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**The 2nd International Scientific Conference „Animal Biotechnology“**

# Slovak Journal of Animal Science

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RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

# Slovak Journal of Animal Science

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Slovak Journal of Animal Science (ISSN 1337-9984) is an international scientific journal that publishes original scientific papers, reviews, short communications, chronicles of important jubilees, reports of participation in important international conferences on animal science in English language.
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# ANIMAL BIOTECHNOLOGY 2014

NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Institute of Genetics and Reproduction of Farm Animals
NPPC - Research Institute for Animal Production Nitra
Slovak Republic

FAKULTA BIOTECHNOLÓGIE A POTRAVINÁRSTVA SPU NITRA

Faculty of Biotechnology and Food Science
Slovak University of Agriculture in Nitra
Slovak Republic

# The 2nd International Scientific Conference „Animal Biotechnology“

*Main topic areas of the conference:*

Animal Genetics and Reproduction
Animal Cell Biotechniques
Cell Cryopreservation
Epigenetic effects
Rabbit Model – Genetics, Reproduction, Nutrition and Health

**December 11th, 2014**

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**Dear Participants and Colleagues,**

We are very pleased to welcome you to the 2nd International Scientific Conference **„Animal Biotechnology“** focused on the area of animal genetics and reproduction. The conference is organized by the Research Institute for Animal Production, NAFC Nitra.

The aim of our conference is a presentation of topical research results and some legislative aspects from the field of farm animal biotechnology, with an actual point on Animal Genetics and Reproduction, as well as a meeting of researchers in the adjacent field of research. We would appreciate an attendance and participation in this conference of colleagues from various research institutes and universities.

We are very pleased to organize this conference in time when our colleague Prof. h. c. Prof. Ing. Jozef Bulla, DrSc. celebrates his 70th anniversary. We would like to wish him a happy birthday and to express our honest acknowledgement for his long-year research activity and great contribution to the development of farm animal science.

We would like to wish you nice atmosphere at our conference for presentation, creative and fruitful discussion and stimulating ideas for future research.

Nitra, December 11th, 2014

Prof. Ing. Peter Chrenek, D.Sc.

# The Jubilee of Professor Jozef Bulla

Significant Slovak scientist and teacher Professor Jozef Bulla, member of the editorial board of the Slovak Journal of Animal Science, celebrates his 70th anniversary on 8th December 2014.

Professor Bulla was born on 8th December 1944 in Bobrovec, distr. Liptovsky Mikulas. He graduated from the University of Agriculture in Nitra in the field zootechny at the Faculty of Agronomy. In 1968 he started his post-graduate study (CSc) in the Research Institute for Animal Production in Nitra. He passed this study in cooperation with the Institute of Experimental Biology and Genetics at the Czechoslovak Academy of Sciences in Prague and at the Department of Genetics at the Faculty of Natural Sciences at the Comenius University in Bratislava in 1972. In 1974 he defended his dissertation thesis in the field of genetics and obtained the scientific degree "candidate of biological sciences (CSc)". He defended his doctoral dissertation "Genetic and epigenetic factors of phenogenesis regulation in birds' liver" and the board of the Slovak Academy of Sciences awarded him scientific degree "Doctor of Sciences" in 1987. He was appointed associate professor (MSMTV SK) in 1984, and the Scientific board of the Faculty of Agronomy conferred him the scientific-pedagogical degree "associate professor" for the branch General zootechny at the Faculty of Agronomy on the basis of his habilitation in 1992. President of the Slovak Republic promoted

Jozef Bulla to a professorship in the field General zootechny-genetics in 1993.

Prof. Bulla worked at the Research Institute for Animal Production as head of the Department of Genetics (1980 – 1986) and director of the Institute for Genetics and Reproduction of Farm Animals (1987 – 2000). During the period 1986 – 1990 he was also director of the International Laboratory for Animal Biotechnologies of COMECON Countries with its headquarter in the RIAP Nitra. In 2001 he was appointed to establish of the Faculty of Biotechnologies and Food (FBF) and was elected as a dean for two functional periods from 2002 to 2010. At present he is

a professor at the Department of Physiology FBF of the Slovak University of Agriculture in Nitra.

His 46 years lasting scientific and research activity was and is aimed at the study of genetic, physiological and biotechnological methods to increase performance in farm animals; at creation, utilization and protection of their gene pool and at application of molecular-biological methods for breeding and selection work. He published more than 400 scientific, professional and popularization publications at home and abroad. He was supervisor of 72 graduate students, 27 bachelors, and 25 post-graduate students (6 from abroad). He participated in scientific, pedagogical and social education of 8 associate professors and 6 professors.

Prof. Bulla is a member of several scientific boards; academic and expert committees and editorial boards; he is holder of significant domestic and international awards.

His activities and results of scientific, pedagogical and scientific work were appreciated by granting him membership of the Learned Society of the Slovak Academy of Sciences and title "honoris profesor" at the University of Natural and Ecological Sciences in Wroclaw, Poland.

The reality of life of Professor Jozef Bulla was and still is consisted in the effort for creation of worthy and generally recognized scientific-pedagogical institutions as an incubator for development of knowledge in line with his character and continuity of work of his teachers. The Faculty of Biotechnology and Food Science at SUA Nitra as well as the former and mainly existing functional structures of the RIAP NPPC in Nitra are such institutions.

On the occasion of his jubilee we wish Professor Bulla in the following years a lot of optimism, health, creative scientific invention and joy in work activity and family life.

Prof. Ing. Peter Chrenek, D.Sc.

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*Review*

# THE ROLE OF MICRORNAS IN RABBIT PREIMPLANTATION EMBRYOS AND PLURIPOTENT STEM CELLS

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## ABSTRACT

Although several groups are able to generate rabbit embryonic stem cells (ESCs) and induced pluripotency cells (iPS) cell lines using different derivation methods, it is still difficult to establish it, and the pluripotent lines, therefore, remain poorly characterized. Since ESCs are derived from early blastocysts, they can reflect the potential characteristics of their embryonic founder population. Therefore, it is important to compare the expression pattern of both miRNAs and proteins known to play regulatory roles during early lineage specification. Our group aimed to explore ESC-specific miRNA expression pattern from early embryonic stages to rabbit embryonic stem-like (ES-like) cell for the first time to get more insight into their potential regulatory mechanism during embryonic development and in stem cell properties.

**Key words:** rabbit; embryo; epiblast; hypoblast; stem cell; microRNA

## INTRODUCTION

### Overview of rabbit embryonic stem cell derivation

Human ESCs are potential source for regenerative medicine, tissue engineering, cell-based transplantation, disease pathology and drug discovery (Thomson *et al*., 1998). In spite of their great medical potential, human ESCs generation requires the destruction of developing human embryos which restrict their intended application ethically. By generation of iPSCs from human somatic cells, it was expected to overcome the ethical controversy associated with human ESCs research (Takahashi *et al.*, 2007; Park *et al*., 2008). To overcome the limitations associated with human ESCs, many efforts have been put to generate the stable animal ESC lines in order to use in regenerative medicine. The rabbit ESCs represent an alternative animal model to study human diseases since rabbit ESCs resemble primate ESCs more closely than mouse ESCs (Wang *et al*., 2007). The establishment of rabbit ESCs has been reported by several groups (Honda *et al.*, 2009), (Catunda *et al.*, 2008; Intawicha *et al*., 2009). Although there are reports indicating that rabbit ESCs might be passaged for longer period over 20 passages (Intawicha *et al*., 2013), but it needs to be maintained manually and pick the only colonies which still keep the typical ESC morphology and stayed undifferentiated (Honda, 2013; Osteil *et al*., 2013;

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Tancos *et al.*, 2012). Rabbit pluripotent stem cells were defined as EpiSC-type or primed state stem cells; these are rarely competent to contribute to blastocyst chimeras and are, therefore, developmentally and functionally distinct from naive state mouse ES cells (Osteil *et al*., 2013), (Intawicha *et al.*, 2013). The first live born ES-derived rabbit chimera was reported in 2011 (Zakhartchenko *et al.*, 2011), but there are no reports about the germ line transmission.

