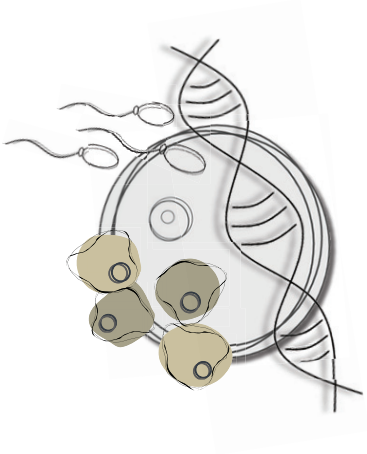

WINTER SCHOOL

ANIMAL BIOTECHNOLOGY 2025

PROGRAMME AND ABSTRACTS

February 6th 2025, Nitra, SUA in Nitra, Slovakia

DOI: https://doi.org/10.15414/2025.9788055228303

Proceeding of the Winter School "ANIMAL BIOTECHNOLOGY 2025"

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SCIENTIFIC PROGRAM of the Winter School

6th February 2025, SUA Nitra, Slovak Republic

„Animal Biotechnology 2025“

Topic: CELL CULTURES:

BASIC RESEARCH AND PRACTICAL APLICATIONS

8.00 – 9.15	**Registration (AgroBioTech RC, Slovak University of Agriculture Nitra)**
9.15 – 9.30	**Opening ceremony**
9.30 – 12.30	**Invited speakers** *(25 min/person)*
Chairmen:	**Peter Chrenek**
9.30 – 10.00	**Jaromir VASICEK** *NPPC RIAP Nitra and FBFS SUA Nitra, SLOVAKIA* **Animal stem cell bank: a novel tool for biodiversity protection**
10.00 – 10.30	**Petr SLAMA** *Mendel University, Brno, CZECH REPUBLIC* **3D cell culture: basic research and practical aplications**
10.30 – 11.00	**Coffee break**
11.00 – 11.30	**Luisa BOGLIOLO et al.** *Department of Veterinary Medicine, Sassari, ITALY* **In vitro assessment of the adverse effect of environmental contaminants on the female gamete**
11.30 – 12.00	**Adriana KOLESAROVA** *AgroBioTech Research Centre , SUA Nitra, SLOVAKIA* **The use of cellular models in the prediction of the role of phytonutrients in the regulation of ovarian functions**
12.00 – 12.30	**Elena GOCZA et al.** *Hungarian University of Agriculture and Life Sciences, Gödöllő, HUNGARY* *Agrobiotechnology and Precision Breeding for Food Security National Laboratory, Gödöllő, HUNGARY* **Potential applications of animal stem cells as *in vitro* model systems in toxicology studies**
12.30 – 13.45	**Photo and Lunch (Mladost dormitory)**

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13.45 – 14.30 Session I.
Cell cultures - Health care & Biodiversity protection *(15 min/person)*

Chairmen: Jaromír Vašíček

13.45 – 14.00 **Petra BOROTOVÁ**
Slovak University of Agriculture in Nitra, SLOVAKIA
Anticancer potential of selected essential oils: evidence gathered from the MDA-MB-231 cell line

14.00 – 14.15 **Tomáš JAMBOR**
Slovak University of Agriculture in Nitra, SLOVAKIA
Survey of neonicotinoids interactions with reproductive cells in vitro

14.15 – 14.30 **Andrea NESVADBOVÁ**
IVF Clinic a.s., Olomouc, CZECH REPUBLIC
Social freezing of reproduction cells

14.30 – 14.45 **Eva ŠEBOVÁ**
Institute of Experimental Medicine, Czech Academy of Sciences, Prague, CZECH REPUBLIC
Mineralization Dynamics in the Osteogenic Differentiation of Mesenchymal Stem Cells

14.45 – 15.15 Coffee break

15.15 – 16.45 Session II.
INTERREG HU-SK *(15 min/person)*

Chairmen: Alexander Makarevich, Ľubomír Ondruška

15.15 – 15.30 **Andrea SVORADOVÁ**
NPPC Research Institute for Animal Production, Nitra, SLOVAKIA
Conservation and Management of Animal Genetic Resources: A Collaborative Approach for Biodiversity Protection „INTERREG HUSK/2302/1.2/018“

15.30 – 15.45 **Kitti BUDA**
The National Centre for Biodiversity and Gene Conversation, HUNGARY
Conservation of the female genetic material in indigenous duck breeds

15.45 – 16.00 **Bence LÁZAR**
The National Centre for Biodiversity and Gene Conversation, HUNGARY
Stem cell-based techniques in avian biotechnology

DOI: https://doi.org/10.15414/2025.9788055228303

16.00 – 16.15 **Barbara VÉGI**
The National Centre for Biodiversity and Gene Conversation, HUNGARY
Spermatological and sperm freezing studies in Hungarian Giant rabbit

16.15 – 16.30 **Jakub VOZAF**
NPPC Research Institute for Animal Production, Nitra, SLOVAKIA
Slovak University of Agriculture in Nitra, SLOVAKIA
ProAKAP4 as an accurate predictor of sperm freezability: A preliminary study.

16.30 – 16.45 **Francesco VIZZARI**
NPPC Research Institute for Animal Production, Nitra, SLOVAKIA
Biodivezity project: a cross-border tool for *Lepus europaeus* population protection

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18.00 – 00.00 **Social evening (Mladosť dormitory)**

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SPONZORS

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ORAL PRESENTATIONS

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Animal stem cell bank: a novel tool for biodiversity protection

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Abstract

The biodiversity of animals may be protected by conservation of genetic material under *in situ* conditions (animals reared in their natural habitats) and under *ex situ in vitro* conditions by cryopreservation of animal reproductive cells e.g., spermatozoa, oocytes and embryos. In recent years, a cryopreservation of embryonic and adult stem cells became an important tool for the conservation of animal genetic resources. A gene bank, established at our institute in 2015, contains reproductive cells and DNA as well as stem cells from Slovak animal breeds. In addition, some of these breeds have already become endangered. Due to the great effort of several researchers as well as due to financial supports of different research grants, until now we have collected mesenchymal stem cells (MSCs) from the bone marrow, amniotic fluid or adipose tissue and endothelial progenitor cells from peripheral blood and the bone marrow of Nitra, Zobor and Zemplin rabbit breeds. Furthermore, we have cryopreserved primordial germ cells and the bone marrow derived MSCs from the Oravka hen. Recently, hematopoietic progenitor cells from Slovak grey-blue rex and Holic blue rabbit breeds have been stored in the gene bank. At the present, we are working on the preservation of rabbit spermatogonial stem cells and ovine MSCs from the bone marrow, muscle and adipose tissue.

Keywords: animal gene bank; cryopreservation; stem cells

Acknowledgments

This work was supported by grants: APVV-23-0141, VEGA 1/0011/23 and INTERREG, HUSK/2302/1.2/018.

3D Cell Culture: Basic Research and Practical Applications

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Abstract

Cell culture is a fundamental tool in biological and biomedical research, providing valuable insights into cellular behaviours and disease mechanisms. Traditionally, two-dimensional (2D) cell cultures have been widely used, but they fail to replicate the complex three-dimensional (3D) structures and cellular interactions present *in vivo*. The emergence of 3D cell culture techniques has significantly advanced our understanding of cell behaviour, tissue development, and disease pathology. This lecture explores the differences between 2D and 3D cell cultures, focusing on the advantages of 3D models in more accurately mimicking the natural tissue environment. We discuss the applications of 3D cell culture in drug discovery, disease modelling, and tissue engineering. Special emphasis is placed on the use of nanofibers in 3D cultures to support cell growth and provide a biomimetic scaffold for tissue formation. Nanofiber-based cultures are particularly promising in recreating the extracellular matrix (ECM) structure and enhancing cellular interactions. Finally, it is introduced the organ-on-a-chip technology, a cutting-edge tool that integrates 3D cell cultures into microfluidic systems to mimic organ functions. This system has shown great potential in studying complex organs, such as the mammary gland in cattle, where it can be used to investigate mastitis, a prevalent inflammatory disease in dairy cows. By simulating the mammary gland environment in a controlled 3D setting, we aim to improve the understanding of mastitis pathogenesis and identify new therapeutic approaches. Through the integration of these advanced 3D culture techniques, we are advancing basic research and offering practical applications in veterinary medicine and biotechnology, particularly for livestock health management.

