

**TETRASPANINS EXPRESSION ON BOVINE AND PORCINE OOCYTES**J. ANTALÍKOVÁ<sup>1\*</sup>, J. JANKOVIČOVÁ<sup>1</sup>, M. SIMON<sup>1</sup>, P. SEČOVÁ<sup>1</sup>, K. MICHALCOVÁ<sup>1</sup>, L. HOROVSKÁ<sup>1</sup>, E. CHMELÍKOVÁ<sup>2</sup>, P. POSTLEROVÁ<sup>3</sup><sup>1</sup>Centre of Biosciences Slovak Academy of Sciences, Institute of Animal Biochemistry and Genetics, Bratislava, Slovakia<sup>2</sup>Faculty of Agrobiolgy, Food and Natural Resources, Prague, Czech Republic<sup>3</sup>Institute of Biotechnology, Czech Academy of Sciences, v.v.i., BIOCEV, Laboratory of Reproductive Biology, Vestec, Czech Republic

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Tetraspanins are multifunctional molecules located in specific microdomains on the plasma membrane. Thanks to their ability to enter into molecular partnerships with other members of tetraspanin family or other proteins, they can form tetraspanin web and thereby affect many cellular functions. Well-described involvement of tetraspanins in somatic cell immune response, cell migration, viral infections, metastasis formation etc., definitely suggests their participation in similar processes occurring during gamete interactions. Up to this date, although an extensive study proved the fact that tetraspanins CD9 and CD81 are directly involved in gamete interaction of mammals, their precise role in the fertilization process is not clear yet. In the present study, we compared the localisation of these two tetraspanins on bovine and porcine oocytes at different stages of development. We investigated a possible role of CD9 and CD81 molecules in the fertilization process of cattle using polyclonal antibodies to CD9 and CD81 in *in vitro* fertilization assay. In our experiment, no significant reduction in the number of fertilized eggs and cleavage of zygotes after pre-treatment of oocytes with CD9 and CD81 antibodies was observed. The obtained results provide additional data on comparison of fertilization process in cattle and pigs.

**Key words:** CD9; CD81; pig; cattle; fertilization

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**THE EFFECT OF THIACTOPRID ON PREIMPLANTATION EMBRYO DEVELOPMENT IN MOUSE AND RABBIT**J. BABELOVÁ<sup>1\*</sup>, Z. ŠEFČÍKOVÁ<sup>1</sup>, M. FÖLDEŠIOVÁ<sup>2</sup>, L. OLEXÍKOVÁ<sup>2</sup>, A. BALÁŽI<sup>2</sup>, A. MAKAREVICH<sup>2</sup>, P. CHRENEK<sup>2</sup>, J. KOPPEL<sup>1</sup><sup>1</sup>Slovak Academy of Science, Institute of Animal Physiology, Košice, Slovakia<sup>2</sup>NPPC – Research Institute for Animal Production Nitra, Lužianky, Slovakia

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The aim of this *in vitro* study was to evaluate the potential toxicity of different doses of active neonicotinoid compound thiacloprid on developmental capacities and qualitative parameters of preimplantation embryos in mice and rabbits. Embryos isolated from superovulated dams at the 2-cell stage of development in mouse / the pronuclear stage of development

in rabbit were cultured in media supplemented with different concentrations of the insecticide until blastocysts formation. Stereomicroscopic examination of *in vitro* developed mouse embryos showed that thiacloprid, given at the highest concentration (100 µM), negatively affected development of 2-cell stage embryos and quality of obtained blastocysts, as shown by significantly decreased average cell number and increased incidence of dead cells in the pesticide-exposed mice embryos (assessed by fluorescence microscopy). Moreover, thiacloprid influenced qualitative parameters of blastocysts also at 10 µM concentration. Lower doses of tested substance had no detrimental effect on mice embryos. The presence of thiacloprid at concentration 100 µM had negative effect also on rabbit embryonic development. Thus, in the blastocysts derived from rabbit eggs, significantly decreased cell numbers and increased percentages of dead cells were recorded. Thiacloprid at 10 µM slightly increased incidence of dead cells, but it did not affect average cell numbers in the pesticide-exposed rabbit embryos. The results of the present study indicate that thiacloprid can influence development and quality of mouse and rabbit preimplantation embryos. The serious toxic effect of this neonicotinoid was observed in a dose-related manner, with significant impact at a concentration of 10 µM and above. Sensitivity of embryonic cells to thiacloprid in two evaluated species (mouse and rabbit) was similar. The neonicotinoid-induced changes during preimplantation period of development have to be considered a potential risk factor in mammalian reproduction.

**Key words:** thiacloprid; preimplantation embryo; toxicity

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**SAFEGUARDING DIVERSITY – THE AUSTRIAN GENE BANK FOR FARM ANIMALS 1997 TO 2017**

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The Austrian Genebank for Farm Animals was founded in 1997 at the former Institute of Organic Farming, now Institute of Organic Farming and Biodiversity of Farm Animals. It belongs to the Austrian Government and is funded by the research budget of the AREC Raumberg-Gumpenstein. The Genebank is a registered AI centre for cattle, goats and sheep. Originally planned as support for *in situ* conservation programs for highly endangered Austrian breeds it has developed into a comprehensive archive of Austrian animal breeding. Today the Genebank consists of semen collections of rare cattle, goat, sheep, pig and horse breeds, backup collections of cattle and pig artificial insemination centres, a collection of commercial goat breeds and the Farm Animal DNA Bank with isolated genomic DNA. The collection of rare breeds is divided into a working collection supporting the ongoing *in situ* conservation programs for highly endangered Austrian breeds and a duplicate collection for safety reasons. All collections are continually replenished and completed. Currently an extension of the Farm Animal DNA Bank to supply genomic DNA of sufficient quality

for single nucleotide polymorphism genotyping is planned. Sires of rare breeds chosen for semen collection should be as little related as possible to each other and to the breeding population. Until now, relatedness was calculated according to pedigree data, now genomic analysis is employed increasingly. The main genomic material in store is semen. To be able to re-establish breeds in case of catastrophes, a supplemental storage of embryos, ova and tissue for cloning would be advisable. The oldest material in storage is about 50 years old. To ensure the continuing use of the Genebank, a national derogation for use of old genebank material not complying with modern regulations is in preparation.

**Key words:** Genebank; rare breeds; farm animals; semen

#### MANAGEMENT AND CONSERVATION OF ANGR IN SLOVENIA

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The most important milestone in the field of Animal Genetic Resources (AnGR) conservation in Slovenia was the ratification of the Convention on Biological Diversity (CBD) in 1996 and the preparation of the Biodiversity conservation strategy, where AnGR conservation was revealed. In 2002, the Livestock Breeding Act protected the Slovenian local breeds and established the institutional framework for their conservation. Management and conservation of AnGR is carried out by the Public service for AnGR conservation (Public service) authorized by the Ministry of Agriculture, Forestry and Food (Ministry). The role of the Public service is to carry out all the necessary measures and actions to protect the AnGR in Slovenia, such as preparation of the long-term and yearly National programmes for AnGR conservation, which form a part of the global efforts to conserve AnGR. In the past, many breeds have been lost, however today we managed to conserve all the local breeds which were almost lost 30 years ago. The Public service is actively working on awareness rising amongst wider public, schools and farmers; it establishes ark farms, centres and parks as well as maintains gene bank of cryopreserved material. In addition, different mechanisms to support and conserve local breeds are used in the country, such as incentives from Rural Development Plan and dedicated “*de minimis*” aids. Despite the efforts in the past 30 years, among the twelve local Slovenian breeds, seven breeds are critically endangered, three are endangered and one is vulnerable. The outline of the past and current conservation measures and activities on the AnGR clearly shows that there is still a considerable need for the careful planning of their management and conservation. Their conservation will contribute to the global and national food security and would better respond to the climate changes, different consumer demands and modern animal breeding needs.

**Key words:** animal genetic resources; local breeds; conservation; Slovenia; endangerment

#### NEED FOR A PROGRAMME FOR ANIMAL GENETIC RESOURCES

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Biodiversity is useful to humankind for economic, cultural and ecological purposes. Not only does it give us many primary materials and draws great benefit from it, it regulates the biosphere as well. Animal genetic diversity is a part of the earth's natural heritage, and humankind depends on animals for food, for clothing and for work. Animal genetic resources allow farmers to select stocks or develop new breeds in response to changes in the environment, threats of disease, new knowledge of human nutrition requirements, changing market conditions and social needs, all of which are largely unpredictable. What is predictable is the future human demand for food. Given the above facts, domestic animal diversity is critical for food security. It is important to stop and reverse the erosion of this diversity. For thousands of years, livestock have been intimately associated with human life. They are one of the special characteristics of human culture. Livestock are essential elements of the economy at local, national and international levels. Livestock management systems respond to economic conditions. Genetic variation is the raw material for animal improvement, its conservation provides options for uncertain and unknown future economic needs. Conserved genetic variation will offer the resources to respond quickly and economically to changes in the market. Breeds with unique traits are of great scientific interest in a number of ways. Some obvious examples are the unique DNA sequences of species, breeds, strains and populations, the specialised physiological and adaptive functions and the opportunity to study animals as biological models. Investigations at the molecular level enter a new era, when society will benefit from manipulation with biological material. Genetic variation will be of increasing interest and importance to science both at the animal and molecular or sub-molecular levels in the future. Domestic animals not only share DNA as the common basis of their genetic nature with all other species, but they are integrated also in many management systems with pasture and forest species and wild animals. The overall approach to conservation of biological diversity should ideally embrace all these species as well as the animal breeds, which provide diversity within the domestic animal species. A primary goal of the management of local, national and global animal genetic resources is to ensure the sustainable development of livestock in particular and agriculture in general.

