PARTHENOGENETIC ACTIVATION OF PORCINE OOCYTES IN *IN VITRO* CONDITIONS

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Effective oocyte activation is a key point in an embryo technology. Activation of *in vitro* matured (IVM) oocytes is essential for successful production of transgenic animals, parthenogenetic production of embryonic stem cells and nuclear transfer. Knowledge of parthenogenetic activation also contributes to better understanding of the mechanisms of fertilization, early embryonic development and general principle of cell signalling pathways.

Present methods for parthenogenetic embryo production depend on the use of *in vitro* maturated oocytes with full meiotic competence and combination of different chemical, physical and enzymatic stimuli. The goal of our study was to compare the success of electrical and chemical activation. This study compared the rates of blastocysts following parthenogenetic activation with electrical pulse with the use of chemical activation (ionomycin). IVM oocytes with a first polar body and high quality were randomly allocated into two groups. The first oocyte group (electrical) was activated by combination of electrical pulse (38 V and 100 μs) and 6-dimethylaminopurine (6-DMAP). The second oocyte group (chemical) was activated by combination of ionomycin (10 mM) and 6-DMAP (2 mM). Activated oocytes were cultured for 7 days in PZM3 medium at 38.5 °C in 5 % CO_2. In both types of activation we reached a high level of cleavage (electrical activation - 77.5 % vs chemical activation - 80 %), which represents successful oocyte activation. Among two types of activation, significant differences ($p < 0.01$) in the blastocyst formation (electrical activation - 15 % vs chemical activation - 21 %) were found. In conclusion, our results demonstrated that the optimal activation protocol of *in vitro* matured porcine oocytes was chemical activation consisted of combination of 10mM ionomycin and 2mM 6-DMAP.

Key words: oocyte; embryo; parthenogenetic activation

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MEIOTIC COMPETENCE OF BOVINE OOCYTES INFLUENCES MITOCHONDRIAL GENE EXPRESSION DURING *IN VITRO* MATURATION

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For the *in vitro* studies, oocytes are usually collected from antral follicles independently of the follicular development stage. Such heterogeneous population of the oocytes differs in meiotic and developmental potential. It is generally accepted that the meiotic and developmental competencies of bovine oocytes can be expressed by both size and health of follicles from which the oocytes are recovered. The objectives of the present study were to assess the changes in expression of mRNA transcripts playing an important role in mitochondrial DNA metabolism during *in vitro* maturation of bovine oocytes with different meiotic competence. Meiotically more and less competent oocytes were obtained separately either from medium (MF) or small (SF) follicles, categorized into healthy and light-atretic categories according to oocyte morphology and matured using a standard protocol. They were examined at 0 and 24 hours of maturation. Either total RNA or poly(A) RNA was extracted from oocytes and the evaluation of gene expression of three mitochondrial factors (TFAM, TFB1M, and TFB2M), MATER, and Luciferase, as external standard, was performed by an RT-PCR. The level of TFAM, TFB1M and MATER poly(A) RNA transcripts significantly decreased in both MF and SF oocytes after maturation compared with that before maturation. The healthy MF and SF oocytes contained significantly higher amounts of TFB1M at the GV stage than light-atretic oocytes. However, no differences in the transcript abundance were found among these categories at the MII stage. While in healthy and light-atretic MF oocytes TFB2M abundance revealed no differences among the GV and MII stage, in SF oocytes TFB2M was increased at the MII stage. In light-atretic SF oocytes the increase was significant ($p < 0.05$) The level of TFAM total RNA significantly increased after maturation compared with that before maturation in all oocyte categories. On the other hand, no significant differences in the transcript abundance were found for TFB1M, TFB2M and MATER total RNA between the GV and MII stages in any oocyte category. The significantly higher amount of MATER total RNA was detected only in healthy MF oocytes in comparison with that in light-atretic MF oocytes after maturation. It can be concluded that differences in mitochondrial genes, MATER poly(A) and total RNA expression were revealed among the maturing healthy and light-atretic meiotically more and less competent bovine oocytes.

Key words: bovine oocytes; meiotic competence; *in vitro* maturation; gene expression

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RECOMBINANT HUMAN LACTOFERRIN PRODUCED IN MILK OF TRANSGENIC GOATS

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Technology for the production of human recombinant proteins from milk of animals allows to obtain various proteins at large amount. On their basis this provides a possibility (including economical reason) to involve into commercial production not only medical products, but also nutritional ingredients to special food products that will open a new area of application of recombinant proteins in human life.

It is known, that lactoferrin is the protein with many major properties and functions, such as anti-viral, antibacterial, antiphlogistic, antineoplastic, immunomodulatory, anticancerogenic, bacteriostatic and antipathogenic. This protein will probably become one of the main and affordable preventive mean for various illnesses of the century. Therefore, human lactoferrin gets a special value in amount that will satisfy all-age people needs. The technology has been implemented with the recombinant human lactoferrin (rhLF) produced in goat milk. As a result, the herd of goats producing active recombinant human lactoferrin in milk was created. Integration of human lactoferrin construct into the goat genome had no negative effect on health and reproductive traits of animals. Human lactoferrin gene, ensuring synthesis of protein in the mammary gland of producing animals, is transmitted steadily from generation to generation. The identical main physical and chemical characteristics of human recombinant lactoferrin and natural lactoferrin in woman's milk were proved, and biological activity of the protein was shown.

The obtained data will contribute to the development of nutritional supplements and drug forms of rhLF with various functional application.

Key words: transgenesis; goats; milk; protein; lactoferrin

BOVINE MODEL FOR THE STUDY OF MOLECULES IMPORTANT FOR REPRODUCTION

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The presence and specific distribution of membrane proteins in gametes are crucial for the process of fertilization. Our main objective was to study these molecules both in male and female reproductive tract, as well as in early embryogenesis, preferentially using the bovine model. Although, currently we concentrate on detecting members of tetraspanin superfamily proteins (CD81, CD9), in our previous papers we characterised complement regulatory proteins (CD46, CD52) in bull sperm and reproductive system. The changes in expression and localisation of molecules of interest are studied in gametes at different stages of their development (testicular, epididymal and ejaculated sperm, germinal vesicle oocytes, metaphase I- or metaphase II- oocytes). The physiological role of targeted molecules is examined during processes of sperm capacitation and acrosome reaction, oocyte maturation, fertilization and early embryogenesis using different methodological approaches. By comparing our results with other findings on murine and porcine models, we provide the knowledge related to mammalian fertilization.

Key words: reproduction; fertilization; gametes; sperm; oocyte

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QUAIL CAM AS A TOOL FOR DIAGNOSIS OF MICROBIOLOGICAL AND ONCOLOGICAL DISEASES

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Avian chorioallantoic membrane (CAM) is an excellent *in vivo* experimental model. It is affordable, easily accessible and it develops quickly, which can be an advantage in many kinds of research. CAM serves as a respiratory organ during development of avian embryo; it is richly vascularized and immunodeficient. During *ex ovo* cultivation CAM is easily observable, with good access for applying experimental substances topically or internally. Quail CAM, in comparison with more commonly used chick embryos, has an advantage in the shorter development time, their sexual maturation is quicker, their egg production is higher and less breeding space is needed. CAM structure is similar to many tissues like lung, bladder, retina or tissue of hemato-encephalic barrier. These options open the possibilities for research in various fields. In our research we used quail CAM to study angiogenesis, pharmacokinetics of drugs and nanoparticles. We also used it for photodynamic diagnosis and therapy of tumours and microbial diseases.

On the third day of embryonal development (ED3), fertilized eggs were disinfected, opened and the embryos were transferred into six-well tissue culture plates and incubated at 37 °C and 60-70 % humidity. On ED7 the studied substances, tumour cells or microorganisms were applied onto CAM surface, depending on the type of experiment. On ED9 the photosensitizer was added and after 1, 3, 5 and 24 hours CAM was illuminated using near-UV light and induced fluorescence was recorded by a digital camera. CAM tissue was evaluated by fractal analysis, histological techniques, real time PCR and other methods.

Our results indicate that Japanese quail CAM model is a useful tool for the study of anti-vascular therapy, tumour angiogenesis, development of new biophotonic techniques as well as novel drug testing.

Key words: Japanese quail; chorioallantoic membrane; angiogenesis; photodynamic diagnosis

Acknowledgements: This research was supported by VEGA 2/0096/18 and APVV-15-0485.

EXPRESSION OF HUMAN MESENCHYMAL STEM CELL MARKERS (CD73, CD90 AND CD105) IN RABBIT ADIPOSE-DERIVED STEM CELLS

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Mesenchymal stem cells were for the first time identified in the adipose tissue (ADSCs) in 2001. There are many studies that tried to characterize ADSCs using surface marker expression analysis by flow cytometry. Studies point out to differences in the expression of individual markers among species. According to the literature, human ADSCs express typical mesenchymal CD markers, such as CD13, CD29, CD44, CD63, CD73, CD90 and CD105, while are negative for hematopoietic markers CD14, CD34, CD45. In our previous studies, we confirmed positive expression of CD29 and CD44 and negative expression of CD34 and CD45 markers on rabbit ADSCs using flow cytometry. In this study, rabbit ADSCs were isolated and cultured until the third passage as described in our previous study. Then, cells were stained with following monoclonal antibodies: anti-mouse CD73 in PE-Cy^7 (clone TY/11.8; eBioscience, Austria), anti-rat CD90 in PE-Cy^7 (clone OX-7; BD Biosciences, USA) and anti-rabbit CD105 in FITC (clone SN6; GeneTex, USA). Samples were assessed using FACS Calibur (BD Biosciences, USA) and CellQuest Pro software (BD Biosciences, USA). At least 50 000 events were analyzed for each sample. The experiment was replicated three times. Obtained data were statistically evaluated using a SigmaPlot software (Systat Software Inc., Germany) and expressed as the mean ± SEM. We observed low expression of all selected markers: CD73 (3.62 % ± 1.91 %), CD90 (9.15 % ± 8.01) and CD105 (4.8 % ± 0.93). Our results are comparable to those of other authors, who examined the expression of these surface markers using flow cytometry on other sources of rabbit stem cells, such as the bone marrow or amniotic fluid. Low expression of these markers could be due to the low affinity of the antibodies. Therefore, it is recommended to use PCR as another method for detection of gene expression, since these markers are probably characteristic also for rabbit mesenchymal stem cells.