Keywords: 3D cell culture; nanofibers; organ-on-a-chip; mammary gland; mastitis

In vitro assessment of the adverse effect of environmental contaminants on the female gametes

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Abstract

The detrimental effect of environmental pollutants such as heavy metals and micro-nanoplastics (MNPs) on female fertility and reproductive health in both humans and animals are a concerning issue. Cadmium (Cd), as one of the most toxic heavy metals, is known to adversely affect the oocyte quality even at low concentrations. Using different mammalian models, previous *in vitro* studies highlighted the negative impact of the Cd exposure on the meiotic competence of oocytes during *in vitro* maturation (IVM) and on their ability to successfully undergo *in vitro* fertilization (IVF) and support preimplantation embryo development. In particular, *in vitro* Cd exposure led to defective nuclear and cytoplasmic maturation in animal oocytes via the impairment of cytoskeleton assembly, spindle organization, chromosome alignment, actin polymerization, distribution of mitochondria, cortical granules, gene expression and epigenetic modifications. The cytotoxic effect of Cd on IVM also led to increased levels of reactive oxygen species and lipid peroxidation, which adversely affected the ability of the oocyte to be fertilized. The potential effects of MNPs on the mammalian female gamete are only recently being studied and there are not enough results on this subject. Studies in mouse showed that MNPs enter the ovary. Depending on the size of MNPs, a dose-dependent effect on oocyte nuclear maturation has been reported, and smaller MNPs appear to be taken up by the oocyte. Recent pilot data in our research group proved that *in vitro* exposure of ovine oocytes to MNPs during IVM caused a decrease in the oocyte meiotic progression, induced apoptosis in cumulus cells and a decrease in the ability of oocytes to develop to the blastocyst stage after fertilization. Currently, there is an insufficient information on the joint effects of MNPs and hazardous metals on the female gamete.

Keywords: cadmium; plastic; oocyte

Acknowledgments

This work was funded by the Ministero dell'Università e della Ricerca PRIN PNRR 2022 - P20222H7J7- CUP J53D23018160001 and PRIN 2022 2022WEPMKX- CUP J53D23010490006.

The use of cellular models in the prediction of the role of phytonutrients in the regulation of ovarian functions

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Abstract

The most common diseases affecting the female reproductive system include benign and malignant tumours (fibroids, cervical carcinomas, ovarian tumours), endometriosis, endocrine disorders, polycystic ovary syndrome, and premature ovarian failure, with a negative impact on women's quality of life. Natural substances in plants, spices, and fruits can effectively modulate key elements of the signaling pathways of viability, steroidogenesis, proliferation, and apoptosis in the female reproductive system. In our study, cellular models such as the human ovarian granulosa cell line (HGL5), human ovarian granulosa tumor cell line (COV434), and human ovarian carcinoma cell line (OVCAR-3) were used in the prediction of the role of phytonutrients in the regulation of ovarian functions. Pomegranate, apricot seeds, sea buckthorn, black elder, honeysuckle and their biological active substances such as resveratrol, isoquercitrin, punicalagin, isorhamnetin, glycyrrhizin, amygdalin, and cyanidin-3-glucoside were evaluated for their effects on ovarian functions *in vitro*. The targeted application of phytosubstances verified by the use of human ovarian cell models is a promising source for supporting hormonal regulation, improving reproductive functions, as well as preventing female reproductive alterations.

Keywords: ovary; phytonutrients; prevention; tumour

Acknowledgments

The work was supported by the projects of the Ministry of Education, Research, Development and Youth of the Slovak Republic project APVV-18-0312, APVV-21-0206, VEGA 1/0620/24 and KEGA 035SPU-4/2023.

Potential applications of animal stem cells as *in vitro* model systems in toxicology studies

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Abstract

Global climate change has created the right conditions for moulds to proliferate and produce mycotoxins. Mycotoxins can accumulate over time and can cause serious health problems at higher concentrations. Zearalenone (ZEA) and Trichothecene-2 (T-2 toxin) are the most frequent mycotoxins produced by various *Fusarium* species. In our work, we investigated the effects of these mycotoxins and toxin combinations on the development of rabbit embryos, rabbit embryonic fibroblast cells, domestic chicken embryos and primordial germ cells (PGCs). Different toxin solutions were injected into the bloodstream of 3-day-old domestic hen embryos or added to the culture medium. We investigated the effects of these toxins on embryo development and on cell proliferation. The accumulation of T-2 toxin in the walls of the tubular parts of the gonads could be detected by staining the tissue sections with anti-T-2 toxin specific antibody. We examined gene expression changes for superoxide dismutase (SOD) and catalase (CAT) by qPCR technique. For SOD, both ZEA and combined treatments resulted in significant increases in the gene expression compared to the control group. Mycotoxins impact animal health and pose risks to human health through the consumption of contaminated products. Additionally, insights gained from researching mycotoxin-induced damage *in vitro* can be applied to other species, including humans.

Keywords: mycotoxin; primordial germ cells; embryonic fibroblast

Acknowledgments

The research was supported by the grants ÚNKP-21-4-I-MATE/19, RRF-2.3.1-21-2022-00007 and HUSK/2302/1.2/018.

Anticancer potential of selected essential oils: evidence from the MDA-MB-231 cell line

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Abstract

This study aimed at evaluating the potential anticancer effects of three selected essential oils (EOs: Wintergreen, Lemongrass and *Litsea cubeba*) on the MDA-MB-231 cell line, representing highly aggressive and invasive triple-negative breast cancer. The cells were cultured in the absence or presence of 0.3, 3 and 30 µl/mL of each EO for 48 h. Membrane integrity was quantified with 5-carboxyfluorescin diacetate while the mitochondrial potential was evaluated with the JC-1 dye. Reactive oxygen species (ROS) levels were quantified using luminometry. Our data indicate that particularly higher concentrations of all EOs had cytotoxic effects, although the specific mechanism of action differed. Wintergreen EO administration led to a significant ROS increase particularly at 3 µl/mL ($P < 0.01$) and 30 µl/mL ($P < 0.001$), however no differences were observed in case of the membrane integrity. The presence of Lemongrass EO led to a significant decrease of the membrane integrity, when compared to the control ($P < 0.01$ regarding 0.3 and 3 µl/mL; $P < 0.001$ in case of 30 µl/mL), but the mitochondrial potential was not affected. The most notable effects were recorded in case of the *Litsea cubeba* EO, administration of which resulted in significant changes to all parameters when compared to the control. These promising preliminary outcomes shall be validated by further analyses.

Keywords: essential oils, MDA-MB-213 cell line, breast cancer

Acknowledgments

This study was supported from the grants no. APVV-20-0058 and APVV-21-0095.

Microgreens: a novel perspective in human health protection

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Abstract

Over the past ten years, interest in fresh, functional, and nutraceutical foods has been on the rise, driven by society's growing interest in healthy eating. From this point of view, microgreens, as a new class of speciality crops, have begun to gain significant popularity. This is primarily due to a rich profile of biologically active compounds including in different microgreen crops, herbs, and wild plant species. The present *in vitro* study was aimed to evaluate the potential impact of *Trigonella foenum-graecum L.* microgreens on human adrenocortical carcinoma cell line (H295R). Cells were cultured in the presence of different doses of experimental microgreens (from 10 µg/mL to 1000 µg/mL) during 48 h exposure. The mitochondrial activity was evaluated by MTT assay, cell membrane integrity was determined by CFDA-AM assay, and steroid hormone release was quantified by ELISA method. Gained results showed, that mitochondrial activity of H295R cells was not significantly affected until 250 µg/mL, followed by radical ($p<0.0001$) decreased at 300 µg/mL and 1000 µg/mL of experimental extract. Similar tendency was observed in case of cell membrane integrity, when the highest applied doses significantly ($p<0.0001$) decreased this parameter. Testosterone and progesterone release was significantly ($p<0.01$, $p<0.001$) stimulated at 150 µg/mL, 200 µg/mL and 250 µg/mL, followed by significant ($p<0.01$, $p<0.0001$) inhibition at 300 µg/mL and 1000 µg/mL of Trigonella microgreens. In conclusion, further experimental research is necessary to determine the biological significance of microgreens and their protective effects on human health.

Keywords: microgreens; H295R cell line; mitochondrial activity; membrane integrity; steroid hormone release

Acknowledgments

The research was financially supported by Slovak Research and Development Agency APVV-SK-PL-23-0037, by the grant Scientific Agency of the Slovak Republic VEGA No. 1/0555/25, as well as by the Polish National Agency For Academic Exchange, namely BPN/BSK/2023/1/00043/U/00001: "Health benefits of microgreens: crops of modern agriculture".

Social freezing of reproduction cells

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Abstract

Social freezing is a term that refers to the process of freezing reproductive cells. The method is mainly used by women, who do not yet feel ready for parenthood or do not have a suitable partner but want to preserve the possibility of having their own child in the future. The aim of our study was to obtain an information on the feasibility and success of oocyte cryopreservation to prolong women's reproductive health. Based on professional literature searches in the Web of Science and PubMed/Medline databases, 93 oocyte retrievals from women with oocytes for vitrification and long-term storage were evaluated. Survival rate (SR %), fertilization rate (FR %) and utilization rate (UR %) in two groups of women with frozen oocytes (patients under 35 years and over 35 years) were determined to estimate the success of the oocyte vitrification method. The results of the comparison of the success rate of oocyte vitrification method showed that the lowest values of SR % and FR % were in the group of older patients with low quality oocytes at the time of oocyte retrieval. Significantly lower UR % value was seen in the group of older patients with higher number of vitrified oocytes retrieved compared to other groups. In conclusion, the woman age at the time of oocyte freezing and the number of stored oocytes are the key factors determining the success rates to secure the possibility of having their own child in the future.

Keywords: social freezing, oocytes

Acknowledgement

This research was funded by the internal grant of the Palacky University Olomouc (IGA_PrF_2024_029).