**Key words:** biodiversity; animal genetic resources; livestock; molecular and economic needs; sustainability

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**ALTERATIONS IN HAEMATOLOGICAL PARAMETERS IN DIABETIC RATS**

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The aim of this study was to determine the effect of chronic high-energy diet feeding on development of *diabetes mellitus* complications in ZDF rats focusing on haematological parameters. Male Zucker diabetic fatty (ZDF) rats (a fatty *fa/fa* mutation (-/-); n = 20) and their lean controls (ZL, non-diabetic, +/- or +/+, not display expression of *fa* phenotype, n = 10) at the age of 3 months were involved in the experiment. The animals (Breeding Facility of the Institute of Experimental Pharmacology and Toxicology Dobra Voda, Slovak Republic, SK U 25016) were housed at number of two rats per plastic cage (800 m<sup>2</sup>) and under specific pathogen free conditions at 23 ± 2 °C and 55 ± 10 % relative humidity with a 12 h light-dark cycle. Rats were provided with water and diet on *ad libitum* base. Rats were divided into three experimental groups as follows: lean untreated rats (C) fed KKZ-P/M (10 MJ.kg<sup>-1</sup>), obese rats fed KKZ-P/M (10 MJ.kg<sup>-1</sup>, E1) and obese rats fed enriched high energy diet (E2, enriched KKZ-P/M, 20 MJ.kg<sup>-1</sup>). Enriched diet was served to induce *diabetes mellitus* symptoms earlier. At the end of the experiment (after 2 months), animals were anesthetized by intraperitoneal injection with chloral hydrate. Blood samples were collected into EDTA-treated tubes. In a whole blood, chosen haematological parameters [total white blood cell count (WBC), granulocyte count (GRA), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), platelet count (PLT), mean platelet volume (MPV)] were measured using Abacus junior VET haematology analyser (Diatron®, Vienna, Austria). One-way ANOVA test was performed to calculate basic statistical characteristics and to determine significant differences. GRA count increased significantly (P < 0.05) in E1 against the control and E2 group. The high-energy diet caused significant decrease (P < 0.05) in MCV in comparison with the control group. Significant increase (P < 0.05) in MPV was observed in E1 group against the control. The results of other haematological parameters were without significant differences (P > 0.05) among the groups of rats. The rise in the secondary symptoms of diabetes complications by high-energy diet was accompanied by disturbed haematology parameters, which is a potential marker of angiopathy. Extended research on diabetes is needed.

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

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*Invited paper***PRESERVATION AND CONSERVATION OF SHEEP GENETIC RESOURCES IN NORTHERN SERBIA, VOJVODINA**

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Nowadays, the largest number of sheep breeds in Vojvodina belong to breeds imported from abroad, primarily oriented to the production of meat. In the past decades, the number of autochthonous sheep breeds has been declining. The main factors affecting the number of autochthonous sheep breeds are low productivity and unprofitability of their production. The areas under the pastures in Vojvodina have been significantly reduced, which further complicates the breeding of endangered sheep breeds, whose nutrition is mainly based on grazing. An additional problem is their unplanned crossing with other breeds. Endangered autochthonous breeds of sheep in Vojvodina are Wallachian sheep and Choka Tsigai sheep. Currently, in the Republic of Serbia only *in situ* conservation is being performed, while *ex situ* or cryopreservation is not implemented. *In situ* conservation includes conservation or the breeding of live animals in production systems which have arisen or in which they are located. *In situ* conservation allows the preservation and maintenance of animal population in their natural environment, leaving the evolutionary processes to shape the genetic divergence and continue the adaptability process of the population, which we preserve. Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia decided to grant subsidies to breeders involved in the breeding of endangered sheep breeds. This decision had a positive effect, to a certain extent, on the number of animals, which has been stabilized. In the future, there is a need to find new ways to achieve a sufficient number of animals by introducing new conservation technologies and additional stimulating measures for breeders.

**Key words:** Autochthonous sheep breeds; Wallachian sheep; Choka Tsigai sheep; *in situ* conservation

**OCCURRENCE OF CHOSEN TRACE METALS DEPENDS ON THE GENDER OF FRESHWATER FISH (CYPRINUS CARPIO)**

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Nowadays a significant increase in heavy metal contamination in the environment has become a worldwide problem due to anthropogenic activity such as industry, agriculture and metal processing. Chronic exposure to heavy metals may result in various alterations of animal and human health, especially when

their dietary content exceeds the permissible levels. Heavy metal toxicity may result in neurological disorders, cancer, liver and kidney damage, as well as numerous other health complications, which have significantly increased the mortality rate over the past decades. Therefore, our study was aimed to compare the concentrations of chosen heavy metals in male and female carp blood and to detect the possible associations. Blood samples were taken from males ( $n = 18$ ) and females ( $n = 24$ ) of freshwater fish (*Cyprinus carpio*). The coagulated blood was centrifuged for 20 min. at 3000 rpm, the blood serum was collected and stored at  $-20\text{ }^{\circ}\text{C}$  until analyses. The concentrations of chosen heavy metals (Mo, Al, Ba, Li) were determined from blood serum by inductively-coupled plasma optical emission spectrometry (ICP-OES). Basic statistical analysis as well as analysis of variance (one-way ANOVA) following Tukey's HSD procedure and Pearson's correlations were performed using STATGRAPHICS Centurion. Our results showed significant negative correlation between Ba and Li ( $r = -0.588$ ;  $P < 0.05$ ) concentrations in male carp blood samples. Moreover, in the female blood samples significant correlation between Mo and Li content ( $r = -0.631$ ;  $P < 0.001$ ) was detected. However, no noticeable differences in concentrations of chosen heavy metals between genders were observed. Obtained data indicate possible correlations between selected heavy metals and their increased content in blood, which may have significant impact on physiological processes in animal organism. Therefore, it is highly recommended to monitor the content of environmental contaminants in organisms.

**Key words:** heavy metals; fish; gender; blood

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## SLOVAK BREEDS OF HORSES

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Horse breeding has undergone significant changes in recent years. From the basic element of agricultural production, horse breeding has become only an additional part of landscaping, forestry and usage of agricultural products. For these reasons, breeding of traditional breeds of horses belongs to a marginal area and is seriously endangered. In Slovakia, this group includes Norik of Muran, Hucul horse breeds and Slovak sport pony. Norik horse is the most typical representative of a horse western breed group. This breed has undergone long-term development due to external conditions and now it differs from eastern type of horses with a more robust body structure. Today the Norik breed is fixed and clearly defined. One of the important breeders of coldblood horses not only within Slovakia is the "Forests of the Slovak Republic", s.e. Horse breeding is realized since 1950 in Forest enterprise Revúca of Horse breeding resort Dobšiná. An incentive for its establishment was the need for horses for forestry.

The importance of coldblood horses will also increase during approaching timber from the protected areas and national parks. The Hucul horse is the typical representative of a mountain horse breed. The home of this horse is Hucul - a distinctive region in the Eastern Carpathians of Romania-Ukraine border. Skeletal remains and hoofs print of the right horseshoe in the underground area of about 700,000 to 245,000 years old allow us to mark a Hucul horse as the autochthonous breed of the Carpathians. The base of Hucul horse breeding in Slovakia has been built in 1922 in the National Stud Farm Topoľčianky. Hucul horse breeding in Europe is currently concentrated mainly in Romania, Poland, the Czech Republic, Slovakia and, to a lesser extent, in Hungary and Austria. There are 7 lines kept in the breeding and they are recognized as international lines of the Hucul horse: Gurgul, Goral, Oušor, Hroby, Prislop, Pietros, Polan. For the rescue and development of the Hucul horse, the HIF (Hucul International Federation) was established in 1994. The Slovak sport pony is a product of a systematic interbreeding crossing of mares of the noble warmblood breeds of horse breed in Slovakia. In particular, there are breeds of Arabian and Slovak warmblood with stallions of pony breeds: the Welsh pony, the German riding pony, and in the second phase of the English halfblood horses. The Slovak sport pony is a multipurpose utility, driving-riding type, with a predominance of riding type. It is designed for riding training for children and teenagers aged 8 to 16, sports, recreational riding and hiporehabilitation. Pony breeding in Slovakia has been carried out since 1980 and currently the breeding core consists of 55 breeding mares and 4 breeding stallions.

