). Here we present a newly developed efficient strategy for targeted rapid SINEC_Cf identification by RFLP analysis of the MITF gene.

Key words: MITF; SINE; coat color; canid

EFFECT OF AMYGDALIN AND APRICOT KERNELS ON DEVELOPMENTAL RATE AND QUALITY OF RABBIT EMBRYOS

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The aim of our study was to examine the effect of amygdalin and apricot kernels on developmental rate and quality of rabbit embryos. In our experiments 12 rabbit females (Californian rabbit) were used. The animals were divided into three experimental groups (IM, D1, D2) and the control group. The IM group was intramuscularly injected with amygdalin isolated from apricot kernels (≥ 99 % purity; 0.6 $mg.kg^{-1}$ of live weight); D1 and D2 groups were fed by a mixture of commercial diet and apricot seeds (D1: 60 $mg.kg^{-1}$ of live weight, D2: 300 $mg.kg^{-1}$ of live weight) during 4 weeks period. A total of 135 pronuclear stage zygotes were collected from superovulated rabbit females 19 h *post coitum* and cultured

under *in vitro* conditions (38.5 °C, 5 % CO_2) for 72 h to reach blastocyst stage. Afterwards, these embryos were analyzed for the developmental rate, embryo cell number (DAPI) and incidence of the dead (propidium iodide, PI) or apoptotic (Yo-Pro-1) cells using fluorescence labelling. A one-way ANOVA were used to analyze differences between groups, and data shown are least squares means ± standard error of the mean. No significant differences were found in the blastocyst stage between control (C; 77.14 ± 19.04) and experimental (IM – 60.6 ± 22.8; D1 – 73.53 ± 30.12; D2 - 66.97 ± 20.91) groups. In regards to the embryo quality, average total cell number in the experimental groups (IM – 100.42 ± 16.16; D1 - 56.07 ± 15.65; D2 - 102.02 ± 29.11) did not differ significantly from control group (83.3 ± 13.15). Also, no any differences were noticed in the incidence of dead or apoptotic cells between these groups. In conclusion, amygdalin had no effect on developmental potential and quality of rabbit embryos.

Key words: rabbit; embryo; amygdalin; apoptosis

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CHARACTERIZATION OF MACROPHAGES IN RABBIT SEMEN

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We aimed at evaluating the occurrence of macrophages in rabbit semen and investigating their impact on the spermatozoa quality. We compared the detection methods using Neutral Red dye, fluorescently conjugated acetylated low-density lipoprotein (AcLDL) and monoclonal antibody CD14. Fresh semen samples collected from broiler rabbit lines M91 and P91 (n = 30) were used. Subsequently, the rabbits were divided into two groups according to semen macrophage concentration, and semen quality was compared in two heterospermic samples. We applied Computer Assisted Semen Analysis (CASA) system to determine motility parameters. Sperm viability parameters, such as occurrence of apoptotic (Yo-Pro-1) and dead/necrotic (propidium iodide) sperm and damage to sperm plasma membrane integrity (PNA) were determined using flow cytometry. Obtained results were evaluated statistically by t-test using SigmaPlot software and expressed as the means ± SEM. The concentration of macrophages in the control group was 0.24 ± 0.06 x 10^6.mL^{-1} (n = 16), whilst in the experimental group it was 9.02 ± 0.29 x 10^6.mL^{-1} (n = 4). Concerning the used methods, no significant differences in the number of identified macrophages either by Neutral Red, AcLDL or CD14 methods were found: 0.21 ± 0.11 vs. 0.25 ± 0.07 vs. 0.23 ± 0.02 in the control, and 8.82 ± 0.38 vs. 9.15 ± 0.32 vs. 9.08 ± 0.19 x 10^6.mL^{-1} macrophages in the experimental groups, respectively. The total motility and progressive movement were decreased in the experimental group ($P < 0.001$). Significantly increased proportions of the apoptotic and necrotic spermatozoa and spermatozoa with disruptions of the plasma membrane integrity in the experimental group were noticed. In conclusion, staining of semen macrophages using either of the methods (Neutral Red, AcLDL dyes or CD14 monoclonal antibody) is similarly reliable. Based on our results, higher presence of macrophages in rabbit semen may have negative effect on some parameters of spermatozoa evaluated *in vitro*.