Mineralization Dynamics in the Osteogenic Differentiation of Mesenchymal Stem Cells

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Abstract

Mesenchymal stem cells (MSCs) are multipotent progenitors capable of differentiating into three primary lineages: osteogenic, adipogenic, and chondrogenic. This study investigates the osteogenic differentiation of bone marrow-derived MSCs under controlled conditions. We cultured MSCs in a basic medium (DMEM supplemented with 10% FBS and 1% penicillin/streptomycin) or an osteogenic medium moreover containing 10 nM dexamethasone, 10 mM β-glycerol phosphate, and 50 µg/mL ascorbic acid-2-phosphate for 35 days. Key experimental time points included days 1, 7, 14, 21, 28, and 35, during which we assessed metabolic activity using the MTS assay, alkaline phosphatase (ALP) activity, dsDNA quantification via Picogreen, and scanning electron microscopy (SEM) combined with energy-dispersive X-ray spectroscopy (EDS) for calcium and phosphorus measurements. Our results demonstrated that ALP activity significantly increased in the osteogenic group on day 7, indicating early-stage maturation towards osteoblasts, while dsDNA levels were higher in the osteogenic group on day 1 but showed no significant differences by days 28 and 35. Metabolic activity peaked on day 7 in the osteogenic medium before declining towards day 35. SEM analysis revealed the growth of crystal-like structures with increased calcium and phosphorus deposition in the osteogenic medium starting from day 14. Hydroxyapatite staining confirmed mineralization only in the osteogenic medium on the final day of culture. These findings suggest that while both media support cell growth, osteogenic conditions significantly enhance differentiation and mineralization potential of MSCs. The implications of this research extend to potential applications in tissue engineering and regenerative medicine, where understanding MSC differentiation can inform strategies for in vitro manipulation and in vivo transplantation to repair or regenerate damaged tissues.

Keywords: mesenchymal stem cell; osteogenic differentiation; calcium phosphates

Acknowledgments

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Conservation and management of animal genetic resources: A collaborative approach for biodiversity protection „INTERREG HUSK/2302/1.2/018“

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Abstract

The preservation of animal genetic resources (AnGR) is critical for ensuring food security, biodiversity and quality of life, both now and in the future. These efforts are guided by international and national frameworks including the European Action Plan for Biodiversity in Agriculture, the UN FAO Global Strategy for the Management of Animal Genetic Resources, Council Regulation (EC) No 870/2004 and Act No 194/1998 Coll. on animal breeding. Conservation serves as a safeguard against climate change, disease outbreaks, genetic issues, social changes and other unexpected events. This project focuses on the identification, selection and genotyping of native Slovak AnGR, emphasizing the collection and analysis of sperm, stem cells and other genetic materials. Advanced methodologies, such as fluorescent markers, PCR analyses and bacteriological profiling, will ensure the evaluation and preservation of high-quality samples. These will be cryopreserved in a gene bank, alongside the establishment of *ex situ in vivo* collections for key species including rabbits, poultry, sheep and brown hares, at the National Agricultural and Food Centre. Cross-border collaboration with Hungary will enhance the project impact by sharing animals, genetic materials and methodologies ensuring robust genetic conservation strategies. Reciprocal storage of Slovak and Hungarian gene samples will provide a dual insurance mechanism against unforeseen events supporting biodiversity preservation in both countries. This integrated approach combines cutting-edge techniques and international cooperation to strengthen the conservation of AnGR for future generations.

Keywords: biodiversity; conservation; protection

Acknowledgments

This research was supported by the APVV-23-0089 and INTERREG, HUSK/2302/1.2/018.

Conservation of the female genetic material in indigenous duck breeds

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Abstract

Currently, *in vitro* poultry gene conservation is not only focusing on semen cryopreservation. In avian species, the female is heterogametic, and the inability of freezing eggs and embryos necessitating alternative methods for preserving the female genome. One promising approach is the cryopreservation and transplantation of gonadal tissue in day-old chicks. This method has been developed in domestic fowl, enabling the recovery of donor-derived progeny through grafting frozen/thawed ovarian tissue. In waterfowl species, only a few reports exist of donor-derived offspring produced via transplantation of native gonadal tissue from Muscovy duck donors into Pekin duck recipients. However, since the recipient's own gonad often cannot be completely removed, both donor- and recipient-derived progeny may be produced. To ensure all offspring are donor-derived, the use of sterile recipients can be a solution. Previous studies have identified the Mulard duck as a suitable recipient due to its anatomically normal but non-functional gonads. The aim of this study was the transplantation of frozen/thawed Pekin duck ovarian tissue to Mulard duck. In case of vitrification and surgical procedure, the same technique was used as in goose. In our pilot study, by grafting native ovarian tissue in Mulard recipients, donor ovaries adhered, started to ovulate, but not into the oviduct. In the next step, frozen/thawed ovarian tissue was grafted in the same combination. Investigating the difficulty of the ovulation process, progesterone (P4) and estradiol (E2) levels were checked by ELISA. It was found that 66 % of the donor ovaries were adhered and 22% of them started to ovulate, but still not into the oviduct. In case of adhesion of donor gonadal tissue, P4 and E2 levels were close to the levels of control Pekin group. Assuming that the GnRH treatment may affect the oviduct function, we repeated the previous group setup with addition of a GnRH analogue (buserelin)-treated group. In this group, 31 % of the grafted ovaries adhered, but only 50 % of them had elevated hormonal levels and developed oviducts. In the untreated group the adhesion rate was 15 %, of which 50 % had elevated hormonal levels. In conclusion, the cryopreserved ovarian tissue transplantation can be feasible in Mulard duck recipients; the ovaries can adhere, but challenges in influencing the function and regulation of the oviduct require further study. The investigation was supported by Interreg HUSK/2302/1.2/018 and KDP-2021.

Keywords: gene conservation; domestic duck; sterile recipient

Stem cell-based techniques in avian biotechnology

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Abstract

The biotechnology research conducted on birds can be broadly classified into two areas of investigation. Firstly, there is a focus on developing techniques that can be applied to the livestock sector, for example, through the creation of an *in vitro* gene bank for the conservation of economically important and indigenous species. Secondly, there is an increasing emphasis on research that can contribute to the protection of endangered bird species, particularly in the context of the ongoing loss of global biodiversity. The domestic fowl (*Gallus gallus domesticus*) is the most widely used avian model, and most of the methods employed in biotechnological research on birds have been developed for this species. However, as some of the methods are species-specific, it follows that for other species these techniques are lagging significantly, thus hindering their wider applicability (endangered wild bird species). In contrast to mammals, where cloning by somatic cell nuclear transfer and embryo cryopreservation are techniques that can be used, these are not feasible for birds due to the specificities of their reproductive biology. As a result, alternative methods are required. These are based on primordial germ cells (PGC), which are precursors of mature gametes. These cells can be efficiently isolated during embryonic development, and in the case of domestic chickens, they can be maintained *in vitro* for long periods of time. They are the most important cells for gene conservation and they can be used to generate germline chimeras and then donor progeny. Additionally, the most advanced method of editing the avian genome is through stem cell modification. To realise the current promise of avian biotechnology, new methodological developments will be needed in the coming years to enable, among other things: *in vitro* cultivation of stem cells from diverse species; the use of alternative cell sources that can generate germline chimeras; *in vivo* genome editing without cell culture; and the use of universal recipients.

Keywords: avian biotechnology; primordial germ cell; germline chimaera; gene conservation

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Spermatological and sperm freezing studies in Hungarian Giant Rabbit

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Abstract

The Hungarian Giant Rabbit, Hungary's sole native rabbit breed, is facing a critical population decline, highlighting the importance of conservation efforts. A nucleus population has been established at the National Centre for Biodiversity and Gene Conservation, and creating an *in vitro* sperm bank is deemed essential for safeguarding the breed's genetic resources. As a first step, we evaluated the spermatological parameters of Hungarian Giant bucks, including sperm volume, concentration, motility, and viability. The results aligned with values reported for other rabbit breeds, confirming the breed's suitability for cryopreservation studies. Subsequently, we assessed sperm cryopreservation protocols focusing on extender effectiveness, dilution ratios, freezing protocols, and the use of dimethyl-sulfoxide (DMSO) as a cryoprotectant. Three extenders were tested: a modified Lake-extender, BotuCrio, and InraFreeze. Post-thaw total motility was higher with the modified Lake-extender (6.4%) and BotuCrio (7.9%) compared to InraFreeze (3.3%), with no significant differences in live, intact sperm ratios. Consequently, the modified Lake-extender was chosen for further studies. Testing dilution ratios (1:1, 1:2, 1:3) and one- versus two-step static freezing protocols revealed no significant differences in motility or sperm viability, favoring the simpler one-step freezing method. When DMSO was applied at the onset of equilibration, post-thaw sperm exhibited reduced viability (9.5% vs. 14.5%) and motility (2.7% vs. 7.4%) compared to delayed application. Overall, while fresh sperm quality was suitable for cryopreservation, frozen/thawed outcomes were suboptimal. Our ongoing efforts aim to optimize protocols to establish a robust *in vitro* gene bank for the Hungarian Giant Rabbit.