Key words: rabbit; adipose-derived stem cells; CD markers; flow cytometry

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ANALYSIS OF THE ESTROGEN RECEPTOR (ESR) GENOTYPES IN WILD BOAR

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The wild boar (*Sus scrofa*) is the main ancestor of domestic pigs, with which it is in a close genetic affinity. The commercial pig breeds were assessed for the polymorphism of the ESR gene, associated with the increased litter size. Our study aimed at investigating the existence of the ESR candidate gene polymorphism in a population of wild boar in Slovakia. Genomic DNA was isolated from the muscle tissue 23 of unrelated wild boars by phenol-chloroform extraction and ethanol precipitation. Polymorphism was detected by a PCR-RFLP. Primers were designed from published porcine ESR sequence and were used to amplify 120 bp fragment. PCR products were digested with a restriction endonuclease PvuII. Restriction fragments were separated by electrophoresis on a 4 % agarose gel. Testing the ESR genes, we found the presence of the AA genotype in all tested individuals. This indicates the absence of PvuII polymorphism in a population of wild boar. On the other hand, in our previous study on domestic breeds of pigs, mainly in Large White and Landrace, the polymorphism of the ESR gene was demonstrated. The frequency of the B allele was in the range of 0.25 – 0.33.

Key words: wild boar; polymorphism; ESR; PvuII

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DEVELOPMENT OF *DIABETES MELLITUS* IN ZUCKER DIABETIC FATTY RATS FED BY HIGH-ENERGY DIET

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The incompletely controlled *diabetes mellitus* (DM), especially concerning the diet, can cause serious complications. Insulin resistance, relative insulin deficiency and elevated glucose level are major symptoms of the type II diabetes (T2DM), considered as one of the most common diseases. The suitable animal model of T2DM is Zucker diabetic fatty (ZDF) rats, which are deficient in the leptin receptors. These animals show fa/fa genotype, they are obese with glucose intolerance, hyperlipidemia, hyperglycaemia and hyperinsulinemia. The goal of this study was to analyse the effect of chronic high-energy diet (live weight, intake of feed and water, glucose and insulin metabolism) on DM complications in ZDF rats. Male ZDF rats (n = 20) and their lean controls (non-diabetic, n = 10) at the age of 3 months were used in the experiment. Animals were supplied with water and diet on *ad libitum* base. Rats were divided into three groups as follows: lean untreated rats (C) fed by KKZ-P/M (10 $MJ.kg^{-1}$), obese rats fed by KKZ-P/M (10 $MJ.kg^{-1}$, E1) and obese rats fed by enriched high-energy diet (E2, enriched

KKZ-P/M, 20 MJ.kg^{-1}). The consumption of feed and water, the live weight, the glucose and insulin levels were determined. ZDF rats in both experimental groups (E1 and E2) showed hyperphagia, obesity, insulin resistance and high hyperglycaemia. High-energy diet induced hyperglycaemia followed by earlier onset of other diabetic symptoms and complications.

Key words: *diabetes mellitus*; Zucker diabetic fatty rats; high-energy diet

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CELL RECEPTOR-MEDIATING COMMUNICATION BETWEEN A PREIMPLANTATION EMBRYO AND SURROUNDING ENVIRONMENT: CLUES FROM MOUSE AND RABBIT MODELS

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Preimplantation period of embryo development is one of the most sensitive phases in mammalian ontogeny, and disturbances at this developmental stage can result in poor pregnancy outcomes (resulting both from natural conception and from biotechnology procedures). Experimental studies have shown that maternal physiological condition and external environmental factors can significantly influence preimplantation embryo development, indicating a communication between the early embryo and its environment. The study of communication between the early embryo and surrounding environment has been mainly focused on protein signaling molecules, such as growth factors and cytokines. However, small-molecule ligands, such as biogenic monoamines, have been shown to influence preimplantation embryo development as well, and results obtained in mouse and rabbit models indicate that biogenic monoamine receptors are expressed in preimplantation embryos. Several adrenergic, dopamine, serotonin and histamine receptors were detected in mouse and rabbit ovulated oocytes and preimplantation embryos, as well as in mouse embryonic stem cells. Although the physiological role of biogenic monoamine receptors in early embryonic cells is not fully understood, experimental data indicate their involvement in the regulation of cell proliferation, differentiation and survival under physiological as well as unfavorable or pathological conditions (e.g. during maternal stress).

Key words: enviroment; embryo; development; receptor; proliferation

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POTENTIAL EFFECTS OF PLANT ALKALOID BERBERINE ON OVARIAN CELLS *IN VITRO*

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Berberine (BBR) is a plant isoquinoline alkaloid with a long history in Chinese medicine, present in root and shoot systems of clinically important medicinal plants. This phytochemical compound is found in different plant families such as *Berberidaceae*, *Ranunculaceae* and *Papaveraceae* families. BBR is a widely used as a natural product that showed pharmacological activities in modern and traditional medicine with non-toxic effect on humans. Current clinical research on BBR points out several medical applications, beneficial for therapies of chronic diseases, such as diabetes, hypertension and hypercholesterolemia. However, BBR effects on human reproduction remain unknown. The aim of the *in vitro* study was to examine the effect of BBR treatment (at the concentrations: 5; 10; 25; 50; 100 µg.ml^{-1}) for 24 h on viability of ovarian cells *in vitro*. Cultures of human ovarian granulosa-lutein cells (HGL5) and human ovarian carcinoma cells (OVCAR-3) were used, and the metabolic activity was determined by *AlamarBlue™* cell viability assay. The viability of HGL5 was not influenced by the berberine treatment at all concentrations used in the study. On the other hand, BBR addition significantly inhibited the viability of OVCAR-3 cells at all doses tested ($P \leq 0.05$). In conclusion, our study suggests potential anti-cancer effect of BBR on OVCAR-3 cell line. BBR might be one of such potential agents for ovary cancer therapy. However, further studies are necessary to define a therapeutic potential of BBR.

Key words: berberine; ovarian cells; viability; cancer

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PRESERVATION AND PRODUCTIVITY CONTROL OF MANGALITSA, AUTOCHTHONOUS PIG BREED IN NORTHERN SERBIA, VOJVODINA

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Mangalitsa is an autochthonous breed of pigs in Serbia, which is mostly raised in the northern parts of the country. Like many other autochthonous breeds of animals, Mangalitsa was on the verge of extinction. Although in the past this was a widespread breed in Serbia, its number was significantly reduced in the 20th century, because the breeders accepted an intensive form of raising of pigs, for which the Mangalitsa was not adequate. Also, many meadows and forests, in which the Mangalitsas are fed, have been destroyed. Lately, the number of Mangalitsa was increased and stabilized but its current status is still defined as endangered. The reason for the increased interest in the raising of Mangalitsa lies in the fact, that consumers recognized the quality of the Mangalitsa meat products. Mangalitsa meat contains more monounsaturated and less saturated fatty acids in

comparison with meat of commercial pig breeds. Since the Mangalitsa pigs are adapted to an extensive way of keeping and have a high disease resistance, this makes them ideal for organic production. A good example of the organic raising of Mangalitsa pigs is applied in the special nature reserve "Zasavica". Currently, only *in vivo* conservation is carried out in Serbia. Another reason for increasing the number of Mangalitsa are subsidies provided to the farmers by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. According to the data from the Main Breeding Organization for Vojvodina, the number of Mangalitsa pigs under control during the period 2013-2017 is on the rise, both for males and females. Implemented selection measures include the control of the productivity of sows and boars. The main goal of the breeding is to preserve and increase the population as well as to improve the genetic basis of the Mangalitsa population in the Republic of Serbia. In the future, new modern methods of conservation and biotechnological methods should be introduced in order to preserve the purity of the breed and increase the number of Mangalitsa pigs.

Key words: mangalitsa; autochthonous pig breed; *in vivo* conservation; productivity control

RAPID GENOTYPING OF THE SHORT AND HIGHLY VARIABLE REGION IN MTDNA OF HERBIVORES

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Mitochondrial DNA is found in nearly all eukaryotic organisms. mtDNA is a short, circular and relatively conserved DNA molecule transmitted maternally. It is one of the most useful tools in population genetics or phylogenetics for maternal inheritance. Screening of polymorphisms of mtDNA is suitable for comparisons among individuals from the same population as well as among distantly related species. mtDNA has an extremely variable region acquired by 2 billion years of mtDNA evolution, and is more variable than the nuclear genome itself. Furthermore, it is an ideal genetic marker for analysis of problematic, low quality and low quantity materials i.e. faeces. Here we present the application of universal primers for rapid genotyping of the highly variable region of mtDNA in cattle, chamois, deer, goat and sheep. DNAs of cattle, deer, chamois, goat and sheep from our DNA bank were used for validation of the method. DNA was extracted from hair roots or blood using Wizard Genomic DNA purification Kit (Promega). PCR reactions were performed with 20-40 ng DNA, 35 cycles and 53°C annealing temperature (Thermo-Start PCR Master Mix, Thermo Scientific). PCR fragments were cycle-sequenced using BigDye Terminator Cycle sequencing Kit version 1.1 and were run on an Avant 3100 Genetic Analyser (Applied Biosystems). Sequences were aligned using Geneious software (Biomatters) and BLAST (NCBI). In summary, we have validated a method for rapid genetic screening of the highly variable region in mtDNA of cattle, chamois, deer, goat and sheep, which can be used for species determination and population studies among even wider variety of herbivore species.