**Key words:** gene reserves; Hucul horse; Norik of Muran; Slovak sports pony

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## THE EFFECT OF CONSERVATION MEDIUM ON STALLION SPERMATOZOA MOTILITY

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Horse breeding in the last decades is mainly related to leisure and sporting activities. Horses are in this respect related to great international quantities. Breeders invest significant funds to obtain the most powerful individuals. Top individuals are used for long time in sport and at the same time, application of biotechnological methods is implemented in their reproduction. The objective of this study was to evaluate and compare qualitative differences in motility parameters of ejaculate with the addition of commercial preservation medium (at the ratio 1:3) and ejaculate diluted with physiological solution (NaCl 0.9 %, Braun, B. Braun Melsungen AG, Germany) at the ratio 1:3. Fresh semen was obtained from 12 breeding stallions at the National Stud Farm in Topoľčianky at age of 5–26 years composed of following breeds: Lipican, Arab

thoroughbred, Hucul, Selle française, Holsteiner, Shagya-arab. Semen was collected by lubricated pre-warmed artificial vagina at the start of the breeding season in February. CASA analysis (Sperm Vision; Minitube, Tiefenbach, Germany) was used for determination of spermatozoa motility. Analysis was performed at seven time periods of sampling, after the 0, 1, 2, 3, 6, 24 and 48 hours. The ejaculate diluted with physiological solution of selected stallions showed rapid reduction of spermatozoa activity in all measured parameters and viability 6 hours after the collection. Analysis of spermatozoa with an added preservation medium showed that motility achieved significantly higher values. From these results, it can be concluded that the addition of preservation medium helps to prolong the viability of stallion spermatozoa and increase the reproductive quality of breeding stallions.

**Key words:** stallion; spermatozoa; preservation medium; CASA

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## PRESERVATION OF ANIMAL GENETIC RESOURCES IN CROATIA

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Animal genetic resources are Croatian heritage with values visible on the economic, social, natural and cultural level. Local breeds include within genome numerous events that occurred in their environment, with or without human influence. They are a living monument to past times and they preserve the identity of the area. Their genes contain a possible potential for safe food production in future times when the importance of their resistance and adaptability will reach its full extent. Main reasons for the disappearance of a part of local breeds in Croatia are: globalisation, industrialisation and intensification of agricultural production, market liberalisation, import of “more productive” exotic breeds, depopulation and urbanisation of rural areas. Program for protection of autochthonous breeds in the Republic of Croatia started three decades ago. Protection is mainly performed through *in situ* models, in which local breeds present a part of the agro-ecosystem, economy and culture of the local community. Six years ago the Croatian government adopted “National programme for the protection of autochthonous breeds”. This programme includes three cattle, nine sheep, three goat, three donkey, four horse, two pig and two poultry breeds. The Croatian “Gene bank” was established four years ago. Programs for preservation of Istrian and Slavonian Strymian Podolian cattle started twenty-five years ago, while Busa cattle is included in a protection programme since 2003. Sheep production in the Republic of Croatia is based on native and protected breeds and, therefore, in a better position in terms of sustainability. Croatian spotted and Croatian white goats are economically active, while Istrian

goat has been included in protection program since 2013. Local pig breeds (Black Slavonian and Turopolje pig) are protected primarily with *in situ* program as well as local horse and donkey breeds. Breeding structure ensures sustainability for the Lipican, Posavina and Croatian Coldblood horse, while the Murinsulaner horse is critically endangered breed. Population of the Littoral Dinaric donkey is the most numerous, while Istrian and North Adriatic donkey belong to the endangered group. Two local breeds of poultry are protected through *in situ* programs.

**Key words:** local breeds; protection programme; genetic resources; Croatia

## GENETIC SCREENING FOR THE MUTATION C.1332 C>T IN THE SLC37A2 GENE ASSOCIATED WITH CANINE CRANIOMANDIBULAR OSTEOPATHY

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Canine craniomandibular osteopathy (CMO) is a non-neoplastic and non-cancerous, proliferative disease in dogs causing extensive developmental changes in the bones of the jaw and skull. It manifests between 4 to 8 months of age with typical clinical signs including lack of appetite, pain, swelling of the jaw, periodical fever, difficulty opening the mouth and dysphagia (Hytönen *et al.*, 2016). CMO corresponds to an infantile cortical hyperostosis (Caffey disease) in humans. It is an autosomal incompletely dominant disease caused by the mutation c.1332 C>T in the SLC37A2 gene in terriers, particularly Scottish, West Highland White and Cairn terriers. SLC37A2 is a glucose-phosphate transporter in osteoclasts and the mutant variant (T allele) of SLC37A2 eliminates a potential binding site for the splicing factor ASF/SF-2. DNA from our canine DNA bank was used for the validation method. DNA was extracted from buccal swabs using Wizard Genomic DNA purification Kit (Promega). PCR reactions were performed in 10 µl volume consisting of 1 µl of extracted DNA (~40 ng), 0.5 mM of each primer, 1x Thermo-Start PCR Master Mix (Thermo Scientific) following PCR amplification protocol 15 min. at 95 °C, 30 s. at 95 °C, 30 s. at 55 °C and 1.30 min. at 72 °C for 35 cycles and final extension 10 min. at 72 °C. Primer sequences were designed according to the reference sequence JH373163.1 from NCBI. The PCR fragments were cycle sequenced using the BigDye Terminator Cycle sequencing Kit version 1.1 and were run on an Avant 3100 Genetic Analyser (Applied Biosystems). Sequences were aligned using the Geneious software (Biomatters). In summary, we validated the method of rapid genetic screening for the mutant allele c.1332 C>T by targeted sequencing of the SLC37A2 gene. This method of genetic identification of the mutant T-allele should help to reduce the prevalence of the causative mutation and decrease the risk of CMO in the three related breeds: West Highland White Terriers, Cairn Terriers and Scottish Terriers.

**Key words:** canine; SLC37A2; CMO; sequencing

### CD81 TETRASPANIN EXPRESSION ON BOVINE GAMETES

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Sperm-egg interaction and fusion represents a key moment of fertilization, and it would not happen in mammals without the interaction of the tetraspanin superfamily members including protein CD81. A detailed immunohistochemical localization of CD81 was monitored on bovine oocytes at different maturation stages, as well as during early embryogenesis. A CD81 protein was also characterized on bovine sperm. On bovine eggs, CD81 was detected on the plasma membrane of the germinal vesicle, metaphase I and metaphase II oocytes. During fertilization, an accumulation of CD81 molecules in the perivitelline space of fertilized oocytes, appeared as vesicles associated to plasma membrane, was observed. In majority of bull ejaculated sperm and caput, corpus and cauda epididymal sperm, CD81 was found on the plasma membrane covering the apical acrosome. Although the process of capacitation did not influence the localization of CD81, it was lost from the surface of the acrosome-reacted spermatozoa in bull. Presented results document certain aspects of CD81 expression in bovine gametes suggesting their possible importance in fertilization process in cattle.

**Key words:** sperm; egg; fertilization; cluster of differentiation 81; tetraspanin

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### ANIMAL BREEDING IN LATVIA

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In 2016, 153,927 dairy cows were registered in the Latvian animal register. The number of dairy farms as well as the number of dairy cows is decreasing in recent years. There are quite many small farms in Latvia – the average number of dairy cows in farm is 8.6 cows. However, the average milk yield is increasing steadily and reaches 6,182 kg per cow per year. The development of the pig breeding sector is negatively affected by the various trade restrictions posed by the outbreak of the African swine fever (from 2014) and by the Russian embargo. Beef cattle breeding is developing quite fast. The number of beef cattle and suckling cows is increasing. In 2016, in animal register 412,084 animals were registered, including 64,332 beef cattle. The poultry sector in Latvia is developing steadily, because this sector has a perspective and is profitable. In Latvia the demand for poultry meat and eggs, especially demand for free-range kept poultry meat and eggs, exceeds the supply. Sheep breeding sector is developing – we can see rapid increase in sheep number as well as live sheep export to other EU countries. Sheep are kept for meat production. Last year a number of sheep in farms increased by 4.2 % compared with 2015. Goats are kept mainly for milk production. In this year, one milk processing

company started to process goat milk and produce semi-hard cheese, so it is possible that this will be a good incentive for goat breeding industry. State aid is currently granted for the conservation of six native breeds – Latvian Brown cow, Latvian Blue cow, Latvian Darkheaded sheep, Latvian Native goat, Latvian White pig and driving type of Latvian horse breed. The number of all native breeds is decreasing, with the exception of the Latvian darkheaded sheep breed. The Latvian white pig is in critical situation. A gene bank is established at the Latvian University of Agriculture to store material from native breeds; also 60 local bee colonies are kept at the Latvian University of Agriculture.

**Key words:** animal breeding; animal genetic resources; native breeds; conservation

### EFFECT OF PERGA CONSUMPTION ON GLYCAEMIA OF ZUCKER DIABETIC FATTY RATS

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The aim of this study was to determine the effect of bee bread (perga) consumption on glycaemia of Zucker diabetic fatty (ZDF) rat model on high-energy diet. Male Zucker diabetic fatty (ZDF) rats (a fatty *fa/fa* mutation (-/-); n = 20) and their lean controls (ZL, non-diabetic, +/+ or +/-, not display expression of *fa* phenotype, n = 10) at the age of 3 months were involved in the experiment (Breeding Facility of the Institute of Experimental Pharmacology and Toxicology, Dobra Voda, Slovak Republic, SK U 25016). Rats were divided into three groups as follows: lean untreated rats (C) fed by KKZ-P/M, obese rats fed by enriched high energy diet (E1, enriched KKZ-P/M) and obese rats fed by enriched high energy diet (E2, enriched KKZ-P/M) and perga given orally by gastric feeding tube at a dose of 250 mg.kg<sup>-1</sup> body weight – for 30 days. A drop of blood was collected from the tail vein for blood glucose determination with digital glucose meter and test strips (FreeStyle, Abbott Diabetes Care Ltd., UK). One-way ANOVA test was performed to calculate basic statistical characteristics and to determine significant differences. High energy diet (E1 and E2 group) caused massive increase (P < 0.05) in the blood glucose level in comparison to the control. Conclusion: Perga consumption had no influence on glucose level in experimental groups when compared to the control. Further experiments with longer consumption of perga are reasonable.