Keywords biodiversity; sperm freezing; gene bank

Acknowledgments

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ProAKAP4 as an accurate predictor of sperm freezability: A preliminary study

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Abstract

Cryopreservation of spermatozoa is a critical tool for biodiversity conservation, enabling the long-term preservation of genetic material from endangered or important species. However, individual variability in sperm freezability poses a significant challenge to the success of cryopreservation programs and standard predictors, such as sperm motility after collection, often prove to be inaccurate. This study represents the first step in a broader series of experiments designed to validate the potential of proAKAP4, a precursor of the sperm-specific protein AKAP4 involved in sperm motility and structural integrity, as a biomarker for predicting sperm cryotolerance. Our initial efforts were to validate the methodology and determine the correlation between proAKAP4 levels and sperm quality indicators such as viability, total and progressive motility after collection. Sperm samples from rabbit males (n=8; 3 replicates) were analysed using computer-assisted sperm analysis (motility and concentration), flow cytometry (viability, apoptotic changes, acrosomal status, oxidative stress) and enzyme-linked immunosorbent assay (proAKAP4 levels) before cryopreservation. Initial findings did not show a correlation between these values. However, verification of the correlation after thawing will be more important, as these results may suggest that proAKAP4 has potential as a predictive marker for sperm freezing, offering a means to pre-select high-quality samples for cryopreservation. This approach could ultimately enhance the efficiency of assisted reproductive technologies in biodiversity conservation initiatives.

Keywords: biodiversity; cryopreservation; sperm; proAKAP4

Acknowledgments

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Biodiverzity project: a cross-border tool for *Lepus europaeus* population protection

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Abstract

The European brown hare (*Lepus europaeus*) is an important small game species, which has suffered such a dramatic decline in western and central Europe since the 1960s. Considering its highly adaptable to a great number of different habitat types, reasons of declining are not clear; probably the most important threat for this species is the intensification of agriculture. The National Agriculture and Food Centre (NPPC, Luzianky, Slovakia) is currently running several monitoring activities of free-ranging brown hare population for the collection of information on health status and the pathogens currently circulating. These data play a crucial role in the monitoring process of potential risk for wildlife, domestic animals and humans. Recently NPPC-Luzianky has been engaged together with the National Centre for Biodiversity and Gene Conservation (Tapioszele, Hungary) for the development of a Interreg HU-SK project (Biodiverzity, co-funded by the European Union), currently ongoing. The funded Biodiverzity project has the main goal to build up a mutual cross-border cooperation and support in protection biodiversity with the purpose of protecting selected animal genetic resources under the conditions of ex situ, in vivo and in vitro. Specifically on brown hare, this involves mapping, selection and genotyping of wild and reared (only by small breeders) animals from cross-border territories. The result of the project will be cryopreserved samples in gene banks on the Slovak and Hungarian sides, mutual exchange of reserve samples, a collection of animals of national breeds.

Keywords: brown hare; biodiversity; wild management program; Interreg EU

Acknowledgments

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ADDITIONAL ABSTRACTS

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Tetraspanins CD9, CD63 and CD81 as potential quality markers of bovine embryos produced from fresh/vitrified oocytes

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Abstract

Vitrification is a common method for cryopreserving cow oocytes, but its success is influenced by various factors that affect embryo development post-thawing. Cryopreservation can cause intracellular changes, particularly damaging the plasma membrane, which may alter protein and lipid organization. Tetraspanins, such as CD9, CD63 and CD81, are membrane proteins that play a crucial role in the cell membrane organization and protein interactions. They are also key markers for extracellular vesicles (EVs), which facilitate embryo-maternal communication during early development. We used immunofluorescence to compare the localization of the tetraspanins CD9, CD63 and CD81 in early (4-8 cell) bovine embryos produced from fresh *in vitro* matured oocytes and matured oocytes, cryopreserved by an ultra-rapid cooling technique in a minimum volume. We applied anti-CD9 (monoclonal IVA-50, polyclonal MRP-1), anti-CD81 and anti-CD63 antibodies to embryos fixed with paraformaldehyde and permeabilized with 0.5% Triton X-100, followed by an incubation in Alexa 488 conjugated secondary antibodies, covered by Vectashield mounting medium containing DAPI and evaluated under Leica TCM SP8 STED 3X confocal microscope. ImageJ software was used to quantify the total signal intensity and the signal intensity in the *zona pellucida* of individual embryos. CD9 signal was detected as clusters on the surface of individual embryo cells, in the perivitelline space (PVS) and the *zona pellucida* (ZP) in all embryos. The signal belonging to CD63 was evident on cell membranes and on structures traversing the entire *zona pellucida*, while CD81 exhibited small clusters on membranes with less abundance in the perivitelline space and weak signals in the ZP. Quantifying the total signal, we found a significant increase in the fluorescence intensity in embryos produced from vitrified oocytes only for the CD81 tetraspanin. We observed a clear signal in the ZP of embryos labeled with monoclonal antibodies against the CD9 and CD63 tetraspanins, with decreased CD9 intensity in the ZP of embryos derived from vitrified oocytes. These findings may suggest that tetraspanins may be involved in EVs produced by embryos, highlighting their potential role in embryo-maternal communication during preimplantation development. These data provide the basis for further detailed studies to understand the impact of cryopreservation on embryo quality and developmental potential.

Key words: cow; vitrification; extracellular vesicles

Acknowledgments

This work was supported by the grants VEGA-2/0074/24, APVV-19-0111 and APVV-23-0203.

The effect of diet supplementation by Camelina Cake on mouse preimplantation development *in vivo* – preliminary results

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Abstract

The aim of our study was to evaluate the effect of diet supplementation by Camelina cake (CC), a rich source of n-3 polyunsaturated fatty acids, on mouse preimplantation development *in vivo*. Mouse females (outbred CD-1 strain) were divided into two groups: control (fed only standard feed mixture) and CC II (fed a diet supplemented with a Camelina cake containing alpha-linolenic acid at a dose of 200 mg/kg body weight. In our previous experiments, the administration of alpha-linolenic acid at 200 mg/kg of body weight had beneficial effect. CC was administered to female mice for 2 weeks (during approx. 3 oestrus cycles). Embryos were isolated from dams on day 4 of pregnancy. Stereomicroscopic evaluation of the oviduct and uterine content showed that the administration of CC in the diet did not significantly affect an average number of isolated embryos per mother (7.53±0.64 in control vs. 8.94±0.82 in CC II; $P > 0.05$). However, the numbers of collected embryos tended to increase in females fed CC. Analysis of developmental capacity showed no significant difference in the stage-distribution of isolated embryos. Administration of CC to the diet did not significantly affect the general ability of embryos to reach the morula/blastocyst stage. More than 90 % of embryos reached the blastocyst stage in both groups (Control - 90.10%; CC II - 95.92%). However, in the CC II group, the mean number of embryos that reached blastocyst stage tended to increase. These preliminary results indicate that administration of CC at chosen dose could have slightly beneficial effect on average yield of isolated embryos per mother and their developmental rate.

Keywords: preimplantation embryo; mice; Camelina cake

Acknowledgments

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Prediction of ketosis using radial basis function neural network in dairy cattle farming

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Abstract

The purpose of the study was to apply an Artificial Neural Networks with Radial Basis Function to develop an application model for diagnosing a type I and II subclinical ketosis in dairy cattle. While building the neural network model, applied methodology was compatible to the procedures used in Data Mining processes. The data set was created based on the composition of milk samples of 1520 Polish Holstein-Friesian cows. The milk samples were collected during test-day milkings and were available at the Polish Federation of Cattle Breeders and Milk Producers. The milk composition parameters were used as the input variables for RBF network models. The value of the output variable was determined based on the content of β-hydroxybutyric acid in blood of cows. In the next stage of the work, the qualities of the pre-selected models were compared and the best ones were chosen. The sensitivity and specificity as well as the size of the AUC (Area Under the Curve) under the ROC (Receiver Operating Characteristic) were taken as the main criteria for network model evaluation. The model characterized by sensitivity of 0.86, specificity of 0.71 and AUC of 0.89 was selected for the type I ketosis. The optimal for the type II ketosis showed the sensitivity and specificity of 0.81 and 0.75, respectively, and the size of AUC - above 0.85. Chosen models were recorded using the predictive modelling markup language (PMML) for data mining models to be shared and used for the different applications.

Keywords: artificial neural networks; β-hydroxybutanoic acid; dairy farming

Acknowledgments

We would like to thank the Polish Federation of Cattle Breeders and Milk Producers for milk samples from dairy producers in Poland and for their contribution to this study.