Key words: mtDNA; genotyping; herbivores

ANTIBIOTIC RESISTANCE OF ENTEROBACTERIACEAE SPECIES ISOLATED FROM RECTAL SWABS OF SHEEP

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The main objective of this study was to determine antibiotic resistance od *Enterobacteriaceae* species isolated from sheep rectal swabs and identification of isolated species as potential resistant gene vectors. Sixty nine sheep rectal swabs were obtained from different farms of Slovakia. Isolation of *Enterobacteriaceae* species was done on MacConkey agar during 24 h at 37 ± 1 °C under air condition. Cultures were purified by four-way streak plate method. Pure cultures were identified by MALDI TOF Mass Spectrometry using MALDI Biotyper 3.1 software (Bruker Daltonics, Germany). Antibiotic susceptibility testing was performed by disk diffusion methodology according to EUCAST. Ampicillin resistance was confirmed by indirect method using MALDI TOF MS, where beta-lactamase hydrolysis of ampicillin was assessed and resistance mechanism was detected. The following antibiotics were used in this study: streptomycin (10 µg/disc), tetracycline (30 µg.disc), chloramphenicol (30 µg/disc), oxacillin (1 µg/disc), ampicillin (10 µg/disc), nalixidic acid (30 µg/disc), amikacin (30 µg/disc), gentamicin (30 µg/disc), levofloxacin (5 µg/disc), piperacillin (30 µg/disc) and tigecycline (15 µg/disc). Overall, from 69 samples the resistance was revealed in following: 5 strains against tetracycline, 2 strains against tetracycline, 4 strains against chloramphenicol, 14 strains against oxacillin, 7 strains against ampicillin and 9 strains against levofloxacin. Antibiotic resistances against nalixidic acid, amikacin, gentamicin, piperacillin and tigecycline were not found in this study. Seven strains were purified by four-way streak plate method and identified by MALDI TOF MS as *Escherichia coli*, *Serratia odorifera* bv.1, *Enterobacter aerogenes*, *Citrobacter farmeri*, *Proteus vulrgaris*, *Klebsiella* spp. and *Yersinia* spp. Disk diffusion method showed resistance of *Escherichia coli* against three antibiotics: chloramphenicol, ampicillin and levofloxacine. *Serrattia odorifera* bv. 1 was resistant against streptomycin and tetracycline. Oxacillin resistance was detected in *Enterobacter areogenes* and resistance against chloramphenicol in *Klebsiella* spp. Other identified bacteria isolated from rectal swabs of sheep were identified as susceptible to antibiotics, which were used in this study. MALDI TOF MS analysis showed that ampicillin was hydrolysed by beta-lactamases produced by *Escherichia coli*, and its decay products as ampicillin with disrupted amide bound (366 ± 0.6 m/z), its monosodium salt (389 ± 0.6 m/z),

its disodium salt (412 ± 0.6 m/z), spontaneous decarboxyled ampicillin (323 ± 0.6 m/z) and decarboxyled ampicillin sodium salt (344 ± 0.6 m/z) were detected. Therefore, enzyme hydrolysis strategy was confirmed as main strategy of antibiotic resistance in *E. coli*. In conclusion, we determined that the resistance against several antibiotics was found in rectal swabs of sheep and it is spread within sheep breeding. Also, enzymatic resistance mechanism is a main resistant strategy within *Enterobacteriaceae* genera in sheep breeding.

Key words: antibiotic resistance; sheep; *Enterobacteriaceae*; MALDI TOF MS

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CHICKEN STEM CELLS AS A POTENTIAL SOURCE FOR ANIMAL GENE BANK

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Biosecurity and sustainability in chicken production requires reliable germplasm conservation. Germplasm conservation in chicken is more challenging in comparison to other livestock species. Embryo cryopreservation is not feasible for egg-laying animals, and chicken semen conservation in different chicken breeds has variable success. A potential solution is the cryopreservation of the committed diploid stem cell, blastodermal cells (BCs), the precursor of gametes and primordial germ cells (PGCs). BCs and PGCs are the lineage-restricted cells found at early embryonic stages in birds. This research dealt with isolation, characterisation and cryopreservation of chicken stem cells for the animal gene bank purposes. Trypan blue, fluorescence microscopy, flow cytometry and transmission electron microscopy were used for the viability assessment and characterisation of fresh and frozen/thawed chicken stem cells. Our results showed that BCs contain lipid granules, which prevent successful freezing even though different methods of cryopreservation were used. However, in contrast, PGCs contain a smaller amount of lipid granules, and, therefore, PGCs are more suitable for cryopreservation. The present study suggest that PGCs should be considered as more preferable source for animal biobanking, and the choice of proper cell source should be done carefully.

Key words: chicken; blastodermal cells; primordial germ cells; cryopreservation; viability

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INBREDISATION – THE WAY FOR CREATION OF GENETICALLY UNIFORM RABBIT GROUPS

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Utilization of rabbits as experimental animals is oriented to specific populations because of user goals. Human diseases, organ transplantations, physiological experiments, feeding trials or genetic experiments are most frequent examples for exploration of rabbits. These utilizations are associated with homogenous genetic background, which can be reached by inbredisation process. Depending on the type of observed and applied traits, homozygotation can be an effective way to create appropriate populations with unique characteristics.

We used 3 panmictic rabbit populations (New Zealand White – NZW, Californian – C and Nitra rabbit – Ni) for directed selection during 5 generations. In the course of inbredisation, the animals were mated *inter se* (full siblings with each other). Initial (founder) animals were selected from a wider population and they formed the original parental groups with 20 does and 5 males of each breed. At average, the same number of selected animals was mated for next generation. Selection criteria were following: for NZW it were – increase of live weight, for C – high level of untroubled behaviour, and for Ni – long ear shell with clear blood vessel. NZW animals were selected on the basis of regular weekly measuring of live weight. Californian rabbits were tested in open field equipment for peaceful habitus as a number of movements per time unit. Animals in Nitra breed population were selected basing on the ear length and good visualisation of central ear blood vessel. In addition to the selected traits, in all three populations the standard breed characteristics were maintained.

After the 5 year selection process the results are following: in the 5th generation of NZW rabbit population an average initial live weight was increased daily from 25.4 ± 6.2 g to 30.2 ± 3.2 g. It represents difference in 411.6 g at slaughter age in favour of the inbred population. Panmictic Californian rabbits had 25.6 ± 7.5 movements in contrast to 14.5 ± 5.7 units for animals in inbred population. The length of ear in the initial Nitra rabbits was 11.5 ± 1.6 cm in comparison to the inbred population (13.5 ± 1.8 cm). According to these results, inbredisation is an effective process for creating relevant populations.

Key words: rabbit; inbredisation; selected traits; live weight; ear length

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DIFFERENCES BETWEEN EWES AND MOUFLONS IN SELECTED METABOLIC PARAMETERS IN RELATION TO THE YIELD AND EMBRYO QUALITY

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The aim of our research was to study the effect of prevalence the serum cholesterol value, urea and total protein before and after superovulation on the yield and quality of embryos in ewes and mouflons. In ewes, positive correlation was found between the level of cholesterol and superovulatory response (r = 0.54), the total number of embryos (r = 0.01) and number of transferable embryos (r = 0.39). Levels of urea were in negative correlation with the superovulatory response (r = -0.42), the total number of flushed embryos (r = -0.49) or transferable embryos (r = -0.58). In mouflons, positive correlation was found between the level of cholesterol and superovulatory response (r = 0.68), total number of embryos (r = 0.50) and transferable embryos (r = 0.48). Levels of urea were in negative correlation with the superovulatory response (r = -0.37), the total number of flushed embryos (r = -0.56) and transferable embryos (r = -0.64). The influence of total proteins in blood serum of donor ewes and mouflons on effectiveness of embryo transfer was not proved in our research. No statistically significant differences between ewes and mouflons in terms of the evaluated parameters were observed.

Key words: blood; embryos; ewes; metabolic parameters; mouflon females

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EVALUATION OF NOVEL ANTIBODIES RAISED AGAINST THE RABBIT CD34 SYNTHETIC PEPTIDE (G1SJT2)

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The aim of this preliminary study was to evaluate the antigen specificity of anti-CD34 antibodies raised in mice against the specific immunogenic oligopeptide synthesized according to the predicted rabbit CD34 protein (G1SJT2). Experimental procedure was performed as described previously (Vasicek *et al.*, 2017 and 2018). Briefly, peripheral blood mononuclear cells (PBMCs) from three young (four months-old) and clinically health rabbits of New Zealand White (NZW) line were used in the experiment. Isolated cells were single stained with 28 different mouse anti-rabbit CD34 monoclonal antibodies: IgG1 isotype sub-clones (4/118, 4/131, 8/80, 8/98, 8/109, 59/1, 59/2, 59/3, 171/123, 171/124, 171/125, 172/2, 172/47, 391/145, 391/157, 391/186, 398/115, 398/117, 398/124, 740/247, 740/252, 873/193, 873/197, 945/68 and 945/95), IgG2a isotype sub-clone (473/174) and IgG2b isotype sub-clones (369/1 and 369/33). Goat anti-mouse Ig-APC (BD Biosciences, USA) was used as a secondary antibody. According to obtained results from flow cytometry, three anti-CD34 antibodies with higher detectable number of $CD34^+$ cells (369/1 and 369/33 – both IgG2b and 473/174 – IgG2a) were chosen for the double staining with CD45 antibody (clone L12/201, IgG1; Bio-Rad Antibodies, USA). As secondary antibodies, antimouse IgG2a-FITC (Thermo Fisher Scientific, USA) or IgG2b-FITC (BioLegend, USA) and IgG1-PE (Miltenyi Biotec, Germany) were used. To exclude dead cells from the analysis 7-AAD (Bioscience, Austria) was used. Cells (at least 50,000 events) were analyzed using a flow cytometer FACSCalibur (BD Biosciences, USA). Modified flow cytometry method based on ISHAGE society was used to determine $CD34^+$ and $CD34^+CD45^-$ cell counts. Observed results were evaluated statistically (Holm-Sidak method in SigmaPlot software), and expressed as the means ± SEM.
We observed significantly higher ($P < 0.001$) expression (%) of CD34 antigen within PBMCs using antibodies: 369/1 and 369/33 (1.27 ± 0.32 and 1.37 ± 0.47, respectively). Slightly increased expression was also found by using of 473/174 antibody (0.50 ± 0.12) compared to the others. Those three antibodies were used to determine undifferentiated $CD34^+CD45^-$ cells within PBMCs. Antibody 473/174 revealed significantly higher ($P < 0.01$) percentage of $CD34^+CD45^-$ cells (0.25 ± 0.02) in comparison to other antibodies 369/1 and 369/33 (0.18 ± 0.06 and 0.11 ± 0.03, respectively). In conclusion, clone 473/174 seems to be another promising antibody for the detection of CD34 antigen within the rabbit PBMCs. However, further analyses are required in order to determine the true CD34 antigen specificity of all newly prepared monoclonal antibodies.