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

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#### CHARACTERIZATION OF SLOVAK DUAL-PURPOSE CATTLE BREED DIVERSITY BASED ON GENOMIC DATA

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The aim of the study was to make a detailed insight into genetic diversity and nucleus population structure of Slovak Pinzgau and Slovak Spotted cattle and to describe the genetic diversity based on the runs of homozygosity (ROH), linkage disequilibrium (LD) and effective population size (Ne) using genome-wide data. Moreover, Bayesian clustering algorithms and multivariate methods were used to detect the population structure, potential admixture level and relationship between breeds. BAPS is computationally superior to STRUCTURE and is suitable for the analysis of large datasets such as SNP arrays. To determine candidate markers for natural selection an alternative approach, based on principal component analysis (PCA), which uses multivariate evaluation to identify the population structure, was used. Relatively low genetic variability was observed in the population, especially in bulls. Most of the bulls clustered together except one bull and his daughters. More than half of the mating combinations were between highly related individuals. Recent genomic inbreeding reached 0.85 % in bulls and 0.94 % in cows, while historical inbreeding covers 5.69 % of genome in bulls and 6.23 % in cows. Based on the false discovery rate equal to 10 % up to 213 loci were identified as outliers. Most of them were found on BTA6 (118) in the two genomic regions within the sequence of genes encoding Toll-like receptors (TLR1, TLR6, TLR10) and immunoglobulin J chain (RBPJ). On BTA21 the strongest signal was detected directly within the sequence of immunoglobulin superfamily (ISLR, ISLR2). The lowest proportion of outlier loci was found on BTA23 (13) mostly within region controlling major histocompatibility complex (MHC). Despite the low variability, it is possible to design appropriate mating plans derived from observing the fine-scale structure of the population. Our results indicated that the signals of selection in genomic regions responsible for adaptive and innate immune response across both cattle breeds resulted mainly from the correlated selection response in a way to maintain their fitness. In the future, genetic selection for animals characterized by better immune response to environmental conditions and diseases can provide health and productivity advantages and can complement traditional health-maintenance methods.

**Key words:** cattle; autochthonous; endangered; high density data

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#### SERUM LIPIDS IN RABBIT BLOOD AFTER APPLICATION OF PATULIN AND STRAWBERRY LEAVES

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Most herbs and plants have been chemically evaluated and their activity has also been proven in clinical studies. However, the elementary mechanisms of function are still unclear. Mycotoxins are small and quite stable molecules which are extremely difficult to remove or eradicate, and which enter the feed chain while keeping their toxic properties. Patulin is a toxic chemical contaminant produced by several species of mold. It is the most common mycotoxin found in apples, apricots, grapes, grape fruit, peaches, pears, olives and cereals. Patulin has been reported to be a genotoxic, reprotoxic, embryotoxic, and immunosuppressive compound. The aim of the present study was to determine the effect of the inclusion of strawberry leaves and single dose of patulin to the feed mixture on levels of blood serum cholesterol and triglycerides in rabbits. Adult rabbits (n = 32) of Californian broiler line were used in the experiment. Rabbits were obtained from an experimental farm of the NPPC, RIAP Nitra, Slovak Republic. The animals were divided into eight groups: one control group - C (0 % strawberry leaves, 0 µg.kg<sup>-1</sup> patulin) and seven experimental groups: EP (n = 4; 10 µg.kg<sup>-1</sup> patulin, 0 % strawberry leaves), E1 (n = 4; 0 µg.kg<sup>-1</sup> patulin, 0,5 % strawberry leaves), E2 (n = 4; 10 µg.kg<sup>-1</sup> patulin, 0,5 % strawberry leaves), E3 (n = 4; 0 µg.kg<sup>-1</sup> patulin, 1,0 % strawberry leaves), E4 (n = 4; 10 µg.kg<sup>-1</sup> patulin, 1,0 % strawberry leaves), E5 (n = 4; 0 µg.kg<sup>-1</sup> patulin, 1,5 % strawberry leaves) and E6 (n = 4; 10 µg.kg<sup>-1</sup> patulin, 1,5 % strawberry leaves). The animals were fed with a granular feed mixture containing strawberry leaves at various doses, and some groups received patulin (Sigma Aldrich, Germany) intramuscularly in injectable form at 10 µg.kg<sup>-1</sup> two times a week for 30 days. The blood serum was separated from the whole blood by centrifugation (30 min at 3000 rpm). Cholesterol and triglycerides were determined by RX Monza automatic clinical analyser (Randox, United Kingdom). Cholesterol level was insignificantly lower in all experimental groups compared to the control group. In the groups E1, E2 and E3 non-significant decrease (P > 0.05) of triglycerides in comparison to the control group was found. Our results are preliminary and further research is needed to verify this trend.

**Key words:** cholesterol; triglycerides; strawberry leaves; rabbits; patulin

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#### MONITORING OF SELECTED TRACE METALS AND THEIR INTERACTIONS TO INDUCE OXIDATIVE STRESS IN COMMON CARP (*CYPRINUS CARPIO*)

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Aquatic animals receive increased number of agricultural and industrial pollutants, which affect health status and free radical formation. The main pollutants are heavy metals, antibiotics and endocrine disruptors. Typical toxic metals (Cd, Pb, Hg, As) can cause hazardous effect to physiological systems of aquatic animals. Trace metals such as Mo, Al, Li, Ba and Ag are not thoroughly investigated. The induction of oxidative stress is monitored as ROS (reactive oxygen species) products. Contrariwise, total antioxidant capacity (TAC) is considered a cumulative action of all antioxidants. The aim of our study was to investigate the associations between oxidative stress markers and trace metal content in freshwater fish. Freshwater adult fishes (*Cyprinus carpio*) were used in our study (n = 36). 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. The content of trace metals (Mo, Al, Ag, Ba, Li) was determined by ICP-OES (Agilent Technologies Australia (M) Pty Ltd.). Oxidative stress markers (ROS, TAC) were assessed using luminol-based luminometry. Statistical analyses were performed using STATGRAPHICS Centurion. Concentration of Li significantly correlated with TAC (r = 0.452; P < 0.01). Mo, Al, Ba and Li were in negative association with ROS (Mo, r = -0.079; P > 0.05; Al, r = -0.107; P > 0.05; Ba, r = -0.082; P > 0.05; Li, r = -0.276; P > 0.05), and Ag was in positive association with ROS (r = 0.301; P > 0.05). The opposite tendency was observed between trace metals and TAC (Mo, r = 0.105; P > 0.05; Al, r = 0.250; P > 0.05; Ba, r = 0.052; P > 0.05; Ag, r = -0.197; P > 0.05). Analysis also showed statistically significant correlation between ROS and TAC (r = -0.742; P < 0.001) and between Ba and Li (r = -0.423; P < 0.05). Weak and nonsignificant correlations were detected between other metals. In conclusion, our results demonstrated effects of trace metals on antioxidant status under natural conditions. Further studies are necessary to test ecotoxicological interactions between other oxidative stress markers and environmental pollutants.

**Key words:** heavy metals; oxidative stress; Common Carp; biomonitoring

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#### EXPRESSION OF HORMONALLY REGULATED GLUCOSE TRANSPORTERS IN PREIMPLANTATION EMBRYOS OF MOUSE CD-1 STRAIN

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It has been well-documented that insulin stimulates glucose uptake in mouse blastocysts. Glucose is an essential energy source for preimplantation embryo especially at later developmental stages, and facilitative glucose transporters (GLUTs) are most important for glucose transport into early embryo cells. GLUTs exhibit a high degree of sequence homology but differ in their substrate specificity, kinetic characteristics, tissue distribution and responsiveness to endocrine signals. Two insulin- or adiponectin-responsive facilitative glucose transporters have been identified in mammalian tissues. Both GLUT4 (SLC2A4) and GLUT8 (SLC2A8) were found in bovine and rabbit blastocysts, but there is a controversy on their expression in mouse blastocysts. The aim of this study was to examine whether both hormone-sensitive glucose transporters, GLUT4 and GLUT8, are expressed in mouse blastocysts of CD-1 strain. We detected PCR products corresponding to both examined glucose transporters in mouse blastocysts of CD-1 strain. Sequencing the PCR products confirmed the GLUT4 and GLUT8 sequence; 100 % sequence identity for GLUT4 and 99.8 % sequence identity for GLUT8 was found between the PCR products and corresponding GenBank reference sequences. Our immunohistochemical study showed the presence of GLUT4 and GLUT8 proteins in CD-1 mouse blastocysts. The fluorescence signal produced by GLUT4 was evenly distributed in TE and ICM cells. The signal for GLUT8 was slightly stronger in mural TE cells than in other cells. Our results indicate that two insulin and/or adiponectin-sensitive facilitative glucose transporters can be expressed in mouse preimplantation embryos.

**Key words:** preimplantation embryo; GLUT-4; GLUT-8

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#### IMPLEMENTATION OF THE REGULATION 511/2014 IN ANIMAL BREEDING AND RESEARCH SECTOR

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The EU ABS Regulation (No 511/2014 on compliance measures for users from the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization in the Union) entered fully into application on 12 October 2015. The EU ABS Regulation is based on the concept of due diligence. The user, defined as a natural or legal person that utilises genetic resources or traditional knowledge associated with genetic resources, is under the obligation to make sure that genetic resources and the associated traditional knowledge have been accessed in accordance with applicable access and benefit-sharing legislation or regulatory requirements, and that benefits are fairly and equitably shared according to mutually agreed terms (Article 4 of the Regulation). The modalities of application of EU ABS Regulation regarding the register of collections, monitoring user compliance and best practices are defined in the Commission implementing Regulation (EU) 2015/1866.