Classification of nuclei in asynchronous division embryos

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Abstract

During early embryonic development, the blastomeres continually shrink as they divide. The nuclei of the blastomeres, whose size is determined by the amount of blastomere cytoplasm, also gradually shrink. However, the size of the nuclei also varies slightly depending on the current phase of the cell cycle. In bovine embryos, division is predominantly synchronous up to the 4-cell stage, but asynchronous from the 4-cell stage onwards. This leads to a situation, when nuclei of different cell stages (4-cell, 8-cell and 16-cell) are present at the same time in the normal 8-cell embryo. For more detailed analysis of embryo development, these parameters must be taken into account and the nuclei analysed must be divided into appropriate subgroups according to their actual cell stage. In an ideal situation, it would be possible to assign these nuclei to the appropriate cell stage on the basis of their size. In reality, however, we get a variety of different sizes, because the size of the nuclei is influenced by a number of factors. The aim of this work was to develop a procedure for the classification of nuclei in embryos with asynchronous division. For the basic separation of borderline stages, we used mathematical-statistical tests of the samples and identified the occurrence of significant gaps by calculating the mean difference and standard deviation.

$$average = \frac{1}{n-1}\sum_{i=1}^{n-1} diff_i \qquad stand\ dev = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n-1}(diff_i - average)^2}$$

Thresholds for nucleus size in bovine embryos were, therefore, set at ≥452,737 μm^2 for 4-cell nuclei, 257,326 μm^2 - 357,467 μm^2 for 8-cell nuclei and ≤ 195,148 μm^2 for 16-cell nuclei. The region 357,468 - 452,736 μm^2 and the region 195,149 - 257,325 μm^2 form a group of nuclei that could be assigned to one of the groups that delimit them and can then be assisted by nuclear size analysis combined with immunofluorescent labelling of the level of transcriptional activity in the blastomere using EU (5-ethynyl uridine) labelling. The signal intensity should be within the appropriate range for the relevant stage of embryonic development.

Keywords: nucleus; asynchronous division; embryo

Acknowledgments

This work was supported by the Slovak Research and Development Agency under the Contract no. DS-FR-22-0003, VEGA 1/0270/24 and Campus France for the cotutelle PhD funding (FR).

Oxidative profile of bovine spermatozoa collected from Simmental, Limousine and Holstein breed: Comparative study

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Abstract

The aim of our research was to compare the differences or similarities in the oxidative profile of bovine spermatozoa of three selected breeds. As a biological material, fresh semen samples collected from Simmental (n=4), Limousine (n=4) and Holstein (n=4) breeds were used. The motility of spermatozoa was assessed by the computer-assisted sperm analysis (CASA) system. Within the oxidative profile, we focused on the global production of reactive oxygen species (ROS), superoxide and hydroxyl radical production, which were evaluated with combined a spectro-fluoro-luminometer Glomax Multi^{+}. Statistical analysis was performed by a one-way ANOVA and Tukey's multiple comparison test. Based on the results, the motility ranged from 85 to 90 % without significant differences between breeds. However, a significantly highest ($P < 0.05$) generation of ROS was observed in the Simmental breed compared to the Holstein breed. The superoxide production was higher in the Simmental ($P < 0.0001$) and Limousine breeds ($P < 0.0001$) compared to the Holstein breed. On the contrary, a significantly higher ($P < 0.05$) concentration of hydroxyl radical was in the Limousine breed compared to the Simmental and Holstein breeds. In conclusion, these novel findings can be helpful in the development of individual cryopreservation strategies in the future.

Keywords: oxidative profile; spermatozoa; cattle breeds

Acknowledgments

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Development and evaluation of a bull sperm sorting method based on cell size and qPCR validation

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Abstract

A novel approach for sorting bull sperm based on cell size was developed to enhance efficiency in sex-based sperm selection. This technique aimed to segregate spermatozoa by size differences hypothesized to correlate with X and Y chromosome-bearing cells. Quantitative PCR (qPCR) methods targeting Y and X chromosomes, as well as satellite DNA regions, were used to assess sorting efficiency. Two distinct qPCR methodologies from prior studies were evaluated: hydrolysis probe-based qPCR and intercalating dye-based qPCR. Initially, the latter method was selected for its cost-effectiveness. Using three primer pairs to detect Y and X chromosomes alongside satellite DNA regions, specificity issues both general and DNA amount-dependent were identified. Despite optimization attempts, these issues persisted leading to the method's abandonment. Subsequently, a hydrolysis probe-based qPCR method was implemented. This approach demonstrated high specificity for Y-chromosome detection, and current optimization efforts for X-chromosome detection led to promising results. Preliminary findings suggest that sperm sorting effectively separates X- and Y-chromosome-bearing cells. The sorting technique continues to undergo refinement to maximize its efficacy and reliability.

Key words: sperm sex-sorting; X, Y chromosome-bearing cells; hydrolysis probe-based qPCR

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The effect of *Hippophae rhamnoides* L. on cataract in Zucker diabetic fatty rats

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Abstract

Present study aimed on the interaction between diabetes mellitus II. type (DM2) and therapy with sea buckthorn (SB) (*Hippophae rhamnoides* L.) fresh fruits. The investigations proved its potential regarding rich content of bioactive compounds with antioxidant effects in therapy of various diseases. Zucker diabetic fatty (ZDF) rats in the age of 15 weeks were used in the study as a suitable animal model for mirroring human DM2. Animals were treated once daily by gastric gavage with metformin (150 mg/kg, n=10), 500 mg/kg of SB (SB1 group, n=10) and 1000 mg/kg (SB2 group, n=10) directly to the stomach. Untreated control diabetic group (n=10) received only water. The experiment lasted 16 weeks. Opacification of lens was examines using mydriatic drops containing 2% homatropine and 0.5% tropicamide into conjuctival sac and was evaluated in five categories (A-clear lens, B-small vacuoles, C-cortex haziness, D-hazy cortex and dense nuclear opacity, E-mature cataract). In enucleated lens the sorbitol concentration was determined. Cataract developed more frequently in the control untreated group of rats. SB and metformin treatment showed anti-cataract activity. We found decrease in sorbitol concentration in all treated group in comparison to the untreated control. Metformin alone had the most significant effect on decrease in sorbitol accumulation in lens. Regular monitoring of blood glucose from the tail vein showed significant decrease in all treated groups when compared to the untreated control. Probably, the main anti-cataractogenic mechanism consists in hypoglycaemic effect of the SB polyphenols that results in inhibition of the polyol (sorbitol) pathway. Regular consumption of SB primarily in young age could delay onset of metabolic disease, improve diabetic symptoms and are suitable co-alternative in therapy of DM2.

Key words: cataract; *diabetes mellitus*; rats; Sea buckthorn

Acknowledgments

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Effect of nanoplastics and their combination with cadmium on *in vitro* maturation of ovine oocytes

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Abstract

Nanoplastics (NPs) are particles created during plastic degradation and can easily enter our body, rising concerns about their potential impact on human and animal health. Cadmium (Cd) is toxic heavy metal used as a plastic additive. In this study, we used ovine oocytes as a model to investigate the effect of NPs and their combined effect with Cd on oocyte nuclear maturation and apoptosis of cumulus cells (CCs). Oocytes were recovered by slicing method from ovine ovaries and matured *in vitro* (IVM; TCM, 10% oestrus sheep serum, 8 mg/mL pyruvate, 100 mM cysteamine and 1 I.U FSH/LH) for 24 h at 38.5°C and 5% CO_2. During maturation 50 nm NPs (Polystyrene; Bangs Laboratories, Inc.) were added to IVM medium at different concentrations (5, 25 and 50 µg/mL) without or with 100nM $CdCl_2$ (5+Cd, 25+Cd and 50+Cd groups), while Cd was also tested separately (Cd group). Oocytes without any treatment served as a control group (CTR). Our preliminary results showed that maturation rate significantly ($p<0.05$) decreased in 50NPs (67.16%) and 50+Cd (60.87%) groups compared to CTR (83.10%) group, while Cd alone had no effect (72.58%). Apoptosis in CCs after IVM was significantly higher ($p<0.05$) in all NPs (10.44-12.24%) and NPs+Cd groups (11.26-12.20%) compared to CTR (6.68%) with no effect of Cd alone (7.80%). Despite increased CCs apoptosis after exposure to lower NPs concentrations with or without Cd, no effect on nuclear maturation was observed in these groups. In conclusion, our preliminary results showed that the highest concentration of NPs decreased oocyte nuclear maturation and increased apoptosis in CCs. This was also observed in the combination of NPs with Cd, while Cd alone had no effect on oocyte nuclear maturation and apoptosis in CCs. Further studies are needed to accurately evaluate the effect of NPs on oocytes and their combined effect with cadmium.

Keywords: nanoplastics; cadmium; ovine oocytes; *in vitro* maturation

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Fenugreek (*Trigonella foenum-graecum* L.) microgreens potential in bovine sperm preservation: a molecular approach

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Abstract

Microgreens have emerged as rich sources of bioactive compounds including antioxidants, polyphenols and flavonoids that could promote cellular protection during stress conditions. Fenugreek (*Trigonella foenum-graecum* L.) microgreen extract represents a potentially cost-effective supplement due to its unique phytochemical profile with a primary representation of protodioscin and diosgenin. This research studied the effect of fenugreek extract on sperm motility and key apoptotic molecular markers: pro-apoptotic BAX, caspase-3 and anti-apoptotic Bcl-2. Bovine semen samples (n=4) were cultured *in vitro* for 24 hours at room temperature either without (control) or with fenugreek extract at doses ranging from 1 to 300 μg/mL. Sperm motility was evaluated using computer-assisted sperm analysis (CASA), while protein levels were quantified using Western-blot analysis. The results showed that the extract did not alter the sperm motility across the concentration range. BAX and caspase-3 levels significantly decreased ($P < 0.05$) in the group supplemented with 150 μg/mL of extract compared to the control. On the other hand, Bcl-2 levels significantly decreased at 10 μg/mL. A considerably higher BAX/Bcl-2 ratio was recorded at 10 μg/mL, while a substantially lower ratio was observed at higher concentrations (150 and 300 μg/mL). This study suggests that higher doses of fenugreek extract (above 150 μg/mL) may regulate the preservation of apoptosis-associated proteins.