Key words: rabbit; HSCs; CD34 antibodies; flow cytometry

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EFFECT OF DIFFERENT EGGSHELL COLOUR ON EXTERNAL AND INTERNAL EGG QUALITY OF JAPANESE QUAIL (*COTURNIX JAPONICA*)

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Totally 300 egg from Japanese quail breed were used to analyze the effect of eggshell colour on internal and external egg quality. The birds were housed as 1 male and 3 females per cage of 0.12 m^2 area at the Research Institute for Animal Production Nitra and fed with a mixture of 11.7 MJ metabolic energy and 200.0 g of crude protein during the experiment. Feed and water were given *ad libitum*. Analysis of external and internal characteristics of Japanese quail eggs was performed in the laboratory of the Department of Poultry Science and Small Farm Animals of the Slovak University of Agriculture in Nitra. The eggs were divided into five groups according to the colour (each n = 60) based up on pigment

found on the surface of the eggshell: brown (brown pigmentation), black (greyish white eggshell pigmented with various sizes of black pigments), white (non-pigmented eggshell), spotted (small black pin dots on greyish brown eggshell) and blue (slightly blue pigments). This research was conducted to investigate the effects of eggshell colour on the egg weight, egg shape index, eggshell weight, eggshell percentage, eggshell thickness, eggshell strength, albumen weight, albumen percentage, albumen index, Haugh unit, yolk weight, yolk percentage, yolk index and yolk colour. Results revealed that eggshell colour had significant ($P < 0.05$) effect on the egg weight (from 11.66 g in spotted group to 12.98 g in black group). The egg shape index did not express any significant ($P > 0.05$) differences between different eggshell colours. Non-significant ($P > 0.05$) effects were recorded for eggshell weight, eggshell percentage, eggshell thickness and eggshell strength among different eggshell colours. Results also showed that eggshell colour had significant ($P < 0.05$) effect on albumen index (from 9.32 % in brown group to 9.63 % in white group), Haugh unit (from 87.51 in brown group to 89.85 in white group), yolk weight (from 4.11 g in spotted group to 4.36 g in black group) and yolk percentage (from 32.22 % in spotted group to 34.89 % in black group). For albumen weight, albumen percentage, yolk index and yolk colour no significant ($P > 0.05$) differences among eggshell colours were observed.

Key words: Japanese quail; egg; eggshell colour; external quality; internal quality

COMPOSITION OF ALVEOLAR AND CISTERNAL MILK OF TSIGAI AND IMPROVED VALACHIAN BREEDS

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The aim of this study was to investigate the composition of the milk in the cisternal and alveolar compartments of the udder in the most bred breeds in Slovakia [Tsigai (TS), n = 9 and Improved Valachian (IV), n = 9]. Measurements took place at the middle stage of lactation. Cisternal milk was milked out after i.v. administration of atosiban (10 $\mu g.kg^{-1}$ body weight) and alveolar milk after i.v. oxytocin administration (4 IU/animal). Milk composition was analysed for percentage of fat, protein, lactose, solids and solids-not-fat with MilkoScan FT120 (Foss, Hillerød, Denmark). The ratios between cisternal and alveolar milk were 55:45 in TS and 65:35 in IV. No significant differences ($P > 0.05$) in milk composition were found in both milk fractions between breeds in protein content of alveolar milk (5.63 ± 0.57 vs. 5.17 ± 0.37 % in TS and IV, resp.) and milk fat concentration in the cisternal as well as in the alveolar fraction (7.94 ± 1.49 vs. 7.55 ± 0.97 % and 9.38 ± 2.02 vs. 9.27 ± 1.39 %, resp.). In conclusion, in both breeds the large amount of milk is present in the alveolar compartments of the udder, indicating the need of milk ejection reflex occurrence for complete milk removal during milking.

Key words: alveolar milk; cisternal milk; ewes; Tsigai; Improved Valachian

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ASSOCIATION BETWEEN SELECTED TRACE ELEMENTS AND HEPATIC PROFILE IN COMMON CARP (*CYPRINUS CARPIO*)

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Chronic exposure to heavy metals and other trace elements causes several injuries in organism. Biomarkers, such as hepatic enzymes, are good indicators of liver injury. The aim of the present study was to investigate the associations between responses to selected trace elements (aluminium – Al, barium – Ba, lithium – Li, molybdenum – Mo) and hepatic enzymes (aspartate aminotransferase – AST, alanine aminotransferase – ALT, alkaline phosphatase – ALP, bilirubin (Bili) and creatine kinase – CK) in Common Carp blood serum.

Totally, 42 freshwater fishes (*Cyprinus carpio*) were caught by seine net. The blood samples, taken by a cardiac puncture method, were allowed to coagulate and then centrifuged for 20 min at 3000 rpm. Blood serum concentrations of AST, ALT, ALP, Bili and CK were measured using DiaSys (Diagnostic Systems GmbH, Holzheim, Germany) commercial kits and the semi-automated clinical chemistry analyzer Randox RX Monza (Randox Laboratories, Crumlin, UK). The content of selected trace elements (Al, Ba, Li, Mo) in blood serum was determined by inductively-coupled plasma optical emission spectrometry (ICP-OES, Agilent Technologies Australia (M) Pty Ltd.). Statistical analyses were performed using STATGRAPHICS Centurion software (©StatPoint Technologies,Inc., USA). The following scheme of descending concentrations of trace elements in blood serum was used: Al (0.61 $mg.L^{-1}$) > Ba (0.17 $mg.L^{-1}$) > Mo (7.13 $\mu g.L^{-1}$) > Li (5.24 $\mu g.L^{-1}$). Levels of serum markers were comparable with other authors, except higher ALP (6.36 $\mu kat.L^{-1}$). Correlation analysis showed significant positive relationship between ALT and

Mo (r = 0.4032; P < 0.01) and significant negative association between CK and Ba (r = -0.3780; P < 0.05) or Li (r = -0.3925; P < 0.05). Insignificant Pearson correlations were detected between other trace elements and hepatic profile markers. Al and Li were in positive association with AST and Bili (n.s.). Mo and Ba insignificantly positively correlated with ALP and negatively with cholesterol and bilirubin. On the other hand, the analysis showed insignificant positive correlation between Al and cholesterol or bilirubin.
In conclusion, obtained data indicate that trace elements affect hepatic profile markers of Common Carp. However, there were no serious damages observed in the health status except for ALP, which may indicate the bile duct epithelial damage. The correlation analysis confirmed statistically significant interactions.

Key words: trace elements; hepatic profile; Common Carp

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ADDITIVES IN JAPANESE QUAIL NUTRITION

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The aim of this study was to analyse the effect of humic substances (HS) on the quality traits (colour, water-holding capacity and pH), serum minerals (calcium, phosphorus, sodium, potassium and chlorides) and the serum metabolites (glucose, total protein, triglycerides and cholesterols) in breast and thigh meats of Japanese quails. A total of 60 animals (30 males and 30 females) were involved in the experiment. The birds, fed by the standard basal diet, were divided into four experimental groups as follows: probiotic females (PF, n = 10) and males (PM, n = 10) received probiotic preparation at the single dose of 1 g.kg^{-1} of feed mixture, humic acids females (HF, n = 10) and males (HM, n = 10) received humic acids at the single dose 3 g.kg^{-1} of feed mixture. The groups fed basal diet without any additive served as the control groups (CF; n = 10, CM, n = 10). After 210 days the quails were slaughtered and the blood samples and samples of muscles (breast and thigh) were collected. The treatments by probiotic and humic acids caused significant increase in serum calcium levels in the female groups when compared to the male groups. Serum phosphorus was significantly increased in the PF group in comparison to the PM group. Both treatments significantly decreased amount of HDL cholesterol in the female groups in comparison to the female control. 24 hours after slaughter, the meat pH in different muscles showed significant differences. In conclusion, the effect of the treatment with probiotics and humic acids was dose dependent. This suggests that the estimation of an effective dose of additives used in poultry feeding plays an important role.

Key words: Japanese quails; probiotics; humic acids; blood biochemical parameters; meat quality

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CYTOTOXIC EVALUATION OF NONYLPHENOL IN TM3 LEYDIG CELL LINE

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Current widespread research of health effects potentially associated with endocrine disruptors has a high priority nowadays. Alkylphenol ethoxylates, a class of non-ionic surfactants, are degraded into alkylphenol diethoxylates and alkylphenol monoethoxylates. These are subsequently degraded into other sub-products and persist in the environment for a long time. Alkylphenols are common environmental contaminants originating from industrial processes, which are widely used as a component of paints, pesticides and herbicides. The most studied alkylphenols are nonylphenols and octylphenols. They have the ability to mimic effects of reproductive hormones and can interfere with the endocrine system leading to reproductive disorders at different levels of cellular system. A significant body of evidence, based upon laboratory experiments and meta-analysis, indicates that exposure to alkylphenols is associated with male reproductive malfunctions and impairment of spermatogenesis followed by irreversible changes in steroidogenesis. The primary objective of our *in vitro* study is to provide a knowledge about the cytotoxic effect of nonylphenols on TM3 cell line. In our study, the effect of 4-nonylphenol on the Leydig cell functions at lower doses (0.04-5.0 µg.mL^{-1}) was evaluated. Cytotoxicity was assessed by measuring inhibition of metabolic activity (AlamarBlueTM) and loss of membrane integrity (CFDA-AM) in order to identify the mode of toxic action after 24 h of culture. Significant (P < 0.001) increase in metabolic inhibition at 2.5 and 5.0 µg.mL^{-1} was revealed, whereas significant (P < 0.001) loss of membrane integrity was occurred at the highest dose (5.0 µg.mL^{-1}) of 4-nonylphenol. Additional *in vivo* and *in vitro* studies are required to better understand the nature of the effects of alkylphenols and their mechanisms of action in altering male reproductive functions.