Key words: macrophages; rabbit; spermatozoa; CASA; flow cytometry

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QUANTIFICATION OF BOVINE GENES INVOLVED IN LIPID METABOLISM USING REAL-TIME RT-PCR

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Molecular genetic markers underlying the complex traits of meat production in cattle were subjected to extensive scientific research in the last few decades because the genes involved in regulation of growth and quality of carcass are important in terms of profitability for farmers as well as for beef industry. The aim of this preliminary study was to analyze the developmental change in the relative gene expression of both genes involved in fatty acid metabolism (*ACACA, DGAT1, FABP4*) and myogenic regulatory factors *(MYF5, MYOD1* and *MYOG*) in the longissimus lumborum muscle (MLL) of Czech Fleckvieh Cattle throughout its life cycle. Acetyl-CoA carboxylase alpha (*ACACA*), Diacylglycerol O-acyltransferase 1 (*DGAT1*) and Fatty acid binding protein 4 (*FABP4*) take part in triglyceride synthesis and play an important role in lipid metabolism. They are associated mainly with variations in the fatty acid (FA) contents in muscles, intramuscular fat deposition and beef marbling. Myogenic factor 5 (*MYF5*), Myogenic differentiation factor 1 (*MYOD1*) and Myogenin (*MYOG*) belong to the myogenic regulatory factor family of transcription factors that regulate myogenesis, skeletal muscle differentiation and fibre development in Vertebrata. Their genes are suitable candidates for molecular markers of growth, economically relevant body measurement traits and meat production traits in cattle. In this study the biopsy samples of the MLL were collected at age of six or twelve months from three bulls and three heifers of Czech Fleckvieh cattle. The animals were sampled also immediately after slaughtering at the age of 18 mo. Relative levels of target and reference gene mRNA were determined using two-step real-time reverse transcription qPCR with gene specific Taqman hydrolyses probes. Analysis of qPCR experimental data was carried out automatically with qBase+ Premium software (Biogazelle, Belgium). The relative expression was calculated for each sample as a ratio of the target

gene mean *Cq* (threshold cycle) to the 3 reference genes mean *Cq* using the Pfaffl formula. The relationship between the particular gene expressions was evaluated by means of Pearson´s correlation coefficient. Results pointed out the differences among relative levels of mRNA transcripts of selected genes with regard to age and sex of animals. A higher relative gene expression was measured in the biopsy samples from heifers (i.e. at age 6 or 12 mo) compared to their slaughter samples (i.e. at age 18 mo). In addition, the expression levels found out in the 18 mo heifers were the lowest and simultaneously the most balanced throughout the study. Gene expressions ascertained in bull samples were the highest in their early age and at once in the 18 mo of age and lowest in the 2nd biopsy samples at the age of 12 mo. The RNA transcripts of the myogenic regulatory factor genes were expressed at a higher level than genes of fatty acid metabolism (except of *FABP4*) in the first taken biopsy samples. Expression of the FA metabolism genes was consistent in the biopsy samples of heifers but their considerable decrease was apparent in bulls of 12 mo. Then the oldest bulls showed again the elevated expression of the FA metabolism genes. Pearson´s correlation test revealed a strong positive correlation among all myogenic regulatory factor genes, ranging from 0.795 to 0.953, which indicates their synchronized action in the bovine muscle metabolism. Correlations between the relative gene expressions of *ACACA* and *DGAT1* or *FABP4* were positive and reached rather the middle level around 0.5. Although the experimental design of the study was proved as suitable, a further research is needed to expand these preliminary findings.