In 2015, the Commission initiated a process to develop guidance documents for users to support fulfilment of their ABS obligations arising from utilisation of genetic resources and associated traditional knowledge. The set of guidance documents include horizontal guidance on the scope of application and core obligations of the EU ABS Regulation (adopted August 2016), and sectoral guidance for users for sectors such as animal breeding, biocontrol and biostimulants, biotechnology, cosmetics, food and feed, pharmaceutical and plant breeding. These sectoral guidance documents will soon be completed. In March 2017, the preparation of additional guidance for public research and for collection holders has been initiated. Guidance on animal breeding as well as guidance for public research and collection holders will provide much needed advice for users of genetic resources and traditional knowledge from the livestock research and breeding communities. The guidance provides examples of activities, which fall within the scope of the EU ABS Regulation or are considered to be outside the scope of this Regulation.

**Key words:** Access and Benefit-Sharing; EU ABS Regulation; animal breeding and research

#### TRANSFORMING GROWTH FACTOR B1 AND VIABILITY OF OVARIAN CANCER CELLS OVCAR-3 AFTER DEOXYNIVALENOL AND ZEARALENONE TREATMENT *IN VITRO*

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The mycotoxins zearalenone (ZEA) and deoxynivalenol (DON) are formed by fungi of the *Fusarium* genus. These mycotoxins are secondary metabolites found as contaminants in almost all agricultural commodities worldwide. The exposure risk to human is either directly through plant foods or indirectly through foods of animal origin. The gross toxic effects of ZEA and DON cause nutritional losses and represent a significant hazard for growth performance, endocrine regulation of the reproductive functions and fertility in females. Nowadays, ovarian cancer represents one of the most lethal of all gynaecological tumours in women. The failure to improve the prognosis for women with ovarian cancer is directly attributable to the lack of an effective screening test for early-stage disease. The objective of this *in vitro* study was to determine the metabolic activity and secretion of transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) in ovarian cancer cell line (OVCAR-3) after application of ZEA and DON mycotoxins. Ovarian cancer cells were incubated without (control group) or with ZEA/DON toxins at different concentrations (0.5, 1, 2.5 and 5  $\mu\text{g}\cdot\text{mL}^{-1}$ ) for 24 hours. The metabolic activity was determined by alamar Blue™ cell viability assay and the release of TGF- $\beta$ 1 was assayed by ELISA method. Our results showed that the cell viability significantly decreased ( $P \leq 0.05$ ) after DON addition at higher concentrations (2.5; 5  $\mu\text{g}\cdot\text{mL}^{-1}$ ) compared with control untreated group. In contrast, ZEA at any used concentration had no effect

on the number of viable cells. Furthermore, TGF- $\beta$ 1 production did not significantly ( $P \geq 0.05$ ) differ either after DON or ZEA addition. Currently, when all possible ways to decrease cancer risk are being sought, our results showed that deoxynivalenol has ability to decrease the number of ovarian cancer cells *in vitro*. In summary, this *in vitro* study suggests that mycotoxins may affect the viability of ovarian cancer cells, and therefore, could provide important insights for searching of new types of anti-tumor drugs and a better understanding of regulatory mechanisms in ovarian cancer cells.

**Key words:** mycotoxins; ovarian cancer cells; viability; TGF- $\beta$ 1

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#### IMMUNOCYTOCHEMICAL DETECTION OF NUCLEOLAR PROTEINS IN INTERSPECIES NUCLEOLUS EXCHANGED EMBRYOS

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It is well known that nucleoli of fully-grown mammal oocytes, so-called nucleolus precursor bodies (NPBs), are essential for embryonic development and their absence leads to the developmental failure mainly at the time of embryonic genome activation (EGA). Therefore, the embryos originated from previously enucleolated oocytes can cleave only one or two times and then their development ceases. Goal of our study was to analyze the presence of nucleolar proteins, C23 and UBF, in interspecies nucleolus transferred embryos (NuTE). In our study the interspecies (mouse/pig) NuTE were produced and were analyzed using immunofluorescence (C23 and UBF staining) from 2-cell stage to blastocyst stage. We have compared the presence of nucleolar proteins (UBF and C23) in three experimental groups of embryos: 1. micromanipulated by transfer of nucleoli from pig oocytes to pig oocytes (P+P), 2. micromanipulated by transfer of nucleoli from pig oocytes to mouse oocytes (P+M) and 3. control group (intact) embryos. The number of nucleoli in P+P and P+M embryos, labeled with C23, was lower than those of control group. UBF was localized in small foci around the nucleoli of blastocysts in control group and P+P embryos, but in P+M embryos was evenly distributed in the nucleoplasm. In conclusion, our results indicate that the mouse nucleolus can partially substitute porcine nucleolus in enucleolated porcine oocytes, but the localization of some nucleolar proteins in NuTE comparing with control group indicates the aberrations occurring already during *in vitro* oocyte maturation and/or initial embryonic cleavage. We believe that our new micromanipulation approach, together with results presented in this study, will form a solid basis for further studies aimed to elucidate the role

of nucleoli in the process of regulation of mammalian oocyte maturation and early embryonic development.

**Key words:** nucleolus; mouse; pig; embryo; immunocytochemistry

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#### MC4R RABBIT POLYMORPHISM

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The melanocortin 4 receptor (MC4R) is a protein produced from the MC4R gene, which has been found in humans to be involved in feeding intake and body weight, and also has a vital role in the control of energy balance and the genetic basis of obesity. Melanocortin 4 receptor (MC4R) gene is suggested to be a promising candidate gene for weight finishing in rabbits. In the present study we focused on detection of MC4R gene polymorphism in 30 rabbits of New Zealand white inbred line. Rabbits used in this study were obtained from National Agricultural and Food Centre, Research Institute for Animal Production Nitra. Genotyping was carried out by PCR-RFLP using primers that insert a restriction site for *SpeI* (*BcuI*). The pattern of restriction analysis, confirming the presence of two fragments of 100 bp and 27 bp, was not observed. The analysis showed that only one undigested DNA fragment of 127 bp was detected, indicating that all 30 rabbits are monomorphic. Additional research exploring the variability of production traits with potential application in rabbit breeding programs is needed.

**Key words:** rabbit; polymorphism; *MC4R*; PCR-RFLP

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#### SCREENING OF THE CZECH RED PIED CATTLE POPULATION FOR DIVERSITY IN INNATE IMMUNITY GENES

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A program aimed at the screening and evaluation of the diversity

in innate immunity genes in the Czech Red Pied cattle has been carried out since April 2016. Since the repertoire of allelic variants of disease resistance genes is supposed to reflect local infection factors and selection pressure, the results are expected to provide informative material for comparison to other world dairy breeds. The survey of innate immunity gene diversity is supposed to be helpful to the oriented breeding and can be used to counteract gene pool erosion. Comparison to the subpopulation of the breed that is conserved in the genetic resource programme since 2010 and reflects the genetic structure around 2000, will facilitate the detection of the current selection trends. Currently, the survey is based on a set of 150 bulls from the production population and 35 animals of the conserved herd. The screening is carried out with targeted re-sequencing that exploits the Pacific Biosciences platform providing reads of full amplicons up to 2000 bp, thus facilitating phasing of structural variants. The monitored set of genes includes *TLRs* coding for both anti-bacterial and anti-viral Toll-like receptors, genes for the members of their signalling pathway, as well as additional chosen innate-immunity genes with previously reported effects on animal health. The detected structural variants are subsequently validated in individual animals with developed series of genotyping assays based on the primer extension method. In order to rationalise the number of reactions, the multiplexes preferentially include diagnostic SNPs (tag SNPs) that are based on the reconstructed haplotype structure of the population. The currently used genotyping panel consists of 93 reactions. The effect of the found gene variants will be further evaluated in the daughter population of 1500 cows, for which the extensive health data are collected in a two-year span. The diagnoses used approximately follow the recommendations of ICAR (2013). The cow population is genotyped only for the relevant subset of genetic polymorphisms.