**Regulatory function of miRNA during early development**

MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate multiple biological processes. They post-transcriptionally regulate gene expression mostly through binding to complementary regions within 3‘UTRs of their target mRNAs leading to translational repression or cleavage. Increasing experimental evidence implies an important regulatory role of miRNAs during early embryonic development and in embryonic stem cell biology.

Regulation of gene expression in developmental processes is an important aspect of miRNA function. Many experiments have been performed to dissect the miRNA pathway in various organisms demonstrating that miRNAs are essential for proper embryonic development. Mouse pre-implantation embryos express developmental stage-specific miRNAs (Mineno *et al.*, 2006; Viswanathan *et al.*, 2009). Interestingly, several stem cell specific miRNAs, such as miR-290 cluster, were shown to be the first de novo expressed miRNAs in mouse embryos at 2-4-cell stage, with an increasing expression through the blastocyst stage (Zeng and Schultz, 2005), (Viswanathan *et al*., 2009), (Kutter and Svoboda, 2008). Moreover, miR-290 cluster deficiency in mouse embryos causes penetrant embryonic lethality and germ cell defects implying the important role of this cluster in embryonic development (Medeiros *et al*., 2011).

**Regulatory Function of miRNA in ESC**s

MicroRNAs have also been identified as important regulators of embryonic stem cell properties (Cao *et al*., 2008), (Marson *et al.*, 2008). The mmu-miR-290 cluster has been reported as an ESC-specific miRNA which expressed highly in undifferentiated ESCs (Marson *et al.*, 2008) and inhibited the ESC differentiation (Zovoilis *et al*., 2009). This cluster has an important regulatory function in undifferentiated ESCs through direct control of de novo DNA methylation (Benetti *et al.*, 2008; Sinkkonen *et al.*, 2008), cell cycle regulation by suppressing the G1-S transition and targeting cell cycle regulators (Lichner *et al.*, 2011; Wang *et al.*, 2008). In addition, the promoter of this cluster is a direct target of key ESC transcription factors, such as OCT4, SOX2 and NANOG (Marson *et al.*, 2008). The human homolog of mmu-miR-290 cluster, miR-371 cluster was also identified to be expressed in human ESCs and does not reappear in any somatic lineage. This cluster shares the chromosomal region with C19MC cluster (chromosome 19 miRNA Cluster) which is a highly repetitive region containing 46 members of the super-family and shows high sequence similarity to both hsa-miR-371 and mmu-miR-290 clusters (Bentwich *et al.*, 2005; Lichner *et al.*, 2011). The miR-302 cluster is also highly expressed in both human and mouse ESCs and down-regulated upon differentiation (Chen *et al.*, 2007). Similar to miR-290 family this cluster is a cell cycle regulator; cyclin D1 and Cdk4 are post-transcriptionally regulated by miR-302 cluster in human ESCs resulting in positive regulation of ESC self-renewal (Card *et al.*, 2008). Furthermore, this cluster might positively regulate the Nodal/Activin pathway, therefore contributing to the maintenance of pluripotency (Barroso-delJesus *et al.*, 2008).

**A microRNA expression profile in rabbit embryos and stem cells**

By applying SOLID deep sequencing technique, we reported the miRNA expression profile during early rabbit embryonic development and in ES-like cells (Maraghechi *et al.*, 2013). We could identify a total of 1693 expressing rabbit miRNAs based on comparison of obtained sequence reads to the known human, mouse and bovine miRNA databases (miRBase). We detected and characterized ESC-specific miRNAs of early steps of rabbit ESC line establishment and their embryonic founder cell population of attached ICM clumps and embryonic disc. We described and analyzed the expression profile of pluripotency-associated miRNAs in rabbit embryos and embryonic stem-like (ES-like) cells. The rabbit specific ocu-miR-302, ocu-miR-290 clusters and three homologs of human C19MC cluster (ocu-miR-512, ocu-miR-520e and ocu-miR-498) were identified in rabbit preimplantation embryos and ES-like cells. The ocu-miR-302 cluster was highly similar to its human homolog, while ocu-miR-290 revealed a low level of evolutionary conservation with its mouse homologous cluster. The expression of ocu-miR-302 cluster members began at 3.5 days post coitum - early blastocyst stage and they stayed highly expressed in rabbit ES-like cells. In contrast, high expression level of ocu-miR-290

cluster members was detected during preimplantation embryonic development, but low level of expression was found in rabbit ES-like cells.

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*Review*

# AVIAN PRIMORDIAL GERM CELLS AND THEIR APPLICATION

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## ABSTRACT

Primordial germ cells (PGCs) are the precursors of the progenitors of the oocytes and spermatocytes. The unique properties and accessibility of avian PGCs during early development provide an opportunity to manipulate with the avian germplasm. In this review the recent developments of the embryo manipulation techniques and the use of PGCs for the conservation of genetic resources and as a vehicle for the efficient production of transgenic chickens are described.

**Key words:** poultry; avian germ cells; embryo manipulation

## INTRODUCTION

Germ cells play a very important role in the species identification as they transmit genetic information to the next generation. In many animal species, including birds, germ cells emerge from a small population of cells known as primordial germ cells (PGCs). Avian PGCs (Figure 1) arise from the pluripotent epiblast and are initially localized in the central disc of the pellucida area of X stage embryos. Once blood vessels form, PGCs enter the blood vessels and migrate into the forming gonad and differentiate nto mature spermatozoa and oocytes in the adult. Chicken PGCs were collected from the germinal crescent (Naito *et al*., 2001), the embryonic blood vessel (Naito *et al.*, 1994) and from the embryonic gonads (Park *et al.*, 2003). Although no phenotypic changes between the different sources of PGCs were observed, and the expression patterns of specific markers were identical (Park and Han, 2012), however the advantage of using gonadal PGCs compared with another sources of these cells is, that a greater number of PGCs can be retrieved from one embryo.

These cells can be cultured and expanded *in vitro*, without loss of germ cell integrity. PAS (periodic acid-Schiff) reaction is a generally accepted histochemical marker for differentiating PGC from surrounding somatic cells (Chojnacka-Puchta *et al.*, 2012). In addition to PAS, chicken PGCs are stained selectively by specific cell surface antigen SSEA-1, which is often used as a marker for stem cell differentiation (Jung *et al*., 2005).