Keywords: *Trigonella foenum-graecum* L.; sperm; preservation

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Production of „archive sires" via rational cryopreservation

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Abstract

The centuries-old history of the Hungarian Grey Cattle is intertwined with the people living in the Carpathian Basin: for a long time it played an indispensable role in the steppe animal husbandry and was an excellent, leg-driven meat animal for the slaughterhouses of Western countries. Today, the Hungarian Grey Cattle is our national treasure, protected as a Hungarikum. The breed almost became extinct in the 1950s when it was ordered that all Hungarian Grey bulls be sent to the slaughterhouse and the cows were ordered to be crossed with the Soviet Kostroma breed. The breed was survived by few bulls and about 250 cows, which form the basis of the current population. Today, the living population of the bull lines has become uneven. In order to strengthen the rare bull lines, an initiation was accomplished by the Hortobágy Nature- and Gene Preservation Nonprofit Ltd. and Association of Hungarian Grey Cattle Breeders. During the breeding program, which lasted for three years (2020-2022), cryopreserved semen of the breeding bulls was used that born in the 1950-'60s. After artificial insemination with sperm frozen for 50 years, an acceptable fertilization percentage was achieved, because sixty-two heifers became pregnant from 150 synchronized animals and 59 calves were born (2021-2023). From the male calves 11 young bull candidates were selected and submitted to performance test (on-farm and central) which will be completed in early 2025 for the first crop. Next to the revitalization of rare sire lines, the genetic pool of the herd becomes renewed by this investigation. By comparing the individuals born from the applied procedure with those born from contemporary sires, any phenotypic changes occurring in the breed can be detected. Last but not least, the use of the reproductive material from time to time also serves to control the reliability of the sperm freezing technique.

Keywords: cryopreservation; artificial insemination; rare breed conservancy

Animal Genetic Resources in Slovakia

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Abstract

Availability of animal genetic resources has an impact on the present and future life quality and important effect on the food safety. Ratification of the Convention on the Biodiversity in 1992 obliges Slovak Republic to protect biodiversity, to guarantee sustainable use of its components and fair and equal access to benefit sharing from genetic resources. The situation with animal genetic resources in Slovak Republic is a little bit better than 10 years ago due to the fact that DNA samples, semen doses, oocyte, embryos and stem cells are stored in the Gene bank. We cryopreserve the sperm from cattle breeds (Pinzgau, Slovak Spotted), horse breed (Slovak sport pony), sheep breeds (native Wallachian sheep, Improved Wallachian, Tsigai and Slovak Dairy sheep), rabbit breeds (Nitra rabbit, Zobor rabbit, Blue of Holic rabbit, Zemplin rabbit, Liptov Bold-Spotted rabbit), chicken (Oravka) and goose breeds (Slovak White and Suchovska goose). In addition, we cryopreserved oocyte and embryos of cattle as well as stem cells from rabbit breeds. From the last update statuses of animal breeds done at the end of 2011 it is obvious that two breeds of pig in the Slovak Republic are subjected to extinction.

Keywords: animal genetic resources; Slovak animal; cryopreservation; gene bank

Acknowledgments

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Localization of CD63 and CD9 tetraspanins, extracellular vesicle markers, in bull reproductive tissues and spermatozoa

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Abstract

Extracellular vesicles are considered essential mediators of intercellular communication in both physiological and pathological contexts. Their important role in various cellular processes, including those critical for mammalian reproduction, is increasingly being investigated. In this study, we analysed the distribution of two major markers of extracellular vesicles, the tetraspanins CD63 and CD9, which are considered molecular partners, using immunofluorescence and immunoperoxidase assays in bovine reproductive tissues and spermatozoa. In the testis, the CD63 was observed in clusters near germ cells, mainly surrounding spermatogonia and spermatozoa within the lumen of the seminiferous tubules. In all three regions of the epididymis (caput, corpus and cauda), positive staining for CD63 was detected in the secretory epithelium as a distinct line of clusters directed towards the lumen. Immunoperoxidase staining confirmed this distribution of the CD63 in both testicular and epididymal tissues and provided a clearer visualisation of the CD63 in the sperm tails within the lumen, particularly in the corpus and cauda. Immunofluorescence analysis revealed that the CD63 appeared predominantly in clusters within the tails of spermatozoa isolated from all regions of the bull epididymis and ejaculate, with some localisation noted in the post-equatorial region of the sperm head. The CD9 showed a variable distribution pattern in epididymal spermatozoa: it was localised to the equatorial region in the caput, extended over the acrosome in the corpus and covered the entire acrosome in the cauda with significant differences between the caput and cauda. We propose that in addition to the CD9 molecules, carried by bull sperm from the testis, additional CD9 molecules are transported within the epididymis. It is suggested that CD63 and CD9 may be components of specific populations of extracellular vesicles associated with either the head or tail of epididymal and ejaculated bull spermatozoa. These findings contribute to a better understanding of the complex mechanisms underlying sperm maturation and fertility acquisition in bulls and highlight the potential involvement of tetraspanins and their partners in these processes.

Keywords: bull; tetraspanin; extracellular vesicles; sperm

Acknowledgements

This work was supported by the grants VEGA-2/0074/24, APVV-19-0111 and APVV-23-0203.

Impact of cadmium and zinc on oxidative status markers in the hermann's tortoise (*Testudo hermanni*)

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Abstract

The aim of this study was to verify the potential associations between the toxic element (cadmium), the potentially essential element (zinc) and oxidative stress markers (glutathione peroxidase - GPx, superoxide dismutase – SOD and total antioxidant status - TAS) in the hermann's tortoise *(Testudo hermanni).* SOD, GPx and TAS activities were measured using the standard kits (Randox Laboratories) on a Randox RX Monza analyser. Quantification of Cd and Zn in blood serum was done using inductively coupled plasma emission spectrophotometer (ICP Thermo iCAP 7000 Dual, ThermoFisher Scientific, Waltham, MA, USA). Potential associations between the monitored markers were determined using a Spearman correlation analysis. The average concentrations of the monitored elements in blood serum were 0.12 ± 0.05 mg/L for Cd and 6.62 ± 1.53 mg/L for Zn. The average levels of oxidative status markers in the blood serum were 33.89 ± 14.71 U/L for GPx, 1.41 ± 0.89 U/mL for SOD and 0.49 ± 0.29 mmol/L for TAS. The correlation analysis did not reveal statistically significant differences between zinc concentrations and oxidative markers with R-values for all correlations ranging from -0.016 to 0.27. We found a moderately strong negative association between cadmium concentrations and TAS levels (R = -0.41). Correlation analysis did not reveal statistically significant differences between cadmium concentrations and GPx and SOD levels (R-values are ranged from -0.049 to 0.269). These results did not prove an association of zinc, but suggested possible negative relationships between cadmium and TAS.

Keywords: trace elements; *Testudo hermanni*; oxidative status; tortoises

Acknowledgments

This study was supported by the project VEGA 1/0571/23 and by the Slovak Research and Development Agency under the contract no. APVV-21-0168 and GA of FAFR, SUA in Nitra No. 5/24 - Detection of xenobiotics and biogenic elements in selected body fluids and their relation on physiological parameters of turtles and tortoises.

Chili Extract Increases Reactive Oxygen Species Production in *Schizosaccharomyces pombe*

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Abstract

Chili extract, derived from *Capsicum*, is known for its antimicrobial properties, which are likely attributed to bioactive peptides present in the extract. This study aimed to explore the potential role of oxidative stress in mediating the effects of chili extract from Habanero Red (HR extract) on the fission yeast *Schizosaccharomyces pombe*, a model organism commonly used in cellular research. Oxidative stress was assessed by measuring intracellular reactive oxygen species (ROS) and malondialdehyde levels. Our preliminary data suggest that HR extract concentrations of 20 µg/mL or greater significantly increase ROS levels in *S. pombe*, indicating a dose-dependent effect. These findings suggest that higher doses of chili extract may exacerbate oxidative stress within the cells. Further research is necessary to elucidate the molecular mechanisms through which chili extract induces these effects, including the identification of specific cellular targets and underlying signaling pathways. Such research will contribute to a deeper understanding of the biological impact of chili extract and its potential for application in various biological contexts.