Key words: nonylphenol; cytotoxicity; Leydig cells

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VALIDATION OF BOVINE 50K SNP CHIP TRANSFER ABILITY INTO NON-MODEL WILD ANIMALS

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The aim of this study was to apply bovine 50k genotyping array in cross-species genotyping of red deer (*Cervus elaphus*) and to validate its suitability for the studies of genetic diversity in non-model wild animal species. The analysed population of red deer consisted of 27 biological samples of farmed animals, which were male progeny of sires from New Zealand and dams from Hungary and free living trophy animals from legal hunting in Slovakia. All of animals included in this study were genotyped using the Illumina BovineSNP50 BeadChip v2 in a commercial lab. The first scan of obtained genotyping data showed significant decrease in the genotyping quality compared to the species from the *Bovidae* family. However, the observed call rate at the level of 61.66 % was in accordance to previous studies, which clearly confirmed that the cross-species application of commercially developed genotyping array on evolutionary related species can lead to 1.5 % decrease of call rate per each million year divergence between species. The subsequent quality control of genotyping data was performed to retain in database only autosomal SNPs with lower than 10 % of missing genotypes and minor allele frequency greater than 0.01 %. Applied quality control resulted in 53.98 % of SNPs that were successfully genotyped in at least 90 % of animals and up to 1579 markers that could be regarded as polymorphic and informative for the subsequent study of genetic diversity. The analysis of the level of genetic diversity conserved within species as well as potential admixture with non-native deer populations clearly validated that the obtained set of polymorphic markers is very suitable mainly to identify the genetic background of analysed animals. For this purpose we used previously published genomic data for four species from the genus *Cervus*: Axis deer (*A. Axis*), Fallow deer (*D. dama*), Sika deer (*C. nippon*) and Wapiti deer (*C. canadensis*). Our results showed that the allele frequencies varied continuously across the three main regions composed from *Cervus*, *Dama* and *Axis* genera. As expected, due to the phylogeny of analysed species, the Wright's FST index and Nei's genetic distances revealed the closest genetic affinity between the species from *Cervus* genus, whereas the highest genetic distance was found between *Axis* and *Dama* genera. The discriminant analysis of principal components, as well as Bayesian cluster analysis, demonstrated the presence of admixed individuals only between the species Red deer and Wapiti deer, probably as a consequence of the introduction of non-native red deer populations into some parts of Slovakia during the 19th century. Any level of admixture between the Slovak Red deer and Sika was not confirmed. Based on this it can be concluded that alongside with other Central European Red deer populations the Slovak population can provide valuable gene pool. In practical point of view our results can be beneficial not only for the future improvement of deer farming in Slovakia, but also for other local populations, mainly in respect to the conservation of its genetic resources, sustainable management and prevention of genetic diversity loss due to the hybridisation with non-native populations.

Key words: cross-species genotyping; deer; diversity; non-model species

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QUALITY OF CRYOPRESERVED SHEEP SEMEN: COMPARISON OF TWO SPERM CONCENTRATIONS

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Artificial insemination in sheep is a challenge because of particularities of the ovine cervix. Laparoscopic insemination is practised in large sheep-holding countries but not in the countries with small-scaled agriculture because of high costs and time consumption. Sperm concentration of 150 x 10^6 spermatozoa is sufficient for laparoscopic insemination, but too low for pre-cervical insemination. The influence of two different sperm concentrations (300 x 10^6 sperm.ml^{-1} and 600 x 10^6 sperm.ml^{-1}) of cryopreserved semen on the quality parameters was examined. After thawing the acrosomes of the sperm cells were evaluated using fluorescent staining. Density, motility, progressive movement and viability were analysed using CASA. The significance of differences was assessed by using general linear models procedures (IBM Statistics 22). Significant differences in the motility and progressive movement between the two concentrations were recorded. In the vitality, incidence of pathological forms and faulty acrosomes only numerical differences were seen. In all examinations better results were seen in the samples with 300 x 10^6 sperm.ml^{-1}. However, in order to recommend an optimal sperm concentration for production of sperm doses, further examinations of pregnancy rates and fertility parameters under field conditions are required.

Key words: sheep; semen; concentration; cryopreservation; CASA

INFLUENCE OF GRAPEFRUIT SEED-EXTRACT ON THE EXSHEATHMENT RATE OF PARASITIC INFECTIOUS THIRD LARVAE

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Frequent deworming during the last years resulted in higher rates of resistances. Usually, secondary plant ingredients are tested *in vitro* prior to the use in the field. The aim of this trial was to examine the influence of grapefruit seed extract on the exsheathment of parasitic third stage larvae. The third stage larvae were obtained from faeces of naturally infected

goats. The main proportion was represented by *Haemonchus contortus* (66 %) followed by *Trichostrongylus* spp. (28 %). The remainder was composed of *Teladorsagia* spp. (2 %), *Trichochstonrgylus* spp. (3 %) and *Strongyloides* spp. (1 %). The larvae were incubated with different dilutions of grapefruit seed extract (1.6 and 3.2 mg.ml^{-1}), tetramisol hydrochloride [positive control (600 µg.ml^{-1})] and PBS (negative control), respectively. Exsheathment was observed 20, 40 and 60 min after adding the exsheathment fluid. Statistical differences were assessed by using general linear models procedures (IBM Statistics 22).

Grapefruit seed extract showed significant influence on the exsheathment of parasitic third stage larvae *in vitro*. 100 % exsheathment rate was reached in the negative control; positive control showed larval exsheathment under 5 %. Grapefruit seed extract showed low exsheathment rates of 6 % (1.6 mg.ml^{-1}) and 15 % (3.2 mg.ml^{-1}). Statistically significant differences were seen between all groups except the two grapefruit seed groups. Although this trial was conducted with a mixture of parasitic larvae, further examinations should be done with monocultures of parasitic larvae to evaluate the effect in different parasitic species.

Grapefruit seed extract seems to be a candidate tool used for a parasite control. In order to determine an appropriate concentration, the type and duration of the feeding, further studies under field conditions are required.

Key words: grapefruit seed; larvae; *Haemonchus contortus*; goats; parasite control

THE SENSITIVITY OF MOUSE PREIMPLANTATION EMBRYOS TO FIPRONIL MIGHT DEPEND ON MATERNAL BODY CONDITION

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It was previously documented that insecticide fipronil have the potential to affect *in vitro* and *in vivo* embryonic development.

The aim of our study was, therefore, to evaluate whether fipronil, applied during preimplantation period, can influence the developmental capacity and basic qualitative parameters (embryo growth and the incidence of dead cells) of mouse embryos obtained from dams with different fat deposition.

A two-generation dietary model, based on mice overfeeding during intrauterine and early postnatal development, was used to produce two groups of mice: CN – Normal control females with physiological (7 % – 8 %) amounts of body fat and EXF – Fat mice with elevated (> 11 %) amounts of body fat. Under *in vitro* conditions, 2-cell stage embryos were isolated from spontaneously ovulated control and fat mouse dams and cultured in media with or without addition of fipronil at 1 µM concentration until the blastocyst formation. Stereomicroscopic evaluation of *in vitro* produced embryos showed that fipronil at 1 µM concentration decreased developmental capacity of two-cell embryos isolated from normal (CN) as well as from fat (EXF) mice. Fluorescence staining revealed decreased cell numbers in blastocysts derived from the fipronil-treated EXF group. In contrast, quality of blastocysts collected from the fipronil-treated CN group did not show any significance differences in comparison with non-treated CN group.

The obtained preliminary results show the increased sensitivity to insecticide fipronil in preimplantation embryos isolated from obese mice.

Key words: preimplantation embryo; mouse; *in vitro*; fipronil; obesity

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SOMATIC CELL COUNT IN MASTITIS DIAGNOSED EWES

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This study deals with the diagnostics of subclinical mastitis based on the somatic cell count (SCC) in the ewe's milk. The aim was to determine whether high SCC is related to the presence of a pathogen in the milk. The trials were carried out on two farms of Lacaune sheep breeding. Milk sampling on each farm took place at two dates – March and June 2018 during evening milking. A total of 202 milk samples (101 ewes) were taken from udder halves for analysis of SCC and bacteriological examination for the presence of mastitis pathogens. Based on SCC, the ewes were divided into five groups: up to 0.2 x 10^6 ml^{-1}; 0.2-0.4 x 10^6 ml^{-1}; 0.4-0.6 x 10^6 ml^{-1}; 0.6 x 10^6 ml^{-1}; over 0.6 x 10^6 ml^{-1}. In the first group of SCC there were 61.88 % of milk samples, in the second – 5.45 %, third – 3.46 %, fourth – 2.97 % and in the fifth – 26.24 %. The impact of the farm was marked, where in the first farm there were 81 % of samples in the first SCC group; in the last farm – 9 %, while in the second farm the first and the last groups represented 43.14 % of samples. Higher SCC values were found in infected samples (logx 5.78 ± 0.10 ml^{-1}) compared to uninfected (logx 4.86 ± 0.07 ml^{-1}, $P < 0.001$). Two important infectious mastitis pathogens with very low mastitis rates were isolated: *Staphylococcus* (*S*) *aureus* (3.70 %) and *Streptococcus* (*Str.*) *agalactiae* (1.23 %). More frequently were isolated Coagulase negative staphylococci (CoNS), *S. chromogenes* (44.44 %), *S. epidermidis* (39.51 %), *S. xylosus* (22.22 %), followed by *Candida* sp. (2.47 %), *Klebsiella oxytosa* (2.47 %) and *Str. dysagalactiae* (1.23 %). We conclude that the monitoring of the somatic cell counts in milk is a suitable zootechnical tool in an efficient dairy sheep breeding.