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

#### ULTRASTRUCTURE OF VITRIFICATION-INDUCED DAMAGES IN BOVINE OOCYTES

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Cryopreservation of tissue from cow's ovaries can help to conserve reproductive potential of animals. The protocol used must ensure the good quality of preserved oocytes after thawing. Ultrastructure of bovine germinal vesicle (GV)-stage oocytes, frozen in the ovarian tissue fragments by two vitrification techniques, was evaluated. For solid surface vitrification (SSV), ovarian fragments were exposed to 4 % ethylene glycol (EG) in DPBS + 10 % FBS for 15 min. and then rinsed in a vitrification solution composed of 35 % 6 M ethylene glycol and 0.4 M trehalose in DPBS + 10 % FBS. After equilibration (5 min. in ice bath), the fragments will be placed in a minimum volume of vitrification solution onto the surface of a metal plate pre-cooled by partial immersion into liquid nitrogen (LN). For

liquid vitrification (LV) ovarian fragments were equilibrated in a vitrification medium containing 40 % ethylene glycol (v/v), 30 % Ficoll 70 (w/v), 1M sucrose and 4 mg.ml<sup>-1</sup> bovine serum albumin at room temperature for 5 min. Then, the tissues in 1.8 ml cryovials, were placed into LN. Oocytes were isolated from thawed ovarian fragments by a puncture of follicles. Oocytes were then fixed in Karnovsky fixative solution, individually embedded into 2 % agar and post-fixed in 1 % osmium tetroxide and embedded into the Poly/Bed 812<sup>®</sup> Embedding Media (Polysciences, Inc.). Ultrathin sections (70 nm) were examined on a transmission electron microscope operating at 80 kV. Oocytes from control group contained mainly hood-shaped mitochondria, vacuoles, lipid droplets, endoplasmic reticulum and germinal vesicle. In both experimental groups (SSV, LV) the oocytes showed disintegrated ooplasm and high degree of vacuolization and vesiculation. Germinal vesicles showed nucleus precursor bodies and the nuclear envelope was disintegrated. Microvilli of cytoplasmic membrane were disrupted. Following vitrification *zona pellucida* acquired layer-like structure and cells of *corona radiata* showed extensive damages. In conclusion, ultrastructure of bovine GV-stage oocytes following vitrification of tissue fragments by both techniques revealed severe damages in the membrane structures (cytoplasmic membrane, nuclear envelope) and ooplasm disintegration. A protocol for cryopreservation of ovarian tissues requires further optimization to maintain good oocyte quality after thawing.

**Key words:** cow; GV-stage oocytes; vitrification; ultrastructure

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#### ASSOCIATION OF SOME METABOLIC PARAMETERS OF EWES WITH YIELD AND QUALITY OF EMBRYOS

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The aim of the study was the assessment of the effect of ovulatory response and metabolic profile (presence of cholesterol, urea and total protein before and after superovulation) of ewes on the yield and quality of embryos. Positive correlation was found between the level of cholesterol and superovulatory response ( $r = 0.54$ ), total number of embryos ( $r = 0.01$ ) and transferable embryos ( $r = 0.39$ ). Levels of urea were in negative correlation with the superovulatory response ( $r = -0.42$ ), with the total number of flushed embryos ( $r = -0.49$ ) or transferable embryos ( $r = -0.58$ ). The influence of total proteins in blood serum of donor ewes on efficiency of embryo production was not proven in our research.

**Key words:** blood; embryos; ewe; metabolic change; yield embryos

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#### REALIZATION OF GENETIC RESOURCES CONSERVATION PROGRAMS IN POLAND

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Poland is one of the precursors of animal species protection, as already in the first half of the twentieth century began the program of restoring bison and breeding of Polish konik, the descendant of wild Tarpan. In 1996, Poland signed the Convention on Biological Diversity and started the creation of programs to protect animal genetic resources. Initially (1999) there were 29 breeds (horses – 2, cattle – 1, sheep – 9, pigs – 3, fur animals – 4 and poultry – 10). In the field of protection, important roles are played by scientific centres, especially the National Institute for Animal Production, which was entrusted in 2004 by the Minister of Agriculture and Rural Development with the coordination of livestock genetic resource conservation programs. Currently, after 18 years of conservation, the number of protected populations has increased to 83 and includes the following species: cattle, horses, pigs, sheep, goats, hen, ducks, geese, fur animals (8 species) and honey bee. In 2016 the number of flocks exceeded 3.3 thousands and the number of breeding females - 102 thousands (including bee families). The objectives of the Program are: a) preserving the population and increasing its number; b) maintaining the greatest possible genetic variability and c) reconstitution/stabilization of characteristics specific to a given population.

**Key words:** conservation programs; domestic animals; coordination

#### SELECTION PROCESS IN THE BREEDING OF NITRA AND ZOBOR RABBITS

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Nitra and Zobor rabbits belong to the group of national breeds. In contrast to other national breeds, both populations were bred at the Research Institute of Animal Production Nitra as “secondary product” of the breeding process focused on creation of more efficient broiler rabbit populations. These breeds are represented at regional as well as national exhibitions. Rabbit breed “Nitra” (in Slovak – *Nitriansky králik*) was established and certified in 1977. Jaroslav Zelník was the main breeder and organizer of the selection process. The initial parent populations were French light silver rabbit and Californian breed. Main emphasis in the selection was put on traits associated with meat utility characteristics like growth intensity, carcass quality and food conversion. In the course of breeding, specific inbred lines were established and after utility tests were realized; the line FRF was selected for further exploitation in the breeding process. Blue acromelanistic animals with middle live weight were stabilized and after this the population was increased, the new zootechnical individual was called Nitra rabbit. The number of bred animals was increased after dissemination of Nitra rabbits to breeders from practice. In the late 80s,

approximately 600 breeding animals were bred in the area of former Czechoslovakia. Raising interest in broiler rabbit production stimulated more intensive use of Nitra rabbits. Their extraordinary utility characteristics and disease resistance were dominant reasons for using this animal in broiler production process. Nowadays, Nitra rabbits are bred by some dozens small animal breeders in Slovakia. Rabbits are regularly exhibited on regional and national level. “Zobor” rabbit (in Slovak - *Zoborský králik*) is a product of hybrid experiments among different New Zealand White (NZW) breed types and acromelanistic breeds (Himalayan, Californian, Nitra rabbit). In some NZW animals (growing in cold environment nest), shadow Dutch spotting phenotypes were manifested. After genetic analysis, it was determined to be a manifestation of Dutch recessive allele present in genotypes. It is a consequence of albinotic recessive allele expression. The current constellation of two active alleles from different genes (acromelanism and Dutch spotting) causes manifestation of interallelic interaction called recessive epistasis. This interaction induces changes in segregation classes during Mendelian segregation. Phenotypically, the exterior of the Zobor rabbit is characterized by NZW type and incomplete acromelanistic spotting i.e. melanin is present in ear hairs, pigment collar in the tarsals region of hind limb and shaded tail hair. Incomplete acromelanistic phenotype is under activity of many genes determining high level of spot variability. For this reason, the typical Zobor exterior is a challenge for breeders.

**Key words:** Nitra and Zobor rabbit; national breeds; selection process

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#### ACTIVITIES OF THE MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT OF THE SLOVAK REPUBLIC IN THE FIELD OF ANIMAL GENETIC RESOURCES

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The market economy and the ever-increasing request for animal proteins have brought increased demands on livestock productivity. In breeding-advanced countries, their breeding was more intense, with a higher level of specialization in a particular performance, as it was in the territory of today's Slovak Republic, thanks to research in the field of biotechnology, the different development of the farmer's property arrangement, the large livestock populations and the social and political conditions. By the end of the last century, the original breeds of farm animals reared in our country were almost entirely displaced by the breeds with higher yields, which were imported mainly from Western Europe, both in the form of live animals but mainly in the form of germ products. These imports have been intensified with the entry of the Slovak Republic into the European Union. Increasing pressure on the livestock commodity economy has forced farmers in Slovakia to refocus themselves to efficient livestock breeds, the weakness of which is a high degree of breed improvement by the high criteria on breeding conditions, nutrition and health prevention. The state has become aware in time of the importance of the genome of the original livestock

breed that has partly formed the domestic environment, as well as the need to preserve biodiversity in this area also for future generations. The role of the Ministry of Agriculture and Rural Development of the Slovak Republic in the area of animal genetic resources is to provide a legal framework for their conservation, protection and exploitation both from the point of view of the cultural heritage and from the point of view of the nutrition needs of the population. Therefore, the ministry applies the measures of the Common Agricultural Policy of the European Union to compensate the income of native breed farmers who are not financially competitive in terms of their sustainability.

**Key words:** biodiversity; support; competitiveness

#### THE RELATIONSHIP BETWEEN HEAVY METAL LEVELS AND SEASONAL PERIOD IN FRESHWATER FISH (*CYPRINUS CARPIO*)

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Heavy metal environmental contamination is a serious problem nowadays, which directly threatens human health. With increasing industrialization and population growth, the problems associated with heavy metal pollution in rivers and water areas are more and more frequent. Heavy metal pollution in rivers constitutes a threat to the consumers of fishery sources. Therefore, measuring the metal bioaccumulation in aquatic organisms is very important. The aim of the study was to determine concentrations of chosen heavy metals in the blood of European carp in different seasonal periods. Blood samples used in our study were collected from European carp (*Cyprinus carpio*) during two seasons - the spring season (n = 23) and the summer season (n = 19). Blood serum obtained from coagulated blood was used for analyses. The concentrations of chosen heavy metals – Mo (molybdenum), Al (aluminium), Ba (barium) and Li (lithium), were determined from blood serum by inductively-coupled plasma optical emission spectrometry (ICP-OES). Basic statistical analysis as well as analysis of variance (one-way ANOVA) following Tukey's HSD procedure and Pearson's correlations were performed using STATGRAPHICS Centurion. Concentrations of monitored heavy metals showed non-significant differences between spring and summer season. The concentrations of analysed metals showed following variations between seasons (expressed as spring season/summer season): Mo – 8.09/5.96 µg L<sup>-1</sup>, Al – 631.30/591.95 µg L<sup>-1</sup>, Ba – 197.43/143.03 µg L<sup>-1</sup>, Li – 4.79/5.76 µg L<sup>-1</sup>. The correlation analysis of the results in the group of spring season showed statistically significant associations only between Li and Mo (r = 0.6758; P < 0.001). In the case of summer season group, non-significant correlations between all analysed metals were detected. Aquatic organisms, especially freshwater, are a suitable subject of environmental load studies because they are in permanent contact with contaminated environment. Therefore, it is important to check regularly not only the environment but also the animals in which the substances such as heavy metals accumulate.