The unique properties and accessibility of avian PGCs during early development provide an opportunity to manipulate with the avian germplasm. Two uses of PGCs are often reported: these cells can be used for the conservation of genetic resources and the production of transgenic birds (see Nakamura *et al*., 2013). An immediate technological application of PGCs is for the preservation of germplasm from specialised and rare breeds of poultry (see Glover and McGrew, 2012). To generate

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a genetically diverse population it will be necessary to preserve the germplasm from a minimum of 13 individuals of each sex (FAO, 1998).

Various methods have been developed to produce transgenic chickens (Table 1). However, the injection of recombinant PGCs into recipient embryos (see Chojnacka-Puchta *et al*., 2012) is currently one of the most widely used non-viral procedure for creating transgenic birds. Generation of transgenic chickens through chimeric intermediates, produced by the transfer of PGCs, has been attempted in our laboratory. This method is particularly important for studying the development of chimeric and/or transgenic organisms.

However, despite above cited achievements, currently the efficiency of the transgenic method is low

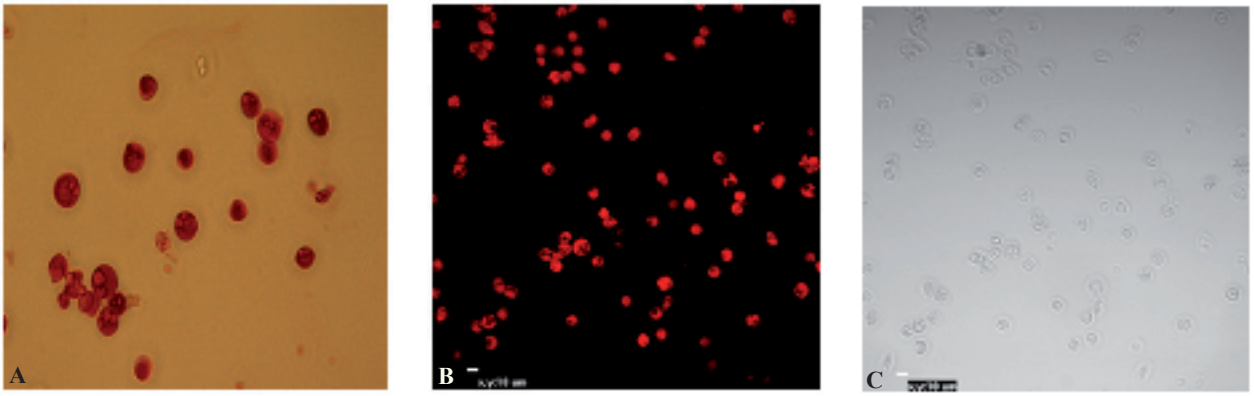


**Fig. 1: PAS reaction indicates the presence of glycogen vesicles and red-stained chicken PGCs (A) (40× magnification). Immunofluorescence staining for the specific cell surface antigen SSEA-1; chicken blood PGCs were found (stages 14–16 HH) to express SSEA-1. Anti-SSEA-PerCp-positive cells are shown in red (B) and bright field (C) (adapted from Chojnacka-Puchta, 2012)**

**Table 1: Milestones of chicken's transgenesis**

| Date of publication | Achievement | Authors |
|---|---|---|
| 1986 | First successful development of transgenic chickens | Salter *et al.* |
| 1990 | First germ line chimeric chicken | Petitte *et al.* |
| 1993 | Transgenic birds produced from transformed PGCs | Vick & Simkiss |
| 1996 | Pluripotent ESC derived from blastoderm | Pain *et al.* |
| 2003 | Expression of a human interferon in egg white | Rapp *et al.* |
| 2004 | Lentiviral vectors, able to infect non-dividing cells | McGrew *et al.* |
| 2005 | Somatic chimeras produce the significant amounts of a therapeutic antibody in the egg white | Zhu *et al.* |
| 2006 | Germline transmission of grown *in vitro*, and genetically modified PGCs | van de Lavoir *et al.* |
| 2007 | Commercially significant amounts of a therapeutic protein in the egg white | Lillico *et al.* |
| 2011 | Suppression of avian influenza transmission in genetically modified chickens | Lyall *et al.* |
| 2012 | Use of transposons to modify the chicken genome | Macdonald *et al.*, Park and Han, Yang and Kim |
| 2012 | Hen's oviduct epithelial cells culture | Kasperczyk *et al*. |

in many cases. On the basis of publications indexed in the Web of Knowledge some major problems concerning the current poultry transgenesis can be defined as following:

- a major problem in creation of transgenic chickens (using any methodology) by manipulating embryos is achieving a sufficient hatchability,
- only a small number of bPGCs and little larger number of gPGCs can be isolated from blood and/or gonads,
- difficult to control differentiation of cells in long-term culture systems,
- very low efficiency of integration of the transgene into the host genome by conventional transfection methods,
- the retroviral construct integrates randomly into the host genome, which may result in transgene expression at various developmental stages and in various tissues.

Some of these problems will be discussed in the light of our research.

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# HEALTH IN CONTEMPORARY CATTLE BREEDING

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## ABSTRACT

The paper deals with the genetic health of cattle. The studies done in the Czech cattle population are summarized. The strict measures taken for BLAD in the 90´s have been efficient, as the frequency of positive sires has decreased rapidly. The diverse approach to Complex Vertebral Malformation (CVM) resulted in different population dynamics. The use of proven heterozygous carriers of lethal alleles in breeding is not absolutely banned, so the rate of heterozygous sires decreases, but persists still considerably, and the occurrence of CVM females remains high. Recessive alleles of bovine citrullinaemia, DUMPS, glycogen storage disease V and II, and factor XI deficiency were not found. The occurrence of congenital defects was analysed. The XX/XY chimerism was found in 0.50 % of Holstein-Friesian sires and in 0.74 % of Czech Simmental sires. By testing for aneuploidy, 5 Charolais sires were found to be carriers of a redundant sex chromosome (61, XXY; 61, XXX). Autosomal aneuploidies were not found. Robertsonian translocations were occurred in 0.82 % of Czech Simmental sires and in 3.57 % of beef sires, whilst Holsteins were not affected. The estimate of genetic variance of stillbirth caused by sires was 1.90 %, and heritability - 0.078, but the sizeable influence of sire´s line was observed. Despite of the low genetic variance, it is recommended that sires with higher incidence of fertility damages should not be used for breeding. In the period of genomics, molecular biology and molecular cytogenetics, the opportunity arises to solve the problem of genetic health principally.

**Key words:** cattle; health; congenital; chromosomal abnormalities

## INTRODUCTION

Fundamentally, the core of the problem is to find the animals that genetically connect high production and good health. The issue is rather intricated, and needs systemic approach.

Firstly, there is some contradiction between breeding for high performance and general state of health. The trait named as fitness, adaptability, and constitution has complex character, and polygene genetic background. It is the main criterion in the natural selection, when the heterozygous animals are preferred. On the contrary, the artificial selection on particular performance traits is mostly directional, homozygous animals are preferred. Thus, it results in lower adaptability, especially when in the breeding process the unsatisfactory attention to health is paid. Then the pressure on the high performance can be resulted in the worse health.

Secondly, the genetics is not clarified sufficiently. In many cases of congenital defects, e.g. even the detection of genetic or environmental aetiology is difficult. The traits with quantitative character, such as reproduction, have low heritability, some characteristics are difficult measurable.

However, in the age of genomics, molecular biology and molecular cytogenetics, the opportunity arises to solve the problem principally.