Key words: ewes; milk; somatic cells; mastitis pathogens

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EFFECT OF TRACE ELEMENTS DETECTED IN CARP SEMEN ON SPERMATOZOA QUALITY

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Male reproductive system may be generally considered the barometer of environmental contamination. There are numerous studies investigating effects of individual chemical elements at environmentally related concentrations, however available literature lacks the information about effects of substance mixtures. The objective of the present study was to determine the effect of trace elements in seminal plasma on spermatozoa quality, taking in consideration motility parameters, RedOx status and DNA fragmentation. Reproductively mature male carps were subjected to the study. Following the milt collection, the trace elements were determined using inductively-coupled plasma optical emission spectrometry (ICP-OES) and cold-vapor atomic absorption spectroscopy (CV-AAS). Sperm motility traits were assessed using computer assisted semen analyzer (CASA). RedOX status was determined by following markers: reactive oxygen species production (ROS), total antioxidant capacity status (TAC), malondialdehyde production (MDA) and protein carbonyl production (PC). DNA fragmentation was evaluated using the APO-DIRECT™ fluorescent kit. Detected chemical elements were put in ascending order according to their concentrations: Hg < Cd < Cr < Pb < Se < Mn < Ni < Sr < As < Cu < Fe < Zn. Positive significant correlations were found between Mn, Se, Sr and Zn and velocity and distance sperm parameters. Cu and Hg showed negative associations with progressive motility. Hg also affected malondialdehyde production. In conclusion, the present study suggests the use of multi-component mixtures of environmentally related trace element concentrations when examining the potential reproductive risk.

Key words: semen quality; trace elements; oxidative stress; DNA fragmentation; bio-monitoring

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THE EFFECT OF HEAVY METALS ON ICHTHYOLOGIC INDICES OF FRESHWATER FISH

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Heavy metals from human activities are considered to be one of the most dangerous pollutants of water ecosystems. Water pollution affects various developmental processes of fish, what can result in deformations, small body size and reduced viability. Different fish tissues are widely used as indicators for determination of water pollution level. Moreover, higher concentrations of heavy metals in water can lead to serious health risks, due to bioaccumulation of these elements in fish body and transmission into a food chain.
In the present study, samples collected from 42 freshwater fish (*Cyprinus carpio*) were analyzed for the presence of heavy metals and their correlations with ichthyologic parameters (standard length – mm, total length – mm, weight – g). The blood samples were taken by cardiac puncture, centrifuged for 20 min at 3,000 rpm and blood serum was collected and stored at -20 °C until analyses. Concentrations of heavy metals (Pb, Cd, Hg, Cr, As) were measured using inductively-coupled plasma optical emission spectrometry (ICP-OES), a modern technique for routine determination of heavy metal concentrations in different matrices. Statistical analyses were performed using STATGRAPHICS Centurion (©StatPoint Technologies, Inc., USA). Average values for ichthyology parameters were 397.5 mm for standard length, 470.6 mm for total length and 2053.86 g for weight. The concentrations of lead, cadmium, mercury, arsenic and chrome varied in ranges 0.0 – 0.302, 0.0 – 0.017, 0.0 – 0.004, 0.0 – 0.330 and 0.148 – 1.362 mg.L^{-1}, respectively. Obtained results showed weak positive linear relationship between arsenic concentration and total length parameter ($r = 0.334$; $P < 0.05$) and between mercury concentration and body weight parameter ($r = 0.316$; $P < 0.05$). At last, weak positive linear relationship ($r = 0.300$) was calculated for arsenic concentration and standard length, but it was slightly over the limit of significance ($p = 0.054$). Other correlations between heavy metal concentrations and ichthyologic indices were not observed. Further analyses including more fish species and more elements are necessary for better understanding of relations between heavy metal pollution and qualitative and quantitative parameters of fish.

Key words: heavy metals; freshwater fish; ICP-OES; ichthyology

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CONTENT OF SELECTED METALS IN FEED AND SHEEP'S MILK FROM DIFFERRENT PARTS OF SLOVAKIA

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Milk and milk products represent an important source of macro and micronutrients including minerals. However, milk and dairy products can also contain chemical hazards and contaminants, which represent a technological risk factor for dairy products, for the related commercial image and, above all, for the health consumer. The aim of this study was to describe the content of selected essential elements and toxic metals in feed and sheep's milk from areas of Slovakia with different character of environment. In the regions of Slovakia -Novoť (undisturbed environment; North Slovakia) and Klátova Nová Ves (widely disturbed environment; Western Slovakia), 11 metals have been analyzed (essential elements - calcium, zinc, selenium, iron, magnesium, copper; toxic elements – arsenic, mercury, lead, cadmium, nickel). Analyses of samples were performed by certified testing laboratory Eurofins Bel/Novamann (Nové Zámky, Slovak Republic). The results showed significantly higher content of selected essential elements in feed in spring season from the area with widely disturbed environment (Klátova Nová Ves). Significantly higher content of essential elements in milk was recorded on the farm of Novoť (undisturbed environment). Occurrence of toxic metals in feed from the area with widely disturbed environment during the spring season did not affect their content in milk. It can be concluded, that the use of milk of sheep from these areas for direct use or for dairy product processing is appropriate, safe and poses no health risk for the consumers.

Key words: sheep's milk; toxic metal; essential elements; feed; environment

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PHYSIOLOGICAL LEVELS OF SOMATIC CELL COUNT IN RAW EWE MILK

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Somatic cell count (SCC) in milk is used as a gold indicator of udder health and could be used for detection of subclinical mastitis in herds. Despite negative impact of high SCC on milk yield and milk components, the physiological values of SCC in raw ewe's milk are still under discussion. The aim of this study was to describe the distribution of ewes of two breeds (Slovak dairy ewe – SD and Lacaune – LC) into SCC groups based on their individual SCC. The ewes were divided into five SCC groups (G1= SCC < 200 × 10^3 cells.ml^{-1}, G2 = SCC between 200-400 × 10^3 cells.ml^{-1}, G3 = SCC between 400-600 × 10^3 cells.ml^{-1}, G4 = SCC between 600-1000 × 10^3 cells.ml^{-1} and G5 = SCC > 1000 × 10^3 cells.ml^{-1}). In total, 771 samples were collected from 90 ewes throughout both milking periods from one experimental herd belonging to NPPC – Research Institute for Animal Production Nitra, Slovakia with minimum four milking records per year in 2016 and 2017. Throughout lactation the most ewes were found out in the first two SCC groups (below 400 × 10^3 cells.ml^{-1}) in 2016 and 2017 (78.89 % and 83.33 %, respectively). Thirteen animals (8SD, 5LC) were in SCC groups over 600 × 10^3 cells.ml^{-1} in 2016, however in next lactation only 6 animals of them did not improve SCC during dry period in following lactation in 2017 (5 of them were LC). Twelve animals (4SD, 8LC) were in SCC groups over 600 × 10^3 cells.ml^{-1} in 2017. Our results indicate that the possible physiological level for raw sheep milk might be considered as the value less than 400 × 10^3 cells.ml^{-1} because of the highest percentage of ewes recorded.

Key words: ewe; milk; somatic cell counts

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DIETARY SUPPLEMENTATION OF VERBASCOSIDE: IMPLICATION ON REPRODUCTIVE ASPECTS

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There is an internationally growing interest concerning the use of natural extract sources in animal production area in order to improve the farming welfare and the animal performance. Nowadays, the application of rabbit as an animal model is widely accepted. In addition, the largely expanding rabbit production is mainly attributable to rabbit's high rate of reproduction, high potential of genetic selection, rapid growth rate, early maturation, efficient feed utilization and high quality of meat. The reproductive performance of male livestock is of economic importance, and improving semen quantity and quality, especially for artificial insemination, additionally helps to avoid the loss of valuable genotypes. The spermatozoa of vertebrates, including rabbits, display high rates of metabolic activity and are also rich in polyunsaturated fatty acids, making them particularly susceptible to oxidation by reactive oxygen species (ROS), especially under stress conditions. Within the endogenous antioxidant system of spermatozoa, many substances extracted from plants are able to improve plasma stability of lipid profile and to monitor ROS production. Natural antioxidants have been widely reported to have potent antioxidant, anti-inflammatory and antimicrobic activities related especially to their phenolic content. *Lippia citriodora*, a plant species in the *Verbenaceae* family, is characterised by the presence of several phenolic compounds, including avonoids, phenolic acids, luteolin derivatives and phenylpropanoids. Phenylpropanoid, particularly verbascoside [2-(3',4'-dihydroxyphenyl)ethyl-O-α-L-rhamnopyranosyl-(l-3)-β-D-(4-O-caffeoyl)-glucopyranoside], is the most abundant compound in *Lippia* extract. Verbascoside contains a rhamnose unit bound to glucose, which acts as a bridge, and exhibits a number of biological activities including anti-inflammatory and antioxidant. The protective

activity may be attributed either to the caffeoyl residue in the molecule acting by direct scavenging of reactive oxygen and nitrogen species, or as chain-breaking peroxyl radical scavengers. Based on the information present in bibliography, our research group tested this natural compound on several animal species, through the inclusion in animal feed. In addition, we evaluated verbascoside with the application on *in vitro* spermatozoa cell model, since to date only few findings are known. Based on our research experiences it can be stated that possible negative effect of verbascoside supplementation into feed mixture on semen quality parameters in rabbit bucks and adult brown hares as well as *in vitro*, obviously considering that target organs of antioxidant activities of phenylpropanoid glycosides are various. In addition, it has to be emphasized that the extract showed a reversible action, since the semen traits of treated animals returned to the normality after the dietary administration period. Due to growing interest in dietary application of natural extract, further research is needed to assess the effect of different doses.