**Key words:** carp; blood; metals; trace elements

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#### THE USE OF STARTING FLUORESCENCE CALCULATION FOR TRANSCRIPT REAL-TIME PCR RELATIVE QUANTIFICATION

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The most widely used techniques for relative quantification of specific transcripts by real-time PCR are delta Ct method and the technique using relative standard curve. Several alternatives to these methods were developed, and we verified the usability of the technique using calculation of a PCR template starting fluorescence. It is assumed that the intensity of reporter fluorescence dye is directly proportional to the amount of PCR amplification product in fluorescent real-time PCR. Based on this assumption, theoretical starting fluorescence can be calculated from amplification efficiency and from fractional number of amplification cycles needed for reaching a selected benchmark. The starting fluorescence is then proportional to the amount of PCR template and represents the quantity of target nucleic acid sequence expressed in arbitrary fluorescence units. We amplified two adiponectin receptor transcripts (AdipoR1 and AdipoR2), isolated PCR products from agarose gel and quantified them spectrophotometrically. We put known amounts of adiponectin receptor PCR products (calculated amount of DNA copies) into PCR reactions and re-amplified these templates in a real-time PCR system. Threshold fluorescence was set in the exponential phase of amplifications and corresponding cycle number was calculated. Amplification efficiencies were measured using LinRegPCR algorithm and theoretical starting fluorescences of DNA templates were calculated. We found that calculated ratios of AdipoR1/AdipoR2 starting fluorescences were very close to ratios of DNA copies of AdipoR1 and AdipoR2 PCR templates put into reactions. These results indicate that starting fluorescence can be successfully used for calculation of the ratio of two different transcript amounts in the sample.

**Key words:** real-time PCR; starting fluorescence; relative quantification

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#### SUSTAINABLE USE OF PODOLIAN COW GENETIC RESOURCES

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Biodiversity preservation is a process of genetic conservation through renewal of degraded ecosystems and natural habitats, and the preservation and recovery of breeds. Sustainable use represents utilization of biodiversity components that does not cause distortion of biodiversity, but represents a rational use of natural resources and maintenance of the potential biodiversity. Rational use of animal genetic resources for the agricultural industry and food production is a good strategy for sustainability of their production. The concept of sustainable use has a great economic importance, but also has an impact on rural development and increase of employment opportunities. Sustainable agricultural production systems are those, which allow the conversion of resources into human food and agricultural products without reducing the availability of these resources in the future or causing degradation of the environment. Sustainable livestock production systems are those in which the animal genetic resources are in accordance with other available resources. According to FAO, achieving this integration is the key to maintaining and developing production through a full range of global resources and ecological production. The aim of this work was to show that the use of Podolian cattle for meat production can be cost-effective, beside the benefits in preserving genetic diversity within an autochthonous breed, also in the social aspects, such as an employment and maintenance of the national traditions. In our work, we will present the sustainable farm production on the example of AnGR farm “Tikvicki” keeping Podolian cows. The Podolian cows are in the herd of 30 reproductively capable females and 20 animals in other categories. Because of the small number of animals there is an intake of new males every two years to prevent inbreeding. In a further analysis, it is necessary to determine the exact revenues and expenditures of the monitored production observed in certain time periods.

**Key words:** AnGR; sustainability; biodiversity; preservation; Podolian cow

#### SLOVAK RABBIT BREEDS

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Nowadays, there are about 70 purebred breeds of rabbits in Slovakia in many color types. Of this number, 12 breeds are generated by Slovakian breeders. Total numbers of breeding individuals kept in Slovakia are different according to the breed. The largest population and the highest number of breeders belong to the following breeds: Slovak grey-blue Rex (200 females and 40 males), the Rabbit of Nitra (150 females and 30 males), the Zemplin rabbit (70 females and 20 males), the Blue of Holic rabbit (60 females and 30 males), the Liptov bold-spotted rabbit (50 females and 20 males), the Slovak pastel Rex (70 females and 30 males) and the Zobor rabbit (50 females and 20 males). Rest of the breeds are represented by very small populations consisting of approximately 20 breeding females and of 10 to 15 males. It is very helpful that a portion of these breeds has farmers interested in its breeding also outside Slovakia and, for example, the population of Blue of Holic breeding rabbits in Germany is estimated to be 30 breeding females and 100 males. Slovak national breeds of rabbits belong to the cultural biological heritage, and our aim is

to preserve and raise it for future generations of breeders. However, without targeted and long-term state assistance this effort is very difficult.

**Key words:** rabbit; Slovakian breed; animal genetic resources

#### MANAGEMENT OF FARM ANIMAL GENETIC RESOURCES IN LITHUANIA

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The main purpose of the first National Programme for the conservation of farm animal genetic resources (AnGR), adopted by the Ministry of Agriculture of the Republic of Lithuania in 1996, was to collect, investigate and conserve the Lithuanian AnGR. At the beginning, only 6 indigenous breeds were included into this programme. The main purpose of the programme was collection, investigation and *in situ* conservation of AnGR. In 1993-1997, herds of indigenous breeds were formed of the animals remaining after expeditions, thus recreating mini-populations of 6 breeds at the Institute of Animal Science (LIAS). Conservation work was supported by the Ministry of Agriculture. Later, unfavourable conditions were created for other 6 breeds developed in 20<sup>th</sup> century, and these breeds were included into conservation programme. In 2008, national programme of AnGR was renewed and the Coordinating Centre for the National Farm Animal Genetic Resources was established at the LIAS. The main activities of the Centre: coordination of animal genetic resources, identification, monitoring, preparation of conservation programs, evaluation of animals, conservation of farm animals *in situ* (selection nucleus) and *ex situ* (conservation of animal genetic material in the form of semen, embryos, samples of blood or hair and DNA). The principles of conservation of AnGR and evaluation of breed status are based on the experience of animal breeding in small conserved herds and on the criteria and global strategy of FAO for the management of AnGR. The first decision in setting up conservation schemes is to carry forward the existing variability in the breeds. The status of Lithuanian AnGR was evaluated by their monitoring. In the period of 1993-2017, a population size of some breeds increased from 30 to 700 animals per breed and has a status of endangered-maintained population. However, some of them are still on the verge of extinction; effective population size is still below 50. Main reasons behind the critical status are small subsidies for breeders, diseases (African swine fever) and limited possibilities for the use of their products. In case a substantial number of animals was lost in different lines and families, the *ex situ* conservation was initiated.

**Key words:** animal genetic resources; conservation; monitoring; indigenous breed

#### PARAMETERS OF SPERM MOTILITY IN ENDANGERED ORAVKA CHICKEN

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The evaluation of semen motility parameters of poultry gives an excellent indicator of their success in cryopreservation and fertilization process. We aimed to evaluate the spermatozoa motility parameters of individual roosters of Oravka breed. Sexually mature roosters (n=6) without infections were used in the experiments. The semen samples were collected twice a week by dorso-abdominal massage during two months (from April to May 2017). The samples were diluted in a saline at the ratio of 1:100 (v:v). The concentration and motility characteristics of diluted rooster spermatozoa were analyzed using the CASA system. Average concentration (10<sup>9</sup> per ml), percentage of total motile spermatozoa (total motility > 5 μm/s), percentage of progressive motile spermatozoa (progressive movement > 20 μm/s), VCL (velocity curved line, μm/s), VSL (velocity straight line, μm/s), STR (straightness - VSL:VAP, velocity average path), LIN (linearity- VSL:VCL), BCF (beat cross frequency, Hz) were recorded. Variability in spermatozoa concentration (3.04 ± 0.36 to 4.12 ± 0.43), total motility (50.71 ± 11.31 to 77.05 ± 5.26) and progressive movement (29.54 ± 8.63 to 58.96 ± 6.82) was observed among individuals. However, no significant differences in concentration and motility parameters among analysed individuals were found. In conclusion, this study provides the first characterization of Oravka spermatozoa motility. Based on our results rooster semen can be used for cryopreservation and fertilization.

**Key words:** semen; motility; Computer assisted sperm analysis; Oravka breed

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#### EFFECT OF DIFFERENT STORAGE TEMPERATURES ON RED DEER SPERMATOZOA MOTILITY

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Intensive red deer breeding in captivity expanded in the second half of the 20<sup>th</sup> century with the purpose of meat production. Red deer breeding recorded a large development including implementation of various biotechnological methods, such as artificial insemination, MOET and *in vitro* production of embryos. Application of these methods leads to intensification of the animal production and enables the use of high quality sires. The aim of this research was to evaluate the motility of breeding bucks (*Cervus elaphus*; n = 10) spermatozoa in different storage

and assessment temperatures, specifically 10 °C and 37 °C. Semen was collected from bucks from different age groups using artificial vagina. Collected semen was extended with conservation medium at ratio 1:1. Conservation medium consisted of demineralized water, fructose, glycerol, citric acid, buffer solution, phospholipids and antibiotics. During the transportation to the laboratory, the semen was stored for about an hour at defined temperatures. The semen was evaluated by CASA system, which determined the following parameters: motility (MOT), progressive motility (PRO), velocity curved line (VCL), beat cross frequency (BCF) and amplitude of lateral head displacement (ALH). Based on the obtained results, the samples, which were incubated at 10 °C showed better motility, progressive motility, VCL, DCL, BCF and ALH than those incubated at 37 °C. In conclusion, the storage of collected semen at 10 °C maintains the spermatozoa properties, which are closely associated with successful fertilization.