The paper summarizes the studies done on the Czech cattle population, and focused on the various aspects of the genetic health.

## RESULTS AND DISCUSSION

### Recessively inherited diseases

Relatively plain are the heritable diseases, lethal and sub-lethal factors caused by monogenic factors.

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The heredity is mostly recessive. In domestic animals, the occurrence of the defect is often breed-specific. In some cases, in different breeds different mutation in various regions of the same gene causes the defect and myostatin could be given as an example. The origin of the breeds is the reason, when they are bred closely over a long period.

Recently, Bovine Leukocyte Adhesion Deficiency and Complex Vertebral Malformation (CVM) in Holstein cattle have become a serious threat. The role of widely used superior breeding males in spreading of the undesirable recessives was described repeatedly. Here, different dealing with a problem of recessive inherited diseases is demonstrated.

In the Czech Republic, the strict measures for BLAD were taken in the 90´s. Every Holstein sire had to be genotyped, and the mating of heterozygous carriers has been absolutely prohibited. During the period of 1993-1998, the occurrence of heterozygous animals in Czech Holsteins exceeded 10 %, whilst in other countries, similar or even higher incidence has been found (Shuster *et al.*, 1992; Jorgensen *et al.*, 1993). Obviously, the measures have been efficient, as the frequency of positive sires has decreased rapidly (Tab. 1), the high occurrence of positive heifers in 2002-2004 was detected by testing females suspected because of a heterozygous parent. More recently positive findings in males are sporadic, but continuous testing is necessary, because in commercial herds, the eradication process is not short-term, as the prevalence of heterozygous animals depreciates by only 50 % each generation. The mating of heterozygous sires in the population may still result in births of affected calves.

A worldwide increase of Complex Vertebral Malformation (CVM) in the Holstein population has been recorded since 2001. The Czech Holstein could not differ of course (Table 1), as the rate of imported sires is approximately 80 %, mainly from the United States, Canada, Germany and the Netherlands. Restrictive measures have been undertaken here too. The CVM status of every sire must be declared, but the use of proven heterozygous carriers of lethal alleles in breeding is not banned, if the breeder agrees. The rate of heterozygous sires decreased, but still persists considerably. The occurrence of CV females remains higher, and similarly in another analysis (Čítek *et al.*, 2006), the frequency of heterozygous females was 19 %. Interestingly, no case of heterozygosity for BLAD and CVM in any individual has been recorded. On the other hand, the probability of contemporary presence is not high taking into account the low number of animals positively genotyped for BLAD since 2001 (n = 14).

Obviously, the diverse approach to the diseases mentioned results in different population dynamics. Of course, the controlled mating of proven non-carriers on the daughters of heterozygous sires can eliminate the birth of affected calves, but the more thoroughgoing breeding would be salutary.

The incidence of other recessives is markedly lower. This we can confirm by our analyses of bovine citrullinaemia, deficiency of uridine–5´-monophosphate synthase (DUMPS), glycogen storage disease V (deficiency of muscle glycogen phosphorylase, or myophosphorylase, *PYGM gene*), glycogen storage disease type II (GSD II, generalised glycogenosis, GAA gene, exons 7, 9, and 13), and factor XI deficiency (exons 9 and 12), the recessive alleles were not revealed.

**Congenital defects**

The congenital defects do not occur often. Involving both hereditarily caused defects and malformations suffered during pregnancy, the definition is rather vague from the genetic point of view. The aetiology of the latter is too often unclear, as many teratogens can be involved. Even the discrimination between acquired and inherited defects can be troublesome, as specific and thorough knowledge is sometimes, regrettably, missing.

Some of the afflictions caused by changes in genetic matter occur only rarely, and a thorough analysis is therefore impossible. However, some, particularly those with a relatively frequent incidence, have been quite well explored. Even in such cases the genetic background can be uncertain, so it is sometimes doubtful whether the condition is monogenic or polygenic, and many disorders have been assigned as definitely caused by one-gene on the basis of data insufficient for such certainty. Similarly, a resolution in favour of dominant or recessive inheritance can be dubious. Hence, because of the number and range of factors in operation in dealing with congenital disorders, any interpretation of the data must be prudent.

Congenital disorders have been a subject to analysis by many authors over the past decades from as early as the 1920s, and there are a number of excellent reviews which summarise their work. A comprehensive list of single locus traits is maintained by the Online Mendelian Inheritance in Animals (OMIA) database and is accessible on the internet.

In a survey done in the Czech cattle, around 8.6 % of Holstein sires and 6.5 % of Czech Simmental sires have born the offspring with congenital disorder (Čítek *et al.*, 2009a).

Summarizing the results, only 18 congenital disorders occurred in the progeny of 10 and more sires, sc. hernia umbilical cong., BLAD, opened hock, contracture of muscles and ligaments of limbs, schistosomus reflexus, stillborn calves, hydrocephalus

congenital, unviable calves, Robertsonian translocation, brachygnathia inferior, atresia ani and recti, ascites, perishing of calves, brachygnathia superior, abortion, spastic paresis, hernia cerebralis, and dystocia. A further 12 disorders affected the progeny of 5 to 9 sires, i.e. different types of atresia, aplasia, cleft, chimerism etc. The remainder of the congenital disorders identified, i.e. 121 (80 % of 151 described disorders) were diagnosed in the progeny of 4 and fewer sires; 88 disorders in the progeny of 1 sire only. Thus, the problem of congenital disorders is rather diversiform and difficult to analyse.

Of particular defects, umbilical hernia was the disorder which occurred most frequently. In literature (Herrmann *et al*., 2001), the influence of the sire and sire line is reported to be significant. Afflictions of limbs were considerably frequent, namely opened hock, and contractures of muscles and ligaments. Quite often schistosomus reflexus and various defects of the head were present. In our analysis, we found only sporadic cases of genital fissures.

Hydrops was found to be relatively common and also hydrocephalus, which has already been referred to as being among the most frequent cranial afflictions. Of defects in female genitals, aplasia was the most common. Male genitals were affected only rarely, cryptorchidism for example, appeared only once.

Even though the genetic analysis of rarely occurring disorders is difficult, sires fathering affected offspring should not be widely used in artificial insemination programmes, but disqualified for fathering of stock bulls or even definitively culled. In this context, it was unfortunate decision, when the control over the congenital diseases in the Czech Republic was abolished.

**Chromosomal abnormalities**

Aneuploidy and chimerism can occur more or less frequently, and for this reason cytogenetic analysis was applied in the Czech Republic by the veterinary survey.

The XX/XY chimerism was found in 0.50 % of Holstein-Friesian sires, and in 0.74 % of Czech Simmental sires (Čítek *et al*., 2009b). By testing for aneuploidy, 3 Charolais sires and 2 heifers were found to be carriers of a redundant sex chromosome (61, XXY; 61, XXX). Autosomal aneuploidies were not found. This does not mean they do not occur. In the group of animals in the analysis, autosomal aneuploidies were evidently lethal early in gestation, and the less harmful gonosomal aneuploidies allowed survival.