Key words: biopsy; cattle; fatty acid; genetic expression; muscle; myogenic factor

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VIABILITY OF RABBIT MESENCHYMAL STEM CELLS DURING EARLY PASSAGING

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Rabbit is a suitable biological model for stem cell experiments due to cellular and tissue physiology which closely resembling human mesenchymal stem cells (MSCs). However, human MSCs are well defined by surface markers; the expression of specific markers is the matter of many studies of rabbit stem cells. The aim of our preliminary study was to evaluate viability and apoptosis of MSCs from early passages (P1-P3) and to detect surface marker proteins that identify MSCs. Briefly, we harvested rabbit mononuclear bone marrow cells of three New Zealand White line rabbits. Cells were separated by gradient density centrifugation. Cells from the suspension were counted, mixed with α-MEM medium supplemented with 20 % FBS and seeded into tissue culture flasks. Medium exchanges were performed every 3-4 days. Upon reaching about 70 % confluence MSCs were trypsinized. MSCs from passages P1, P2, P3 were stained by Annexin-V, Yo-Pro-1 and PI to test the viability and apoptosis by fluorescent microscopy and flow cytometry. Cells from P3 were used for detection of surface markers. Our MSCs were positive to CD44 and CD29 surface markers, commonly used to profile MSCs. We also used CD90 and CD45 markers as a negative control. Fluorescent microscopy revealed that early apoptotic cells had the trend of decreasing incidence from P1 to P3. The proportion of early apoptotic cells stained by Annexin-V was lower in P2 than in P1 ($P < 0.05$) and also decreased from P2 to P3 ($P < 0.01$). Using Yo-Pro-1, we revealed that apoptotic incidence was lower in P3 compared to P1 ($P < 0.05$). On the other hand, using flow cytometry wet observed statistically significant decrease in Annexin-V positive cells in P3 compared to P1 ($P < 0.01$). Fluorescent microscopy revealed a decreasing trend in apoptosis rates related to passaging with more statistically significant results. But this trend was not observed using flow cytometry, which is considered to be more reliable and accurate method, compared to fluorescent microscopy. These observations suggest that the passaging of stem cells does not affect their viability.

Key words: rabbit; MSC; viability; passaging

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TLR4 GENE POLYMORPHISM PRESERVED IN TWO CATTLE BREEDS OF GENETIC RESOURCES

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Allelic variants of disease resistance genes in the historical animal breeds are supposed to reflect local infection pressure. They represent a reservoir for breeding programmes and help to counteract gene pool erosion. Therefore, screening for the diversity of innate immunity receptors belonging to the Toll-like receptors (*TLR*) family was carried out in two cattle breeds. The survey covered limited herds of Czech Red and Czech Red Pied included in the conservation programme. The polymorphism has been discovered with high-throughput sequencing of pooled PCR amplicons from coding regions of all bovine *TLRs* using the PacBio platform. The detected SNPs were subsequently validated with Sanger sequencing and appropriate genotyping techniques (PCR-AFLP, ARMS). In the case of *TLR4*, whose product participates in the early recognition of G-negative bacterial pathogens, eight SNPs were validated in the coding and adjacent regions. In spite of long reads from 700 to 1200 nt, which are characteristic for the PacBio platform, the phasing of SNPs was mostly based on the calculation by the PHASE programme. The probabilistic approach predicted 18 haplotypes, what is a significantly higher diversity than reported for the European production breeds. Although three haplotypes were shared by the two breeds at similar frequencies, nine haplotypes showed preference for the Czech Red and six for the Czech Red Pied cattle. Haplotype B1, common in European breeds, was greatly reduced in Czech Red. Although the haplotype frequencies might have been distorted by the bottleneck in the history of both populations, the ancient breed Czech Red appears to harbour more haplotype diversity than the Czech Red Pied as a Simmental variant. This difference could correspond to the phenotypic features of the local breeds and the speculative association of the Czech Red breed with short-horn aurochs.

A picture will be clearer after inclusion of validated SNPs from the remaining members of the *TLR* family. In view of the presence of production herds of Czech Red Pied in parallel to the conserved nucleus herd, the effect of intensive breeding on the *TLR* diversity can be evaluated in this case. The processing of the results also confirmed the advantages of using the PacBio technology for resequencing of genes of interest. Limited capacity of runs is outweighed by the length of reads, single-strand sequencing and a low error rate, which are helpful in subsequent SNP discovery and direct phasing.