Keywords: chilli; *Schizosaccharomyces pombe*; oxidative stress

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Mitochondrial and lysosomal activities in bovine oocytes following vitrification

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Abstract

Mitochondrial function is crucial for many cellular processes such as oocyte maturation, embryo development and competence acquisition. Mitochondrial disfunction induced by vitrification can be alleviated after warming, when damaged mitochondria are removed, and *de novo* synthesis occurs to restore mitochondrial function. High number of mitochondria in oocytes indicates their quality, but little is known about the recovery of mitochondria after thawing/warming of oocytes and embryos. The aim of the study was to examine the mitochondrial and lysosomal activity in bovine oocytes following vitrification. In our experiments, mitochondrial activity after warming of oocytes reached the level of mitochondrial activity in fresh oocytes after 3-hour recovery period in presence of an antioxidant substance - astaxanthin (Ax). Vitrification resulted in increased lysosomal activity (1.90 arbitrary units - AU) compared to the fresh (control; 1 AU) and Ax group (0.85 AU), while mitochondrial activity (1.31 AU) remained comparable with the fresh control (1 AU) and Ax group (1.59 AU). Interestingly, mitochondrial activity in the Ax group was significantly higher than in fresh control oocytes. Distribution pattern of mitochondria and lysosomes was mostly scattered throughout the ooplasm of oocytes in all tested groups. Vitrification/warming significantly increased lysosomal activity compared to the control and astaxanthin groups. This observation indicates that increased lysosomal activity in vitrified/warmed oocytes can be associated with induced mitophagy as a survival response to vitrification damages.

Keywords: bovine; oocyte; vitrification

Acknowledgments

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Protein partners of CD63 in bull spermatozoa

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Abstract

CD63 is a member of the tetraspanin family, a group of proteins primarily involved in organizing plasma membrane proteins into molecular complexes known as the "tetraspanin web." Tetraspanins are capable of interaction with various partner molecules, including other tetraspanins and lipids, such as cholesterol. This ability allows them to regulate several cellular processes including cell adhesion, morphology, protein trafficking and signal transduction. In this study, we investigated the potential protein partners of CD63 in bull spermatozoa isolated from different regions of the epididymis as well as from ejaculated sperm. To construct a protein-protein interaction network associated with CD63_BOVIN (UniProt identifier: Q9XSK2), we utilized the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING v12.0) database. Based on this analysis, we performed co-immunoprecipitation experiments to identify potential partners of CD63 including tetraspanin CD9, integrin αV and syntenin-1 in bull epididymal and ejaculated sperm. Sperm samples isolated from the caput, corpus and cauda epididymides, along with ejaculated sperm, were solubilized in 1 % (w/v) Brij-97 and precipitated by an anti-CD63 monoclonal antibody. The resulting co-immunoprecipitated complexes were captured using protein G-agarose and analysed via 12% SDS-PAGE followed by Western blotting with anti-CD9, anti-integrin αV and anti-syntenin-1 antibodies. Our co-immunoprecipitation experiments revealed the potential interactions of CD63 with CD9 tetraspanin, integrin αV and syntenin-1 in both epididymal and ejaculated sperm. These proteins were detected in the CD63 precipitates as bands corresponding to approximately 50 kDa (CD9), 130 kDa (integrin αV) and 49 kDa (syntenin-1). These findings suggest that CD63 along with these partners may be integral components of molecular complexes present in the membranes of epididymal and ejaculated bull spermatozoa. However, further investigation is necessary to elucidate their specific roles in the dynamics of sperm membrane-associated molecules during pre-fertilization processes.

Key words: CD63; protein; molecular complexes; sperm.

Acknowledgments

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Evaluating the Impact of Bee Bread Extract on Transcriptional Profiles in Primary Rat Osteoblasts

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Abstract

Bee Bread (BB) is a fermented natural product rich in bioactive compounds with antioxidant and antimicrobial properties. Research in a rat model showed its potential to enhance diabetic bone structure, suggesting benefits for bone disorders. The aim of this *in vitro* study was to analyse the effects of BB extract on gene expression in osteoblasts. BB extract was prepared via methanol extraction, filtration, and evaporation, then dissolved in cell culture medium. It was applied to rat osteoblasts at 10, 100, and 200 μg/ml, with untreated medium as a control. After 72 hours, mRNA was extracted, cDNA synthesized, and the expression of 15 target genes (RUNX2, BMP2, BMP7, TGFB1, TGFBR1, BGLAP, ALPL, SPP1, VDR, COL1A1, IBSP, CDH11, VEGFA, TNFSF11, TNFRSF11B) analyzed using real-time PCR, with five reference genes (TBP, HPRT1, PGK1, B2M, LDHA). BB extract upregulated RUNX2, BMP7, TGFBR1, and ALPL in all groups. Dose-dependent effects included increased TGFB1, BMP2, and VDR in BB10; CDH11 in BB10 and BB100; TNFRSF11B in BB100 and BB200; and BGLAP and VEGFA in BB200. SPP1 was downregulated in BB10 and BB100, while TNFSF11, COL1A1, and IBSP remained unchanged. These findings indicate BB extract promotes osteoblast differentiation, bone mineralization, and osteogenesis while inhibiting bone resorption, demonstrating its potential for bone health and therapy.

Keywords: Bee Bread; osteoblasts; gene expression; *in vitro*

Acknowledgments

This study was financially supported by the Ministry of Education, Science, Research and Sport of the Slovak Republic, grant numbers KEGA 012UKF-4/2023 and 023UKF-4/2025.

Cementarky - population structure and genomic relationships to spotted cattle in Europe

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Abstract

Animal genetic resources, especially the unique characteristics of local breeds, are vital for maintaining biodiversity and ensuring food security. They represent an irreplaceable source of genetic potential, essential for promoting sustainability, enhancing resilience, and addressing future challenges in agriculture. This study aimed to analyse the genomic diversity of animals representing the original phenotypic type of Slovak Spotted cattle, known as "Cementarky," and their genetic relationships with other red and spotted breeds maintained across Europe. The genome-wide data for 35 animals was obtained using InfiniSEEK high-throughput sequencing technology. After data cleaning, the genomic database contained a total of 626,420 informative autosomal SNP markers. The genomic diversity status of the tested group of animals was derived from the level of genome-wide heterozygosity, genomic inbreeding (F_{HOM} and F_{ROH}) and effective population size (N_{eLD}). Genomic relationships to other red and spotted European cattle breeds were quantified by principal component analysis, calculation of genetic relationship matrices, construction of phylogenetic networks and estimation of admixture proportion between them. The results revealed relatively high overall heterozygosity across the genomes of the studied animals (H_o=0.34±0.16), reflected in low genomic inbreeding coefficients (F_{HOM}=0.001±0.04; $F_{ROH>4Mbp}$=0.01±0.01) and a good level of effective population size (N_{eLD}=243.81 animals). As expected due to the genetic background of analysed animals, they showed a strong connection to Slovak Spotted cattle production herd while also sharing genetic variants with Austrian breeds (Tyrol Grey, Braunvieh) and Czech breeds (Czech Spotted, Czech Red). The high heterozygosity and genetic potential found in the analysed group of animals suggest that Cementarky may be a valuable reservoir of genetic diversity, which may help in the conservation and utilisation of genetic resources not only in the case of Slovak Spotted cattle but also other related breeds in Europe.

Keywords: animal genetic resources; cattle; genomic diversity; local breeds

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The fission yeast *Schizosaccharomyces pombe* as a model system for toxicological and cell protective studies on eukaryotes

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Abstract

Schizosaccharomyces pombe (*S. pombe*), commonly known as fission yeast, is a free-living, single-celled eukaryotic organism that taxonomically belongs to the archiascomycete fungi. It was first described in the 1890s and has been extensively studied since the 1950s. *S. pombe* cells are rod-shaped and divide by medial fission during vegetative growth. The mitotic cycle is relatively fast, with a cell doubling time of approximately 3 hours. Fission yeast typically exists in a haploid form, consisting of only three chromosomes, and its genome was fully sequenced in 2002. Due to its ease of genetic manipulation and rapid growth, it has become a widely used model organism for studying cell cycle control, DNA repair, recombination, but also metabolism, and toxicological studies. *Schizosaccharomyces pombe* serves as a convenient model organism to study a variety of biological processes because it shares many functional similarities of biological processes with higher eukaryotes. In our studies, we have utilized S. pombe to investigate the effects of various substances on cell growth, morphology, antioxidant capacity, reactive oxygen species (ROS) generation, ion balance, and cell cycle progression at a molecular level. The substances we examined include heavy metals such as nickel and cadmium, abiotic toxicants like acrylamide, and bioactive compounds such as ascorbic acid, Aronia juice, oyster mushroom extract, and *Cornus* mas extract.