The search for Robertsonian translocations showed a relative frequency of 0.82 % in Czech Simmental sires and 3.57 % in beef sires. The Holstein-Friesian sires and heifers were not affected. Our results are well consistent with other reports, e.g. Seguin *et al*. (2000), who did not find any centric fusion in American Holstein-Friesian sires, but did find chimeric animals. Also, Nel *et al*. (1991) note the lack of chromosomal aberrations in pure dairy breeds found by teams across the world, when the chimerism found in Holstein-Friesians cannot be considered to be a primary chromosome defect.

As the chimeric sires have reportedly decreased reproductive performance, and the presence of Robertsonian translocations has been confirmed, the early

**Table 1: BLAD and CVM heterozygotes in the Czech Republic 1993-2005 (Čítek *et al.*, 2008)**

| Year of testing | BLAD | | | | CVM | | | |
|---|---|---|---|---|---|---|---|---|
| | sires | | heifers | | sires | | heifers | |
| | tested | positive | tested | positive | tested | positive | tested | positive |
| 2005 | 101 | 1 | 8 | 0 | 85 | 7 | 20 | 4 |
| 2004 | 161 | 3 | 10 | 2 | 72 | 6 | 13 | 5 |
| 2003 | 153 | 0 | 9 | 2 | 108 | 12 | 13 | 5 |
| 2002 | 89 | 2 | 9 | 2 | 35 | 7 | 3 | 0 |
| 2001 | 83* | 2 | - | 0 | 10* | 2 | - | 0 |
| 2000 | 69* | 1 | - | 3 | not tested | | | |
| 1999 | 14 | 0 | 2 | 0 | not tested | | | |
| 1993-98 | 568 | 79 | 121 | 13 | not tested | | | |

* number of tested sires and heifers together

cytogenetic screening of sires seems to be advisable; but again, the systematic examination was abolished.

**Polygenic health traits**

The field reveals more sub-traits, and it is hard to say, which is more important or should be preferred. In the Czech Republic, the breeding values for the own sire´s fertility are assessed, for the fertility of its daughters, the breeding value for the delivery difficultness, and for the longevity. In a broad term, also the body formation could be considered to be an indicator of health.

As mentioned in the introduction, the heritability of fertility is low. In our analysis e.g., the population genetic analysis resulted in the estimate of genetic variance of stillbirth caused by sires as $\sigma_s^2$ = 1.90 %. Then the calculated value of stillbirth heritability was $h^2$ = 0.078 (Čítek *et al.*, 2011). However, in our other analyses, the sizeable influence of sire´s line was found. Despite of the low genetic variance, it is recommended that sires with a higher incidence of fertility damages should not be used for breeding.

Breeding for the easy delivery must be carried out advisedly, because birth mass and growth capacity correlate positively, and focusing on the course of the birth results in a reduced body mass. In such cases, genome analysis may be promising. Though many analyses have been carried out already on calving difficulty, stillbirth and birth weight, further extensive studies for the assessment of their reasons, and quantification of genetic parameters are necessary. Recently, quantitative trait loci affecting the traits mentioned have been established with promising results. The QTL affecting dystocia, conformation, and economic merit were described as being related to calf size or birth weight. Grosz and Mac Neil (2001) revealed the QTL influencing the birth weight with no significant effect on growth since birth to weaning. Therefore, potentially the incidence and degree of dystocia can be reduced without compromise of subsequent growth performance. The microarray approach is promising also in other health indicators with polygenic heredity.

## CONCLUSION

The occurrence of inherited disorders is sporadic. However, a massive spread in farm animals could have a serious affect on the economy. Identification of the molecular basis for these diseases enables the quick recovery of the population. Permanent attention must be paid to the congenital defects and to the health indicators with polygenic heredity.

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# VIABILITY OF BOVINE PREIMPLANTATION EMBRYOS FOLLOWING CRYOPRESERVATION

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## ABSTRACT

In programs for the preservation of animal genetic resources a great significance belongs to a long-term storage of biological material (gametes, embryos) at extra-low temperatures. The aim of this study was to determine quality and viability of bovine embryos of Holstein breed cryopreserved by a vitrification procedure (n = 88) in comparison to intact (fresh) bovine embryos (n = 117). The embryos were recovered on 7th day after the first insemination by a flushing out of the uterine horns of superovulated cows with a Bioniche complete flush solution using a silicone two-way Foley catheter. The embryos of morula or early blastocyst stage were subjected to the two-step vitrification procedure using EFS vitrification solution (ethylene glycol - 40 % v/v, Ficoll 70 - 18 % w/v; 0.3 M sucrose in D-PBS + 20 % fetal calf serum and 5 $\mu g.ml^{-1}$ gentamycin), pulled into the open-pulled straws and slowly immersed into liquid nitrogen. Following thawing the embryos were cultured for 48 hours in order to reach advanced developmental stage (expanded blastocyst), afterwards these embryos were analyzed for the developmental rate, embryo cell number and incidence of the dead cells. About 83 % of all control embryos were developed to the expanded blastocyst stage, whilst in the frozen-thawed group only 60 % of embryos reached this stage ($p < 0.05$). Frozen-thawed embryos contained significantly less number of embryonal nuclei when compared with fresh embryos. Dead cell incidence (TUNEL-index) was more than twice higher in the frozen-thawed embryos (9.53 %) in comparison to the fresh embryos (4.32 %), but this value did not exceed 10 %, the critical value which may compromise the embryo viability. Our experiments confirm that cryopreservation may affect embryo viability. Therefore, monitoring of embryo quality following cryopreservation would provide a basis for understanding embryo sensitivity to freezing protocols and help to improve a cryopreservation technique.

**Key words:** embryo; blastocyst; morula; vitrification; apoptosis

## INTRODUCTION

Cryopreservation of preimplantation embryos is an important tool for maintaining animal genetic resources by increasing the usage of reproductive potential of genetically valuable animals. However, cryopreservation procedures may negatively affect survival of gametes and embryos as a consequence of damages to the cell and even cell death (apoptosis or necrosis). Determination of these influences may have a great importance for the explanation of failures in reproductive processes. Research in this area enables us to follow processes running at the cell level and factors regulating these processes.

Reliable information about embryo viability can be provided either by ultrastructural analysis at the level of electron microscopy or by embryo transfer to recipient females, but these methods may not be practically available in every laboratory. Cryotolerance may be a useful indicator of blastocyst quality (Rizos *et al.*, 2001). As a functional criteria for

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the evaluation of embryo viability after cryopreservation, a post-thaw cleavage up to blastocyst stage (Popelkova *et al*., 2005; Makarevich *et al.* 2008), embryo cell number and embryo diameter (Popelkova *et al.*, 2009), proliferation (PCNA) index (Markkula *et al*., 2001) or number of apoptotic (TUNEL) cells (Marquez-Alvarado *et al.*, 2004; Makarevich *et al.*, 2008) and the state of actin cytoskeleton (Tharasanit *et al.* 2005; Makarevich *et al.*, 2008) have been used. Exact knowledge of processes leading to damages and embryonal loss may help to find out approaches for the improvement of embryo quality and survivability following cryopreservation.

The aim of this study was to determine quality and viability of bovine embryos cryopreserved by vitrification procedure in comparison to intact (fresh) bovine embryos. The embryos following vitrification-warming were cultured for 48 hours in order to reach advanced developmental stage (expanded blastocyst), afterwards these embryos were analyzed for the developmental rate, total cell number and dead cell index.