**Key words:** Red deer (*Cervus elaphus*); deer farming; spermatozoa motility; CASA

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#### ANIMAL GENETIC RESOURCES IN THE SLOVAK REPUBLIC

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Slovak Republic supports the conservation of animal genetic resources through a variety of means and tools. Important part of this effort is monitoring of livestock populations. This activity is undertaken by NPPC – Research Institute for Animal Production Nitra. The monitoring of population status is based on the cooperation with Breeding Services of the Slovak Republic, s.e. and authorized breeders' organizations. The monitoring covers not only the endangered breeds, but also other main breeds of livestock. The data are stored and published in the EFABIS database. At the moment, 15 breeds of cattle, 6 breeds of pigs, 23 breeds of sheep, 8 breeds of goats, 11 breeds of horses, 46 breeds of rabbit, 17 breeds of chicken, 3 breeds of geese and 2 breeds of ducks are regularly monitored. Besides the monitoring of population data, we also try to identify the motivation factors, which influence the farmers' decision for the local breed breeding. Another important battery of activities is propagation of local breeds and raising public awareness. NPPC – Research Institute for Animal Production Nitra keeps live animals from several local breeds under *ex situ* conditions. These are used for providing breeding material but also for presenting the local breeds during national and regional exhibitions. The presentation of animals goes hand in hand with presentation of local products from these animals. The close cooperation with media helps to spread the issues of animal genetic resources among the broader public.

**Key words:** animal genetic resources; local breeds; conservation; monitoring

#### SLOW-FREEZING DOES NOT CHANGE CHROMOSOMAL COUNTS IN RABBIT AMNIOTIC FLUID STEM CELLS

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Amniotic fluid stem cells (AFSCs) are frequently used in human regenerative medicine. After collection of amniotic fluid it is important to process and store the cells in the most efficient way for the future use in cell therapy or for the conservation as gene resources. Slow-freezing method is often used for long-term storage of stem cells in many laboratories, however there is a need to control the cytogenetic state of the cells post-thawing. Therefore, the aim of this study was to determinate the changes in chromosomal count in rabbit AFSCs before and after the slow-freezing. Samples of fresh and slowly frozen AFSCs at third passage were subjected to the karyotype analysis. In this study, 30 metaphase plates per sample were observed. A normal rabbit karyotype consists of 22 pairs (2n = 44) of chromosomes. Chromosomal abnormality was defined as following: hyperploidy - > 44, hypoploidy - < 44. Results showed that 60 % of fresh cells were able to maintain the stable karyotype. Abnormal karyotype was present in 40% of cells, of which 10 % were hyperploid and 30 % hypoploid cells. In slowly frozen cells, normal karyotype was monitored in 66.6 % of cells. Chromosomal aberrations were detected in 33.2 % of cells including 6.6 % hyperploids and 26.6 % hypoploids. Stem cell preservation is a necessary step from the view of the therapeutic perspective as well as the perspective of gene resource storage. However, it is strongly recommended to monitor the numeric chromosomal status following the freezing process.

**Key words:** rabbit; amniotic fluid stem cells; karyotype; slow-freezing

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#### ASSOCIATION BETWEEN SELECTED ENVIRONMENTAL POLLUTANTS AND OXIDATIVE STRESS MARKERS IN CARP SEMEN

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Aquatic contamination has become a worldwide concern as the ever-increasing presence of heavy metals, pesticides and endocrine disruptors may have detrimental effects on the natural habitat with subsequent hazard to the food chain. The male reproductive system is a highly sensitive barometer of changes in the internal or external milieu of the organism, and hence may serve as a suitable indicator of environmental pollution.

Heavy metals such as lead, cadmium or mercury are well-known contaminants of the aquatic environment with detrimental effects on the structure and function of male reproductive structures. Less attention is given to minor yet potentially dangerous chemical elements, such as aluminium (Al), lithium (Li) and barium (Ba). As a proper oxidative balance has become a crucial prerequisite for an optimal reproductive performance, the aim of this study was to assess possible relationships between the total antioxidant capacity (TAC), production of reactive oxygen species (ROS) and the content of Al, Li and Ba in carp semen. Semen samples were collected from 15 carp males and lysed using RIPA buffer and sonication. ROS and TAC were assessed using luminol-based luminometry, while the concentrations of Al, Li and Ba were determined using inductively coupled plasma mass spectrometry (ICP-MS). Basic statistical analysis as well as Pearson's correlations were performed using GraphPad Prism, version 6.0. The analysis revealed significant negative associations between ROS and TAC ( $r = -0.652$ ;  $P < 0.001$ ), indicating a direct inverse relationship between these two parameters. Weak and insignificant associations were detected between Al and Li ( $r = 0.258$ ;  $P > 0.05$ ) as well as Li and Ba ( $0.069$ ;  $P > 0.05$ ), however a significant positive relationship was revealed between Al and Ba ( $r = 0.956$ ;  $P < 0.001$ ). All elements were in a positive association with ROS ( $r = 0.310$ ;  $P < 0.05$  in case of Li;  $r = 0.412$ ;  $P < 0.05$  with respect to Ba;  $r = 0.515$ ;  $P < 0.001$  in relation to Al). Inversely, negative correlations were recorded between the concentration of the selected chemical elements and TAC ( $r = -0.280$ ;  $P > 0.05$  in relation to Li;  $r = -0.423$ ;  $P < 0.05$  in case of Ba;  $r = -0.556$ ;  $P < 0.01$  with respect to Al). Our data suggest that the presence of minor chemical elements may represent a threat to the oxidative milieu of male reproductive structures and subsequently decrease male fertility in fish. As such, we may recommend that a regular monitoring system should be established to screen the occurrence of hazardous chemical elements in aquatic environment.

**Key words:** heavy metals; oxidative stress; ROS; TAC; freshwater fish

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#### NEW CD34 ANTIBODY SUB-CLONES FOR THE HEMATOPOIETIC STEM CELL PHENOTYPING IN THE RABBIT BLOOD AND BONE MARROW

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The aim of this preliminary study was to compare the antigen specificity of newly prepared antibody sub-clones against rabbit CD34 peptide for the identification of hematopoietic stem cells (HSCs) among the rabbit mononuclear cells isolated from the peripheral blood and bone marrow. Three young (four months old) and clinically health rabbits of New Zealand White (NZW) line reared in a partially air-conditioned hall of a local rabbit farm at RIAP Nitra were used in the experiment. Mononuclear cells were isolated using Biocoll solution from the rabbit peripheral blood (PBMCs) and bone marrow (BMMCs), aliquoted into prepared tubes and stained with 20 different sub-clones of mouse anti-rabbit CD34 monoclonal antibodies: IgG1 isotype sub-clones (257/77, 257/80, 274/9/76, 274/9/78, 7/1, 7/4, 7/2, 417/55, 417/58, 417/68, 485/71 and 485/72), IgG2b isotype sub-clones (465/1, 465/3 and 465/13) and IgM isotype sub-clones (63/29, 63/45, 63/47, 507/15 and 507/27). Briefly, at least  $1 \times 10^6$  cells were incubated with 100  $\mu$ l of each CD34 sub-clone supernatant that was extracted from the corresponding hybridoma cell line at 4 °C for 15 min. Subsequently, cells were incubated with a secondary antibody - goat anti-mouse Ig-APC (BD Biosciences, USA) at 4 °C for a further 15 min. After washing, cells were stained with 7-AAD (Bioscience, Austria) in order to exclude dead cells from the analysis. All labeled cells were analyzed using a flow cytometer FACSCalibur (BD Biosciences, USA). At least 50,000 events (cells) were analyzed in each sample. Modified flow cytometry method based on International Society for Hematology and Graft Engineering was used to determine CD34<sup>+</sup> (stem and progenitor) cell counts. Observed results were evaluated by a one-way ANOVA (Holm-Sidak method) using SigmaPlot software (Systat Software Inc., Germany) and expressed as the means  $\pm$  SEM. We observed significantly higher expression (%) of CD34 antigen within PBMCs using sub-clones: 465/1 ( $6.2 \pm 0.5$ ), 465/3 ( $5.2 \pm 0.6$ ), 465/13 ( $5.4 \pm 0.7$ ), 63/29 ( $3.2 \pm 0.8$ ), 63/45 ( $5.1 \pm 2.4$ ) and 63/47 ( $11.5 \pm 1.1$ ) compared to other sub-clones. Similarly, the same sub-clones identified significantly higher percentage of CD34<sup>+</sup> cells within BMMCs ( $5.5 \pm 1.6$ ,  $3.6 \pm 0.4$ ,  $3.3 \pm 0.2$ ,  $6.5 \pm 2.3$ ,  $6.8 \pm 2.0$  and  $11.7 \pm 1.7$ , respectively) compared to other sub-clones. In conclusion, some of the tested sub-clones showed increased expression of CD34 antigen within the rabbit PBMCs and BMMCs. However, further analyses are required in order to determine the true CD34 antigen specificity of newly prepared monoclonal antibodies.

**Key words:** rabbit; HSCs; CD34 sub-clones; flow cytometry

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