Keywords: *S. pombe*; metabolomics; ionome; antioxidant capacity; toxicological studies

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Cell cultures improve the preimplantation development of bovine embryos

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Abstract

Culture conditions are a limiting factor in the development of preimplantation embryos *in vitro*. Suboptimal conditions can result in the arrest of development or significant reduction of the embryo quality and viability. The use of cell cultures, so-called nutrient monolayers, which can positively influence the environment for embryos and demonstrably support their development, has also played a very important role here. The main benefit of using cell monolayers in embryo culture is the adjustment of the medium composition by the production of growth factors and the reduction of oxidative stress. For co-cultures, primary cells obtained from the epithelial cells of the fallopian tube or monolayers obtained from follicular cells of the *stratum granulosum* were initially used. Due to the disadvantages of primary cell cultures (laboriousness, possibility of contamination, inconsistent quality, short life span), new methodologies using stable continuous cell lines such as BRL (Buffalo rat liver) or Vero (the kidney of an African green monkey) cells have been successfully introduced. Recently, new continuous line, porcine oviductal epithelial cells (POEC-1), has been established to use for porcine embryo production by *in vitro* fertilization (IVF). In our experiments for bovine *in vitro* embryo production, we used BRL-1 (Buffalo Rat Liver; ECACC) culture. We found that monolayer of BRL cells significantly improves blastocyst development and subsequent embryo hatching until the 10th passage of BRL cells. Currently, there are several options for successful culture of preimplantation embryos, such as a culture at reduced O_2 levels in a precisely defined medium, or the culture with the support of nutrient monolayers.

Keywords: embryo; IVF; co-culture

Acknowledgments

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The influence of the *HTR2A* gene polymorphism on the temperament of Polish Red cows by using Classification and Regression Trees *(CART)*

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Abstract

Temperament is an important behavioural feature in breeding cattle. It is inheritable, and so is a factor in selection programmes in some countries. In recent years, several dozen genes that might significantly affect cattle temperament have been identified, including the serotonin receptor 2A *(HTR2A)* gene, but its polymorphism was only analysed for its production value, especially among high-production breeds. The goal of this study was to analyse the polymorphism of the *HTR2A* gene in a native conservation Polish Red Cattle breed using the decision tree . In this study, 124 Polish Red cows were genotyped using Sanger sequencing. Statistical analyses included the method of data exploration known as the classification and regression tree and the chi-square test of independence, which offered a precise description of the relationship between cattle temperament and genotype. Two mutations, *rs110801604* and *rs43696136*, proved to be closely related to temperament, as animals with extreme temperaments (calm and excitable) had different genotypes in those loci. These promising results indicate that further research into the polymorphism of the *HTR2A* gene is warranted for cattle of different breeds and purpose.

Keywords: behaviour; regression tree; Polish Red cattle

Acknowledgments

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Perga as a treatment for TAG, HDL and LDL in diabetic group

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Abstract

One of the most common and quickly emerging rapidly spreading in the community is *diabetes mellitus*. Bee bread, a lesser-known kind of bee product, was selected as tested substance for our research. The bee bread known as a perga is a fermented type of bee pollen that is easier for the human body to digest and has a higher nutritional content than regular pollen. The purpose of our study was to determine the effects of perga on the serum HDL and LDL. The levels of TAG, LDL and HDL were measured using a Biolis 24i Premium biochemical analyzer. The stomach tube was used to apply 700 mg kg of perga per day to male ZDF (Zucker diabetic fatty) rats. This line naturally develops type 2 diabetes mellitus throughout their lifetimes. ZDF rats with diabetes (Fa/Fa; n = 20) and rats of the same species without diabetes (Fa/Fa; n = 10) were involved to the experiment. The rats were randomly divided into three groups (Z – lean group without treating, D – diabetic group treated with water and DP – diabetic group treated with bee bread, n=10 in each group). The mean TAG level was significantly ($P<0.001$) lower in group Z than in groups D and DP. The TAG level was also significantly ($P<0.05$) lower in group D as compared to diabetic treated rats (DP) group. A significant decrease ($P<0.01$) in HDL levels occurred in the DP group compared with the D group. Significantly lowest ($P<0.001$) blood HDL levels were in Z group compared to DP and D groups. Significantly lowest ($P<0.001$) LDL value was in healthy rat (Z) group compared to D and DP group. The difference between D and DP group was non-significant ($P>0.05$). Based on our experiment's findings, we can conclude that perga was effective in diabetes therapy regarding lipid metabolism. Since diabetes mellitus is one of the most common illnesses in the world, more study is needed to slow and prevent its progression. If incorporated into people's normal diets, the perga is showing itself to be a very good natural option that might aid in the solution of this worldwide issue.

Keyword: bee bread, *diabetes mellitus*, ZDF rats

Acknowledgments

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Expression of genes involved in insulin signalling in early embryos from obese female mice

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Abstract

In our study, the influence of maternal obesity on expression of genes related to insulin signalling in mouse embryos was examined. Expression of transcripts for insulin receptor, insulin-like growth factor I receptor and selected genes representing two main branches of insulin receptor signalling (phosphatidylinositol 3-kinases and mitogen-activated protein kinase 1 and 3) was analysed in the blastocysts recovered from control and obese mice. After total RNA extraction and cDNA synthesis, PCR with specific primers was used to find out, whether the expression of selected transcripts in blastocysts is altered by maternal obesity. Obtained PCR products were verified using a gel agarose electrophoresis. We demonstrated the expression of three PI3K catalytic subunits (alpha, beta and delta) in preimplantation embryos for the first time. Results of our mRNA quantification showed a significant increase in the amount of insulin receptor transcripts in the blastocysts recovered from obese mice. No significant differences were found in the expression of other genes. Further research is needed to reveal the possible mechanisms by which maternal obesity modifies insulin signalling in preimplantation embryos.

Keywords: blastocyst, insulin signalling, maternal obesity

Acknowledgement

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"Cementarky" - yellow original type of Slovak Spotted cattle

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Abstract

Due to its very good milk yield, growth intensity and drag force, Simmental cattle became popular all over the world including Slovakia. After the first imports to the country in the 18th century, more intensive crossbreeding with local breeds followed in 19th century. In the post-war period intensification and crossbreeding continued and Slovak Spotted (SS) cattle represented 56% of the cattle population. Big impulse for genetic and management improvement came in 1990s with the import of Fleckvieh animals with higher milk yield. There is a region in the middle of Slovakia, which avoided process of collectivisation, creation cooperatives and intensive crossbreeding with Fleckvieh. Small farmers kept their land and animals. They have traditionally preferred SS cattle well adapted to this production system where drag force, milk and meat production were needed. Nowadays, farmers in the region still prefer yellow animals of smaller frame that suit their barns. Cows are expected to have udder and teats suitable for hand milking. Animals are expected to be long-living with good fertility traits including easy calvings and robust in order to be productive in a system with limited feeding regime. Based on discussions with farmers, most suitable animals for their production system are the original type of SS cattle, resembling animals from 1980s period. In order to preserve this type of production system and facilitate access to suitable animals, the initiative for recovery of original type of SS cattle started. The cows, resembling the 1980s SS type, were selected and consequently inseminated with the Simmental´ sperm doses produced in 1980s or earlier periods. After the two-year period of activities, newborn animals have been weighted, measured, and samples for genetic analyses have been collected to study genetic relatedness of this group to SS population and genetic background for coat colour.

Keywords: animal genetic resources; endangered breeds; local breeds; Slovak Spotted cattle

Breed-dependent variations in the sperm membrane dynamics: a potential marker for cryosensitivity?

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Abstract

This study attempts to assess and compare selected sperm characteristics indicative of the plasma membrane integrity and dynamics in three selected bovine breeds. Semen samples were collected from stud bulls of the Limousine (LIM, n=7), Simmental (SIM, n=7) and Holstein (HOL, n=7) breeds. Progressive motility was quantified using the computer-assisted sperm analysis system. Membrane integrity was quantified with 5-carboxyfluorescin diacetate while the acrosome integrity was evaluated with a FITC-labelled PNA lectin. Phosphatidylserine dislocation characteristic for early apoptosis was determined with the fluorescent Annexin V-staining. Data analysis revealed that HOL showed the lowest progressive motility, which was significant in comparison to other breeds ($P < 0.01$). Membrane integrity was significantly lower in LIM against SIM ($P<0.05$), while the acrosome integrity was significantly lower in SIM in comparison to the other tested breeds ($P < 0.01$). The lowest apoptotic index was observed in HOL, which was significant particularly when compared to SIM ($P < 0.01$). In summary, each species may be characterized by unique semen features, which may be monitored more closely during sperm cryopreservation.

Keywords: bovine breeds; membrane; spermatozoa

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Hidden Potential of Biologically Active Substances on Avian Bone Health

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Abstract

Egg and chicken meat production is associated with challenges such as osteoporosis, which impact animal health and farm profitability. High egg production and intensive broiler growth lead to bone thinning and increased fragility. This study focused on testing the impact of drone brood homogenate on chicken bone tissue cells *in vitro*. Thigh bones from juvenile chickens were used to isolate progenitor cells. Mesenchymal stem cells, progenitors of osteoblasts, were isolated from fragments of the femoral epiphysis. Progenitor cells of osteoclasts were isolated from bone marrow using gradient separation. Osteoblasts and osteoclasts were cultured in DMEM medium with the addition of FBS and antibiotics, and with various concentrations of the homogenate. Gene analysis and immunocytochemical methods showed that homogenate supplementation can support osteoblast proliferation and increase the expression of *RANKL* and *OPG* genes, which may be crucial for bone tissue cell differentiation. Studies of biologically active substances often examine their effects on various tissues and cells, mostly of plant origin and in the human sphere, while drone brood is traditionally use for male sexual disorders whereas in terms of osteoporosis treatment and prevention is poorly recognized.

Keywords: bone; chicken; drone brood

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