## MATERIAL AND METHODS

**Preparation of cows for flushing, embryo recovery and evaluation**

The cows of Holstein breed were used as donors of embryos. Oestrus of the cows was synchronized by the injection of a $PGF_{2alfa}$ analogue (Oestrophan, Bioveta a.s., Ivanovice na Hane, Czech Republic). The cows were superovulated by application of porcine pituitary gonadotropin (Pluset®- FSHp-LHp, Laboratorios Callier, Barcelona, Spain) twice daily during 5 days at 8.00 and 20.00 hours (given in a decreasing dosage rate; starting with the doses of 150 IU FSH +150 IU LH in the morning at the 11th day to 50 IU FSH+50 IU LH in the evening at the 15th day) of the oestrous cycle. Oestrophan was administered for luteolysis at the 13th day. Insemination was performed two times by the same AI technician with frozen-thawed AI doses from one sire only at 12 hrs intervals started at 12 hrs after the standing oestrous detection.

Embryo recovery was performed on 7th day after the first insemination by a standard non-surgical technique to flush out the uterine horns. Uterine flushing was conducted with a complete flush solution (Bioniche, Belleville, Ontario, Canada) using a silicone two-way Foley catheter (Minitüb GmbH, Tiefenbach, Germany). Flushed ova/embryos were transferred to the holding medium - phosphate buffered solution (PBS) with 20 % foetal calf serum (FCS, Gibco BRL) and assessed using a stereomicroscope. The embryos were classified according to their stage of development as of good quality (i.e., morulas, early blastocysts) and of poor quality (i.e., fragmented/degenerated embryos, unfertilized oocytes). Only good quality embryos were used for a cryopreservation by vitrification procedure.

**Vitrification procedure**

The two-step vitrification procedure was used in our experiments. In the first step the embryos were equilibrated by 3 min incubation in the holding medium (D-PBS with 20 % FCS). In the second step the embryos were placed into the vitrification solution EFS (described by Kasai *et al.*, 1990), consisted of ethylene glycol (40 % v/v), Ficoll 70 (18 % w/v) and 0.3 M sucrose dissolved in a holding medium (D-PBS + 20 % FCS and 5 $\mu g.ml^{-1}$ gentamycin). The embryos were pulled into the open-pulled straw by touching of the narrowed tip of the straw to a microdrop of EFS solution according to Vajta *et al.* (1998) and then the straw was slowly immersed into liquid nitrogen.

**Warming of embryos and recovery**

After two weeks of storage in liquid nitrogen, the embryos were warmed by holding the straw at room temperature for 10 sec. Afterward, the straw with embryos was plunged into the drop with holding medium consisted of D-PBS, 20 % FCS and 0.3 M sucrose for 3 min. The embryos expelled off the straw, then washed twice in culture medium (B2 INRA medium with 10 % FCS and 5 $\mu g.ml^{-1}$ gentamycin) and placed in a fresh culture medium B2 INRA medium for 48 h incubation, until advanced blastocyst stage.

**Analysis of embryos for total cell number and dead cell index (TUNEL)**

Following 48 h culture the embryos were evaluated for the developmental stage and selected for analysis of cell number and dead cell index. The embryos were washed 3-times for 5 min in PBS-PVP washing solution (PBS with 4 $mg.ml^{-1}$ polyvinylpyrrolidone; Sigma-Aldrich Chemie, Steinheim, Germany). Then the embryos were fixed in 3.7 % neutrally buffered formalin (Fluka, Buchs, Switzerland) for 5 min and in 70 % ethanol for 10 min. Permeabilization was done by 15 min incubation of embryos in 0.5 % Triton X-100 in PBS. The embryos were processed for TUNEL using MEBSTAIN Direct Apoptosis Detection Kit (IM3171, Immunotech, Marseille, France) according to the product manual. Briefly, fixed and permeabilized embryos were incubated at 37 °C in TdT-labelling mixture (TdT buffer, FITC - dUTP a TdT) for 1 h. Following this incubation TUNEL-reaction was stopped by three-time washing of embryos in PBS-PVP solution. Thereafter the embryos were

transferred onto coverslip and covered with 5 µl of Vectashield anti-fade mounting medium, containing DAPI stain (Vector Laboratories, Burlingame, CA, USA). The coverslip was attached to microslide using nail polish. All treatments were performed at ambient temperature. The samples were stored at - 20 °C until fluorescence analysis.

TUNEL-indexes were determined on the basis of proportion of TUNEL-positive nuclei (green fluorescence) to total embryo cell number (DAPI-stained nuclei), which have been counted under the Leica fluorescent microscope (Leica Microsystems, Germany) using specific wave-length filters.

**Statistics**

The experiment was performed in 4 replications. A one-way ANOVA and Tukey-test were used to analyze differences in dead-cell index between groups. Since variable "TUNEL-index" was normally distributed, a log-transformation of original values has not been done. The hypothesis of normality was rejected for "Total cell number". For these two variables the log-transformation of original values was used. All calculations were performed using the SAS software package.

## RESULTS AND DISCUSSION

Totally 205 embryos were used in experiments. Both fresh and frozen-thawed embryos were cultured since the morula stage up to 48 hours, when it was expected that most of embryos can reach either blastocyst or expanded blastocyst stage. As it is shown in Table 1, about 83 % of all fresh embryos (control) were developed to the advanced blastocyst stage, whilst in the frozen-thawed group only 60 % of embryos reached this stage, and the difference between groups was statistically significant ($p < 0.05$). Frozen-thawed embryos contained significantly less number of embryonal nuclei when compared with fresh embryos. Dead-cell index calculated on the basis of the number of TUNEL-positive cells was more than twice higher in the frozen-thawed embryos in comparison to the fresh embryos (Table 1), but this value did not exceed 10 %, the critical value which may compromise the embryo viability.

Despite intensive research efforts various cryopreservation procedures still cause significant morphological and biochemical alterations, which may lead to cell death and loss of embryo viability. The loss of viability can be influenced by the type and concentration of cryoprotectant, the freezing protocol, animal species, developmental stage of the embryo and system of embryo production (Fabian *et al.*, 2005). In our study we used the embryos of cattle which were *in vivo* recovered from the hormonally superovulated cows at the stage of compact morula or early blastocyst. Such embryos were frozen by vitrification, where the vitrification solution contained, beside ethylene glycol, also ficoll 70 as cryoprotectant, which was previously validated in several reports. In particular, Ficoll 70 was previously successfully used as a cryoprotectant in many studies on cryopreservation of embryos of different animal species including rabbit (Kasai *et al*., 1992; Papis *et al*., 2005; Makarevich *et al.*, 2008) and cattle (Darvelid *et al.*, 1994; Mahmoudzadeh *et al.*, 1995; Nguyen *et al.*, 2000).

Developmental stage and morphological quality of frozen-thawed embryos are the primary parameters affecting their survival rate. Morula stage embryos following vitrification and warming were in our experiments post-cultured for two days; it is the time required to reach advanced blastocyst stage. About 60 % of vitrified embryos were developed to expanded blastocysts stage after thawing, what is less than the blastocyst rate in fresh control (83 %), but this value is in the standard range of embryo survival

**Table 1: Influence of vitrification-warming on the embryo viability**

| Groups | No. embryos, N | Developed to the advanced Bl stage, n (%) | Total cell number, n x ± S.E.M. | Dead cell (TUNEL) index, % x ± S.E.M. |
|---|---|---|---|---|
| Fresh (control) | 117 | 97 (82.91)[a] | 137 ± 7.50[a] | 4.32 ± 0.52[a] |
| Frozen-thawed | 88 | 53 (60.22)[b] | 122.7 ± 3.98[b] | 9.53 ± 1.49[b] |

[a] versus [b] – significant differences between groups at $p < 0.05$

rate after cryopreservation.