Key words: feed additives; phenylpropanoid glycoside; livestock farming

INTERACTIONS BETWEEN THE CONTENT OF SELECTED TRACE ELEMENTS AND SPERM QUALITY IN COMMON CARP

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The reproductive performance of common carp can be influenced by several factors, such as mineral imbalance and aquatic contamination. Molybdenum (Mo) and other essential elements are correlated with semen quality parameters including sperm density and motility. Less attention is given to minor, but potentially dangerous elements, such as lithium (Li) and barium (Ba). The aim of the present study was to investigate the association between the content of selected trace elements and sperm quality in common carp.
Reproductively mature common carps (n = 16) (*Cyprinus carpio*) were used in our study. The milt was collected from the sperm duct post-mortem. Motility parameters (MOT, PRO, DAP, VAP, STR, BCF, concentration) of the semen were determined by the CASA assay. The samples of biological material were stored in a freezer at -20 °C until the processing. Quantification of the elements (Ba, Li, Mo) present in the milt was done using inductively coupled plasma–optical emission spectrophotometer (ICP OES 720, Agilent Technologies Australia (M) Pty Ltd.). Statistical analyses as well as Pearson's correlations were performed using STATGRAPHICS Centurion. Positive correlations were found between spermatozoa concentration and motility ($r = 0.577$; $P < 0.05$), as well as progressive motility ($r = 0.637$; $P < 0.01$). The analysis revealed significant associations between Ba and sperm quality parameters (DAP, $r = 0.530$; $P < 0.05$; VAP, $r = 0.544$; $P < 0.05$; BCF, $r = 0.590$; $P < 0.05$). Li content was correlated with progressive motility ($r = 0.557$; $P < 0.05$). In conclusion, our findings suggest that evaluated elements affected sperm quality in common carp. In further studies is necessary to test broader spectrum of elements, which may influence motility parameters of common carp semen.

Key words: trace elements; carp semen; CASA; ICP-OES; motility

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TESTING OF NUTRIENT REQUIREMENTS OF RED DEER

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Slovakia has experienced a rapid increase in the number of red deer farms in the recent years and according to the amendment to the Act no. 184/2018 Coll. on veterinary care, red deer is treated as a farm animal. In such a situation, precise feeding of red deer is required, as in the case with other farm animals. We carried out feeding and balance experiments with Slovak origin red deer over several years in the laboratory of ruminant physiology of nutrition at the Department of nutrition. The results of these experiments have been used to prepare tables of nutrient requirements for fawns, females and males evaluated for particular seasons of the year. Daily intake of milk replacer for fawns was markedly increased in the first days of life. At 21 days of age, it was 3.750 ml per animal with body weight (BW) 16.9 kg. In the categories of older animals, without milk replacer, at 95 days of age (BW 42.5 kg) / 195 days (BW 90 kg), the daily requirement of dry matter is (DM) 1550 / 2350 g, crude protein (CP) 301 / 400 g, Ca 15 / 24 g, P 10/15 g, metabolizable energy (ME) 16 / 26 MJ. Nutrient requirements were calculated for females weighing 150 kg. From the point of view of physiological burden on females, we can distinguish two periods during the year. In January – March the nutrient requirements are decreasing and in the period of summer during lactation and autumn the nutrient requirements are increasing: DM 2550 / 3450 g, CP 360 / 560 g, Ca 20 / 29 g, P 12 / 18 g, ME 25 / 37 MJ. Males have the greatest body weight before the rut in summer (275 kg) and during the rut in September – October. With significantly reduced food intake and increased activity the weight can fall to 30 %. Daily nutrient intake for males in summer and during rut is following: DM 5010 / 1490 g, CP 830 / 202 g, Ca 54 / 16 g, P 31 / 9 g, ME 56 / 14 MJ.

In this paper we present the first table values of the nutrient requirements tested on Slovak origin red deer.

Key words: red deer; nutrient; requirements

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BROWN HARE (*LEPUS EUROPAEUS*) AS A BIOINDICATOR IN AGRICULTURAL LANDSCAPE

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Brown hare is a bioindicator game animal, whose health status is endangered by anthropic factors. It lives in agricultural landscape affected by foreign substances introduced by industrial fertilisers, pesticides and industrial emissions. Today, it is essentially impossible to find a hare without pathological changes.

The aim of the study was the research of a xenobiotic presence in the agrarian landscape. The samples (parenchymatic organs, fat tissue) were collected by an autopsies of hares harvested during the hunting season (November – December). Organic pollutants were determined by gas chromatography, heavy metals – by an atom spectrophotometry and aflatoxins by a RIA method. Statistical analysis was performed using the Excel software. DDT content in old hares was significantly higher than in the young hare category (three-times; P = 0.000+++). HCB content in old hares was also significantly higher than in the young hare category (25 times; p = 0.0017++). For α+β-HCH no difference was determined between the young and old hares (P = 0.191) or between the sexes (p = 0.1767). Content of γ-HCH in the fat of old hares was significantly higher than in that of young ones (three-times; P = 0.0123+). The effect of sex on the organic pollutant content was essentially non-significant. PCB content was approximately 2.4 times higher in old hares compared to young and the difference was highly significant (P = 0.000+++). The contents of heavy metals (Pb, Hg, Cd) were also determined. Seasonal differences in the content of Pb and Hg in hares were significant; therefore, a season in which the experimental samples were collected was taken into account. No significant seasonal differences were determined for the Cd content, but significant differences were confirmed based on the age of the hares, which is necessary to consider at the result interpretation. Pb and Hg content in the organs of our hares was relatively low. The examined hares probably consumed a feed contaminated by various concentrations of fungal metabolites-mycotoxins. Feeds displayed no noticeable organoleptic differences. Feeding an infected feeds can lead to hidden uncontrolled intake of mycotoxins and even to subclinical and clinical symptoms of aflatoxicosis. In hares, aflatoxicosis can be the cause of numerous undetermined steatoses of the liver in winter or spring season.

Toxicological examinations to determine residues of the selected organic pollutants point towards the fact that not all locations of the monitored territory are affected at the same level, but the levels of organic pollutant, we determined in the hares, did not exceed the limits set by hygienic norms.

Key words: brown hare; bioindicator; organic pollutants; heavy metals; mycotoxins

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POTENTIAL EFFECTS OF GRAPE PHYTOCHEMICALS ON OVARIAN CELLS *IN VITRO*

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Phytochemicals may play an important role in decreasing the risk of chronic disease including certain types of cancer disease. Grapes, one of the most popular and widely cultivated and consumed fruits in the world, are rich in phytochemicals. Human ovarian granulosa cell line (HGL5) and human ovarian carcinoma cell line (OVCAR-3) represent model systems for understanding the molecular mechanisms of phytocompound action in healthy and pathological cells. The aim of our study was to evaluate the effect of grape pomace extract *(Vitis vinifera* L., cultivar *Pinot gris*) at the doses of 0; 6.25; 12,5; 25; 50 and 100 $\mu g.ml^{-1}$ on the viability of HGL-5 cells, as well as on the secretion of steroid hormones and the viability of human ovarian carcinoma cell line (OVCAR-3). The metabolic activity was evaluated by alamarBlueTM cell viability assay and the release of hormones was assayed by ELISA methods. The viability of HGL5 cells was not significantly influenced ($P \geq 0.05$) by grape pomace extract at all concentrations. The secretion of 17ß-estradiol and progesterone was significantly ($P \leq 0.05$) decreased at the highest concentration – 100 $\mu g.ml^{-1}$. On the other hand, the viability of OVCAR-3 cells was significantly ($P \leq 0.05$) decreased after addition of extract at the concentrations of 12.5; 25; 50 and 100 $\mu g.ml^{-1}$ compared to the control.

Our results suggest that phytochemicals of grape pomace extract could be potential regulators of steroidogenenis and viability of human ovarian cells *in vitro*.

Key words: ovarian granulosa cells; grape; viability; steroid hormones

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ALTERATION OF SOME HAEMATOLOGICAL PARAMETERS OF RABIT BLOOD AFTER MYCOTOXIN EXPOSURE

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The goal of the present study was to analyze the effect of a single dose of mycotoxins (deoxynivalenol DON and T-2 toxin) on selected hematological parameters of rabbit's blood. Experimental group of rabbits received mycotoxins intramuscularly (Romer Labs Division Holding GmbH, Tulln, Austria) at the dose of 0.08 mg per kg of body weight 72 hours before slaughter. Whole experiment lasted 90 days. Selected haematological parameters (WBC – total white blood cell count, MID – medium size cell count, GRA – granulocyte count, RBC – red blood cell count, HGB – haemoglobin, HCT – haematocrit, PLT – platelet count, MPV – mean platelet volume, and PDWc – platelet distribution width) were measured using haematology analyser Abacus junior VET (Diatron®, Austria). Neither DON nor T-2 toxin given at a single dose of 0.08 mg had significant effect on rabbit blood. However, our previous studies revealed that various secondary metabolites exhibit a wide range of immunomodulating activity. High doses of deoxynivalenol influenced the lymphocyte count in porcine blood *in vitro*. Results of this study provide the basis for further research of a mycotoxin impact on blood cells.

Key words: mycotoxins; haematological parameters; immunomodulating activity

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UTILIZATION OF BEE BREAD IN THE MODULATION OF AN INTERNAL MILIEU OF ZDF RATS

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Male ZDF rats (a fatty fa/fa mutation; n = 40) and their lean non-diabetic counterparts (controls; +/+ or +/-, with no expression of the fa phenotype; n = 10) at the age of 3 months were involved in the experiment. The animals were divided into 5 groups as follows: the control group (K), the group of obese rats fed with a high-energy diet (P1), the group of obese rats fed a high energy diet and a daily dose of bee bread (P2), the group of obese rats fed with a normal diet and a daily dose of bee bread (P3), and the group of obese rats fed with normal diet (P4). For experimental groups P2 and P3, bee bread was administered daily orally via a probe at the amount of 500 mg per kilogram of live weight. All rats had an access to water and food *ad libitum*. After 3 months of experiment the blood was taken and selected haematological and lipid parameters were determined. The mean values of total cholesterol (TC), triacylglycerides (TAG), HDL and LDL cholesterol were measured using Biolis 24i Premium Biochemical Analyzer (Tokyo Boeki Medi Sys Inc., Japan). We found significant change ($P < 0.05$) in the total number of erythrocytes (RBC). These changes have been attributed to developing diabetes. We observed a significant increase ($P < 0.001$) of all monitored parameters in the all experimental groups when compared to the control group. The contents of TC, TAG, HDL and LDL cholesterol were without significant changes ($P > 0.05$).
The hypolipidemic effect was not statistically significant in the groups with orally administered bee bread. Further experiments with various doses are needed.