Key words: cattle; genetic resources; innate immunity; Toll-like receptors; haplotypes

EFFECT OF CRYOSTORAGE LENGTH ON FERTILIZING ABILITY OF PINZGAU BULL SEMEN *IN VITRO*

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The aim of the study was to evaluate fertilizing ability of Pinzgau bulls semen after different periods of storage in liquid nitrogen. The semen samples of 15 bulls were divided according to cryostorage length into the three groups: less than 7 years (group 1), 7 to 13 (group 2) and 14 or more years (group 3). Straws with frozen semen were thawed in a water bath and the motile fraction of sperm was isolated by modified swim-up method. Motile sperm ($2x10^6.ml^{-1}$) were co-incubated with matured bovine oocytes in a fertilization drops (IVF-TALP) under mineral oil at 38.5 °C in presence of 25 $\mu g.ml^{-1}$ heparin for 20 h. Totally, 739 oocytes were used for *in vitro* fertilization test. Following fertilization the presumptive zygotes were stained with DAPI nuclear stain and status of chromatin was examined under fluorescence microscope. Penetration of sperm heads into the ooplasm of oocytes and formation of pronuclei were evaluated. No significant difference in fertilizing ability among the experimental groups was found. The total rates of penetrated/fertilized eggs for group 1, 2 and 3 were 79.31, 76.40 and 76.14 %, respectively. Significantly higher pronuclear formation was observed in the group 1, where 72.9 % of eggs have two visible pronuclei at 20 hours post-insemination compared to 65.72 and 47.21 % in groups 2 and 3, respectively. Also, syngamy of both pronuclei was observed in 4 % of fertilized eggs from group 1 (20 hpi), probably due to faster pronuclear formation, whilst no syngamy was observed in groups 2 and 3. In conclusion, length of cryostorage had no direct influence on penetrating and fertilizing ability of long-term cryostored Pinzgau bull semen. However, long-term cryostorage can affect speed of pronuclear formation.

Key words: Pinzgau; bull; sperm; fertility; cryopreservation

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EFFECT OF PUNICALAGIN AND FSH ON PORCINE OVARIAN GRANULOSA CELLS *IN VITRO*

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Punica granatum (Pomegranate) is one of the oldest edible fruits in the world, which contains number of bioactive compounds like ellagitannins, polyphenols etc. Punicalagin is the predominant ellagitannin of pomegranate and is presented in form of two anomers - punicalagin α and β. Punicalagins are contained in husk, pulp or seed. Punicalagin is metabolised to ellagic acid (antioxidant), and microorganisms of colon can metabolize ellagic acid to urolithins. Punicalagin or its metabolites are able to induce changes in intracellular mechanism of ovarian cells. These compounds can be protective agents; however at higher concentrations it can have opposite action on viability of animal cells. The aim of our *in vitro* study was to examine effect of punicalagins or their metabolites in combination with follicle-stimulating hormone (FSH) on the secretion of steroids hormones (progesterone and 17β-estradiol) by porcine ovarian granulosa cells. Granulosa cells from the ovaries of pre-pubertal pigs were cultured at various doses of punicalagin (0.01, 0.1, 1, 10 and 100 $\mu g.ml^{-1}$) and FSH (10 $ng.ml^{-1}$) during 24 h. Steroid hormones of female reproductive system - progesterone and 17β-estradiol were determined by Enzyme-linked immunosorbent assay (ELISA, Multiscan FC, ThermoFisher Scientific, Vantaa, Finland). Secretions of progesterone and 17β-estradiol by granulosa cells were insignificantly ($P \geq 0.05$) affected by punicalagin and FSH treatments at all used doses. In this preliminary study punicalagin in combination with FSH at tested doses did not affect secretion of progesterone or 17β-estradiol by porcine ovarian granulosa cells *in vitro*.

Key words: 17β-estradiol; follicle-stimulating hormone; granulosa cells; progesterone; punicalagin

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INFLUENCE OF POLYMORPHISM G.9476869G>A IN MYELOPEROXIDASE (MPO) GENE ON THE ANTIOXIDANT ACTIVITY IN MILK OF POLISH HOLSTEIN-FRIESIAN COWS

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The milk is a good source of proteins, fat and vitamins, as well as anti-oxidant compounds. The major antioxidants present in milk are vitamins A, C and E, carotenoids, coenzyme Q10, conjugated linoleic acid (CLA), whey protein, peptides and enzymes: catalase, lactoperoxidase, glutathione peroxidase and superoxide dismutase. The enzyme with antioxidant activity is myeloperoxidase (MPO).