The number of cells and the extension of apoptosis are important parameters of embryo development and embryo health (Brison and Schultz, 1998). Total cell number in our experimental embryos (following freezing-thawing) was significantly less than that of the intact (control) embryos. This indicates that cryopreservation procedure inhibits proliferation of the embryo cells (127 cells per embryo versus 137 cells in control), however this inhibition is not crucial for further development of the embryo.

Apoptosis at the blastocyst stage eliminates cells that are damaged, excessive or no longer required or developmentally incompetent. Therefore, apoptosis can be considered to be a normal process in preimplantation embryos to eliminate deviating cells, but a high incidence of apoptotic cells is correlated with abnormal morphology of the embryo (Hardy *et al.*, 1999). In our experiments dead (TUNEL-positive) cells were observed both in cryopreserved and in intact (control embryos), however the incidence of dead cells (TUNEL-index) in embryos was different. In particular, cryopreserved embryos contained more than twice higher proportion of dead/apoptotic cells (9.5 %), than intact control (4.32 %). This difference can be explained by the fact that vitrification and/or thawing procedure can compromise the quality of the embryo by damaging their membranes and organelles and thereby by triggering protective mechanisms to eliminate damaged cells. The proportion of dead cells in the embryos following vitrification in our study does not exceed 10 %, what may suggest that this parameter is still within the physiological range and the embryos following vitrification are principally able to survive and develop further.

Similar results about the quality of vitrified embryos were obtained in the experiments with rabbit embryos (Chrenek *et al*., 2014) using the same cryopreservation medium (EFS). In particular, rabbit embryo development (expanded blastocyst rate) following vitrification was deteriorated from 96 % in the intact control to 72.6 % in the frozen-thawed embryos, even though those authors used, oppositely to bovine embryos, *in vivo* fertilized rabbit embryos (Chrenek *et al*., 2014). This report and our present experiments confirm that cryopreservation may affect embryo viability, as a consequence of the toxic action of cryoprotectants during vitrification or thawing procedure itself.

In conclusion, monitoring of embryo quality following cryopreservation would provide a basis for understanding embryo sensitivity to freezing protocol and will lead to improved cryopreservation technique. At the process of the creation of animal gene banks we should expect possible deteriorating effect of a cryopreservation procedure on the embryo survivability.

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# DEVELOPMENTAL POTENTIAL OF VITRIFIED RABBIT EMBRYOS

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## ABSTRACT

The aim of this study was to examine how vitrification procedure can affect further development of preimplantation rabbit embryos. We evaluated post-thaw development and quality of the embryos following vitrification using morphological characteristics and fluorescent markers of the cell viability. The pronuclear stage eggs were flushed from the oviducts of the New Zealand rabbit females at 19-20 h *post coitum* (hpc) and randomly divided into two groups: first - intended for vitrification (n = 135) and second - non-vitrified (intact) control (n = 135) eggs. Then zygotes were cultured for 72 h, afterwards the embryos of the first group, which reached morula stage, were frozen by vitrification in EFS medium (ethylene glycol, Ficoll70, sucrose), whilst the embryos (morula) of the second (control) group continued in the culture until 120 h (expanded blastocyst stage). Then all the embryos including intact control (n = 270) were processed for total cell number, differential staining and cell death (apoptosis). Following thawing almost 73 % of embryos survived and developed to advanced blastocyst stage versus 96 % in the intact control. In regards to the embryo quality, in the vitrified embryos total cell number (117 ± 36.0) was significantly lower than in the intact control (135 ± 30.2). However, there were no significant differences between the vitrified and intact embryos in the proportion of ICM (inner cell mass cells) to the total cell number and in the embryo diameter. On the other hand, the incidence of dead cells (apoptotic index) was almost twice higher in the embryos subjected to cryopreservation (4.21 ± 1.85) compared to control (2.08 ± 0.50). However, this incidence of dead cells in vitrified embryos is still in the physiological range, and does not compromise further development. Our observation indicates that rabbit embryos after freezing have only slightly altered viability and quality. Therefore, vitrification techniques tested in our study can be used for cryopreservation of embryos of national rabbit breeds for the purpose of long-term storage of embryo samples in the animal gene bank.

**Key words:** rabbit; embryo; vitrification; survival; apoptosis

## INTRODUCTION

Cryopreservation is an important tool for creation of embryo banks for future use in animal breeding, veterinary and human medicine. This technique also enables to protect germ cells of rare or endangered species and strains of farm and wild animals. In certain cases optimization of cryopreservation protocols for more sensitive embryos is relevant. Vitrification technique, introduced for rabbit embryo cryopreservation by Smorag *et al.* (1989) and Kobayashi *et al*. (1990), improved general efficiency of embryo survival. The efficiency of rabbit embryo vitrification evaluated *in vitro* (Papis *et al*., 1993) and/or in vivo (Kasai *et al.*, 1992) depends partly on resistant morula stage embryos obtained from outbreed strains of rabbits.

Rabbit embryos have been successfully cryopreserved either by conventional slow freezing (Naik *et al.*, 2005), classical one-step or two-step vitrification (Kasai *et al*., 1992; Kauffman *et al.*, 1998; Silvestre *et al.*, 2003; Naik *et al.*, 2005], open pulled straw (OPS) vitrification (Naik *et al.*, 2005) or modified (sealed) OPS procedure (López-Béjar and

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López-Gatius, 2000). Cryopreservation of rabbit embryos at morula (Silvestre *et al.*, 2003; Naik *et al*., 2005), blastocyst (López-Béjar and López-Gatius, 2000) and zona-free expanded or hatching blastocyst stages were reported (Cervera and Garcia-Ximenez, 2003), but higher rates of development were achieved when blastocyst (but not morula) stage embryos were used.

Since long-term storage of biological material at ultra-low temperatures is an effective tool for creating and maintaining of animal gene banks, the aim of this short study was to examine how vitrification procedure can affect further development of preimplantation rabbit embryos. We evaluated developmental potential and quality of the embryos following vitrification using morphological characteristics and fluorescent markers of the cell viability.

## MATERIAL AND METHODS

**Egg collection**

New Zealand White rabbit does (4-6 month old), kept on the local farm were superovulated using a single *i.m.* injection of 20 IU.kg$^{-1}$ PMSG (Werfaser, Alvetra und WERFFT, Wien, Austria). The females were given 40 IU.kg$^{-1}$ hCG (Werfachor, Alvetra und WERFFT) *i.m.* 72 h after PMSG administration and does were mated with fertile bucks belonging to the same breed. The pronuclear stage eggs were flushed from the oviducts of the slaughtered animals with PBS supplemented with 5 % fetal calf serum (FCS) both from Gibco BRL (Auckland, New Zealand) 19 to 20 h *post coitum* (hpc). Recovered eggs were evaluated morphologically and the eggs with pronuclei, two polar bodies and compact cytoplasm were selected and randomly divided into two groups: 1) intended for vitrification and 2) non-vitrified (intact) control eggs. Then zygotes were cultured in the $CO_2$ incubator, and after 72 h the embryos of the first group, which reached morula stage, were frozen by vitrification, whilst the embryos (morula) of the second (control) group continued in the culture until 120 h (expanded blastocyst stage).