Key words: bee bread; high-energy diet; *diabetes mellitus* type 2; Zucker diabetic fatty (ZDF) rats; haematological parameters; lipid profile

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GLUCOCORTICOID RECEPTOR SPLICE VARIANTS IN MOUSE PREIMPLANTATION EMBRYOS

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We examined whether glucocorticoids (endogenously produced during stress reactions or therapeutically administered) can directly affect preimplantation embryo. Because of alternative processing of glucocorticoid receptor (GR) five trancripts exist in humans, but only three of them (GRα, GRβ, GRγ) were found in mouse adult tissues. Using RT-PCR we detected GRα, GRβ, GRγ splice variants in mouse oocytes and preimplantation embryos in our study. To ascertain whether truncated mouse glucocorticoid receptor, which is orthologous to the human GR-P splice variant (i.e., "mouse GR-P"), is expressed in preimplantation embryos, we designed specific primers using information on the structure of human "canonical" (GR alpha) and GR-P transcripts. Forward primer was located in the "canonical" GR sequence. Two GR-P reverse primers ("RP1" and "RP2") were located in the GR-P-specific sequence. The RP1 reverse primer was used for analysis of GR-P expression in mouse oocytes and embryos and RP2 reverse primer was used to obtain the mRNA sequence of the mouse GR-P ranging beyond the first in-frame STOP codon. We showed the presence of GR-P splice variant in mouse brain tissue, as well as in the blastocyst for the first time. Our results indicated that the first part of mouse GR-P transcript

sequence is located in the end of exon 6 and in exon 7 and the next part is located in the beginning of original intron 7. Our comparison of human GR-P transcript with the mouse orthologue, identified in our study, revealed significant differences within the coding region of transcripts suggesting possible interspecies differences in GR-P functioning.

Key words: preimplantation embryos; glucocorticoid receptor; splice variants

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BREEDING OF MINIPIGS AS LABORATORY ANIMALS

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Minipigs are one of the most important laboratory animals because of their similarity to human. They are small, thus, much easier for handling. Also, requirements of food, space and pharmacologic products are significantly reduced. There are at least 45 breeds of minipigs available worldwide (Smith & Swindle, 2006). While conventional farm pig breeds are extensively used in pork industry, smaller pigs, named minipigs or miniature pigs, are produced by cross-breeding of various purebred or wild species for special needs (Vodicka *et al.*, 2005). The aim of our study was to describe growth intensity and reproduction parameters of minipigs bred in the Institute of Animal Science Prague. The herd was established in 2009. The minipigs of Minnesota type were the basis for a new herd. The stabilization of a phenotype, especially in body weight, was realised during last years. Only natural breeding is used in minipigs. Piglets are weaned at two months of age. The special feed mixture is used for minipigs to fed them.

The data from 89 litters were collected in years 2009-2017. The number of piglets born, born alive and weaned was monitored and percentage of piglet losses was calculated. The growth parameters were measured in 100 piglets-birth weight, weight at weaning and every month until one year of age. The average birth weight of piglets was 352 ± 85 g, weight at weaning – 3520 ± 743 g and live weight at 12 months of age was 49.4 ± 6.3 kg. Reproduction parameters of monitored litters were as follows: number of total born piglets – 8.6 ± 2.9, number of live born piglets – 7.1 ± 2.3, number of weaned piglets – 6.7 ± 2.4 and calculated piglet losses were 6.1 %.

Farm breeding of minipigs is not common in the Czech Republic; breeding as pet animal prevails. There are no available data for comparison. In comparison with original Minnesota minipigs, more piglets born/litter and lower birth weight were monitored in our study. Minnesota breed reaches at average 6 piglets per litter, birth weight of piglets is 590 g and body weight of adult minipig is 55-70 kg (McAnulty *et al.*, 2005).

Key words: minipig; reproduction; growth

GENETIC MARKERS AS A TOOL FOR TRACEABILITY OF ORAVKA PRODUCTS

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The aim of present study was to assess the level of genetic diversity within three autochthonous chicken breeds, as well as to identify the genetic relationships between them based on microsatellite data. In this study, overall 78 animals belonging to the yellow-brownish Oravka (Ob), the white Oravka (Ow) and the Sumavanka (S) breeds were included. The genomic DNA was extracted from feather samples by using commercial kit according to Wizard® Genomic DNA Purification Kit Protocol (Promega Corporation). A panel of eight microsatellite markers (Lei166, Lei192, Lei228, Lei229, Lei234, Lei254, MCW34, and MCW69) were used for animals' genotyping. The level of genetic diversity within each breed was determined based on the calculation of mean number of alleles (MNA), observed heterozygosity (H_o), gene diversity expressed as expected heterozygosity (H_e), effective allele number (N_a) and Shannon's information index (I) using Genalex v6.1. The amount of inbreeding-like effect within breeds, expressed by the Wright's F_{IS} index, was calculated using FSTAT v2.9.3.2. The genetic relationships among breeds were estimated based on the Nei's genetic distance and Wright's F_{ST} index. In addition, the discriminant analysis of principal components (DAPC) was used to examine the genetic structure of breeds under consideration. The mean number of alleles across breeds ranged from 2.50 ± 0.33 (Ow) to 8.00 ± 1.43 (Ob), which signalized a certain decrease of genetic variability mainly in case of the White Oravka and the Sumavanka breeds. This decrease also confirmed the obtained values of effective number of alleles (2.13 to 3.46) and Shannon's information index (0.75 to 1.36). Similarly, the gene diversity indicated higher proportion of heterozygosity within the yellow-brownish Oravka (0.63 ± 0.08) compared to the white Oravka (0.46 ± 0.08) and Sumavanka (0.44 ± 0.10) breeds. However, despite the higher proportion of heterozygotes within the yellow-brownish Oravka, the Wright's F_{IS} index showed that this population can be affected by a relatively strong impact of inbreeding (F_{IS} = 0.17). The lowest value of F_{IS} index (0.03) pointing to low effect of relatives mating was found in the Sumavanka breed (F_{IS} = 0.03). The average values of Nei's distance (0.93 ± 0.23) and F_{ST} index (0.33 ± 0.08) showed that the populations were genetically differentiated. As expected, due to the genetic background of analysed breeds, the highest genetic similarity was found between the white and yellow-brownish Oravka, while the highest genetic distance showed the white Oravka and the Sumavanka breeds. Moreover, the DAPC analysis indicated that between the white and yellow-brownish Oravka some level of admixture can be found. The obtained three discriminant functions corresponded to 67.4 % of total genetic variance conserved in the analysed dataset. Based on the study

results it can be concluded that the state of genetic diversity within analysed autochthonous chicken breeds should be monitored constantly. Moreover, the increase in a sample size could be beneficial to describe their gene pool in a more detailed level, as well as to identify genetically most important individuals for the sustainable management of animal genetic resources in future.

Key words: diversity; chicken; local breeds; microsatellites; traceability

BIOTECHNOLOGY APPLICATION – ARTIFICIAL INSEMINATION AND EMBRYO TRANSFER IN CONTROLLED BREEDING OF WILD ANIMALS

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Mouflon (*Ovis aries musimon*) is the only wild form of sheep living in Europe. Most of the introduced population into continental Europe come from the islands of Sardinia and Corsica. The aim of the work was to create a gene pool of the highest quality donors from the point of view of genetic variability, the quality of the horns and to create a gene pool of frozen embryos of mouflon. Hormonal synchronization was used to induce a superovulation reaction by combining an intravaginal CIDR G device in combination with the hormonal preparation, Pluset® (FSH / LH), which was administered on day 11 to 14 of progesterone treatment at 12 hour intervals. Artificial insemination was performed by 48 hours after removal of the intravaginal tampon. Embryo gain was performed on the 6th day of ovulation at the compact morula stage, eventually early blastula. The embryos obtained were then frozen by cryopreservation techniques and archived in a liquid nitrogen. Experimental embryo transfer of frozen mouflon embryos was performed on the domestic sheep recipient (cross valaška and lacaune). On the 157th day of pregnancy a mouflon male was born. Application of the MOET (Multiple Ovulation and Embryo Transfer) can help to vital rescue programs for endangered animal species.

Key words: mouflon; embryo; embryo transfer; cryopreservation

MOUSE EMBRYOS FROM OBESE DAMS SHOW HIGHER SENSITIVITY TO OXIDATIVE STRESS *IN VITRO*

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Aim of study was to test hypothesis that maternal obesity can affect sensitivity of preimplantation embryos to oxidative stress.

To produce mice with obesity-like phenotype, a trans-generation model based on the over-nutrition of experimental animals during intrauterine and early postnatal development was used. To assess the sensitivity of preimplantation embryos to oxidative stress, an *in vitro* experiment was performed: the 2-cell stage embryos isolated from control and obese females were cultured in the presence of three different chemicals till blastocyst formation. Following oxidative stress inductors were used: AAPH [2,2'-Azobis (2-methylpropionamidine) dihydrochloride] at 0.01 mM, SNP (sodium nitroprusside) at 0.01 mM and BSO (buthionine-sulfoximine) at 5 mM.

Stereomicroscopic evaluation showed that the presence of SNP and BSO negatively affected *in vitro* development of embryos isolated from obese mothers. In control embryos, similar tendency was recorded only after exposition to BSO. In blastocysts originating from obese dams, decreased cell numbers after treatment by AAPH and BSO, and increased incidence of cell death after treatment by SNP and BSO were revealed. Assessment was performed by means of fluorescence imaging. In control group, negative effect on blastocyst quality was observed only after BSO treatment. In conclusion, the results show that preimplantation embryos isolated from obese mice display higher sensitivity to oxidative stress *in vitro* than embryos isolated from control females.

Key words: preimplantation embryo, mouse, *in vitro*, obesity, oxidative stress

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