The aim of this study was an identification of polymorphism in the myeloperoxidase gene and determination of its influence on the antioxidant activity in milk of Polish Holstein-Friesian cows. In addition, we attempted to associate the MPO gene polymorphism with milk production traits. For this purpose, the antioxidant activity of milk was measured using TEAC (Trolox Equivalent Antioxidant Capacity) method. To identify polymorphism in the MPO gene a PCR-RFLP method was used. g.9476869G>A polymorphism of the MPO gene was identified in the analyzed herd of cows. Statistically significant associations were found between genotype and antioxidant activity of milk and average daily milk yield of cows.

Key words: cattle; Polish Holstein-Friesian; MPO gene; milk; antioxidant activity

CONTAMINATION OF FLUSHING MEDIUM BY STENOTROPHOMONAS MALTOPHILIA AFFECTS THE QUALITY OF SHEEP EMBRYOS

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Increasing use of gametes, progress in micromanipulations with oocytes and embryos as well as advances in gamete cryopreservation and long-term storage in liquid nitrogen represents an increased risk of contamination of germ cells by bacteria *Stenotrophomonas maltophilia*. The main goal of our work was to verify the influence of possible bacterial contamination of flushing facilities and medium in the MOET (multiple ovulation and embryo transfer) system on the yield and quality of sheep embryos. Influence of a possible contamination on a quality and quantity of ovine embryos was monitored on a group of 16 ewes. For our analysis we used the primocultivation method using MacConkey agar and XLD agar. We evaluated the colony growth and microscopic findings by Gram staining. The presence of bacterial contamination was proved by biochemical test NEFERMtest 24. Contamination of flushing medium by *Stenotrophomonas maltophilia* markedly affected the yield as well as quality of embryos. In the group of intact ewes we observed an average number 4.625 of total embryos and 4.125 of transferable embryos. In the group of *Stenotrophomonas maltophilia* infected sheep we observed the average number 1.625 embryos and only 0.625 were transferable.

Key words: bacteria; embryos; flashing medium; sheep

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EVALUATION OF PINZGAU BULL SPERM FOLLOWING LONG-TERM STORAGE IN LIQUID NITROGEN

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Cryopreservation of livestock semen has been used to improve the breeding of animals of genetic importance, and has contributed to the conservation of endangered species. For the cryobank establishment and operation it is reasonable to control long-term storage effects on post-thaw survival of mammalian sperm. The aim of the study was to examine sperm viability of Pinzgau bull frozen-thawed sperm following long-term storage in liquid nitrogen. Insemination doses, provided by Slovak Biological Services Inc. (Lužianky, Slovak Republic), were slow-frozen and stored in containers with liquid nitrogen for 1 -18 years. The sperm samples were divided into three groups according to the length of storage: A (≤ 7 years), B (8-13 years) and C (≥ 14 years). Post-thaw sperm assessment of total motility (TM; CASA analysis), apoptotic and dead/necrotic sperm occurrence (fluorescent probe YoPro-1 and propidium iodide) and sperm morphology (light microscopy) was performed immediately after thawing in water bath at 37 ± 1 °C for 1 min. No significant influence of storage length on the sperm motility of Pinzgau bulls was noted. The post-thaw total motility in all the groups was about 40 % i.e. in accordance to the commercial insemination dose standards (post-thaw TM ≥ 30 %). Proportion of apoptotic sperm ranged from 21 to 23 % and occurrence of necrotic/dead sperm was at the level of 27-30 % with no significant differences among the groups. In terms of morphological changes, most of the individuals examined demonstrated morphology in accordance to the commercial insemination dose standards (malformation rate ≤ 20 %) with no significant differences between groups. The significant differences noted were rather due to inter-male variability in susceptibility of sperm to the same freezing-thawing protocol used, than due to storage itself. Therefore, it can be suggested that individual differences are an important factor that should be taken into account when semen from individual bulls is to be stored for a long period as a genetic resource.

Key words: Pinzgau cattle; sperm viability; long-term storage

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