**Procedure of vitrification-devitrification**

For the vitrification an EFS medium based on a $CO_2$-independent medium (CIM, Gibco BRL) supplemented with 20 % FCS and composed of 40 % EG, 18 % Ficoll and 0.3M sucrose was used. The morula stage embryos (5-8) were equilibrated in EFS medium for 3-4 min at room temperature and then sucked up into 0.25 ml straw in EFS medium. Straws were melted on the tip, exposed for 60 sec to vapour of liquid nitrogen and then plunged into liquid nitrogen.

The embryos were devitrified by keeping the straw on the air for 10 sec and then plunging to water bath at 20 ºC for 7 sec. Then the content of the straw was released into 0.5 M sucrose and after 7-8 min embryos were transferred to CIM medium added with FCS in order to be washed out of the cryoprotectant. After warming the embryos were cultured up to expanded blastocyst (48 h) stage when a developmental rate (at 120 hpc) was determined. All embryos including intact control were processed for total cell number measurement, differential staining of cell compartments (ICM, TE) and cell death (apoptosis) detection.

**Assays of embryo viability**

Total cell number was counted after staining of the embryos with DAPI fluorochrome. Number of ICM cells was counted after the differential staining of blastocysts for ICM and TE cells as described earlier (Chrenek *et al.*, 2011). Apoptotic index was determined by TUNEL-reaction using a MEBSTAIN direct apoptosis kit (Immunotech, Marseille, France). Embryo diameters, excepting zona pellucida, were measured from the same images on the screen of the monitor using a scale bar micrometer (Leica, Germany), which was previously calibrated on a ×10 or ×20 objective and ×10 eyepiece. Total cell number, apoptotic index, and number of inner cell mass (ICM) cells were counted from the embryo images acquired on a Leica fluorescent microscope using appropriate wavelength fluorescent filters.

**Statistics**

Development of rabbit embryos up to blastocyst stage was analyzed using the Chi-square test. Differences between groups in total cell number, ICM cell number and TUNEL index were analyzed using analysis of variance (ANOVA).

## RESULTS AND DISCUSSION

In our study totally 270 rabbit embryos were used, of them 135 were vitrified at the morula stage and the other 135 were cultured further until the blastocyst stage serving as a control (Table 1). Earlier reports (Fabian *et al.*, 2005; Popelkova *et al.*, 2005) documented that post-thaw embryo survival can be a valid tool to evaluate the efficiency of cryopreservation technique. After cryostorage and devitrification almost 73 % of embryos survived and developed to advanced blastocyst stage versus 96 % in the intact control.

In regards to the embryo quality, in the vitrified embryos total cell number was significantly lower than in the intact control. However, there were no significant differences between the vitrified and intact

**Table 1: Survival and quality of vitrified or intact rabbit embryos**

| Group of embryos | Total no. embryos, N | Post-thaw development to expanded blastocyst, n (%) | Embryo quality | | | |
|---|---|---|---|---|---|---|
| | | | Total cell. number (n) x ± SD | Number of ICM cells/ embryo, N, x ± SD (%) | Embryo diameter (μm), x ± SD | Apoptotic index, % x ± SD |
| Control | 135 | 130 (96.0)[a] | 135 ± 30.2[d] | 37 ± 7.5 (32.0) | 129.85 ± 10.9 | 2.08 ± 0.50[e] |
| Vitrified | 135 | 98 (72.6)[b] | 117 ± 36.0[c] | 32 ± 6.5 (23.7) | 123.2 ± 7.2 | 4.21 ± 1.85[f] |

Values with different superscripts within columns are significantly different ($p < 0.05$).

embryos in the proportion of ICM (inner cell mass cells) to the total cell number and in the embryo diameter. On the other hand, the incidence of dead cells (apoptotic index) was almost twice higher in the embryos subjected to cryopreservation (Table 1) compared to control.

Our observation indicates that rabbit embryos after freezing have altered their viability and quality, though not all studied parameters were affected by cryopreservation procedure. The more affected parameters were the total cell number of blastocysts and the dead cell index. This difference between vitrified and intact embryos can be explained by their different dynamics of the development: embryos following devitrification need some time to recover from the deep freezing and, therefore, have the delayed proliferation and the lower cell number compared to the intact embryos.

The number of cells and the extension of apoptosis are important parameters of embryo development and health (Brison and Schultz, 1998). Apoptosis at the blastocyst stage eliminates cells that are damaged, excessive or no longer required or developmentally incompetent. Therefore, apoptosis can be considered to be a normal process in preimplantation embryos to eliminate deviating cells, but a high incidence of apoptotic cells is correlated with abnormal morphology of the embryo (Hardy, 1999). In our study, increased apoptotic index in vitrified embryos evidences that some cells were damaged following the process of devitrification, however, this percentage of dead cells in these embryos (4.21 %) is still in the physiological range. This ratio of dead cells is lower compared to the previous study (Makarevich *et al.*, 2008), where, depending on vitrification protocol used, the proportion of dead cell was 7.5 % and 10.3 %, respectively.

In conclusion, the vitrification procedure using in our study (EG+ Ficoll70+ Sucrose) only slightly altered post-thaw survival, so that embryo viability and quality was not affected seriously. Therefore, this vitrification technique can be used for cryopreservation of embryos of national rabbit breeds (Zoborsky and Nitriansky) for the purpose of long-term storage of embryo samples in animal gene bank.

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## ANIMAL RESEARCH AND CHALLENGES AND NEEDS OF AGRICULTURE

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Animal research has led to many advances in science. Agriculture has been an important beneficiary, where research on genetics, molecular biology, physiology, ethology and some other fields on animal sciences has brought about modern, highly productive species at a lower cost, lending directly improvement in human nutrition. Nowadays, agriculture and research are facing new challenges. Agriculture shall not only provide foods in sufficient quantity and quality but is also intrinsically linked to major societal concerns. For instance, these activities play an essential role in the development of sustainable production systems, preserving resources for future generations while maintain production and meeting societal concerns such as the quality of life of producers and in case of husbandry systems, the welfare of farm animals. Agriculture takes part in this process whereby the sustainability of a production system comprises economic efficiency preservation of the environment and social acceptability for farmers, consumers and citizens. The aims and objectives of future research involving animals will be different. The newest goals will involve the close integration of animal production with the environment. These goals of quality and sustainability will also be achieved by applying the methods and results of the genomic era. High resolution gene maps and markers will boost selection for multiple criteria; marker genes can be monitored to better understand function of the physiological or production traits of animal organism.

Topics were divided into five priority areas: food, animal production, animal health, animal as model in biomedical research and creation of integrated modern experimental techniques that use up-to-date molecular technology. The needs for research can be viewed at a number of levels (Oldham, 2010):

- *Research for better predictive understanding of animal biology*.
  This might be phased as research to enable the prediction of phenotypic expression. Through such research should come, for example, improved abilities to optimise reproductive efficiency, resistance to disease, efficiency of feed conversion.
- *Research for better predictive abilities to manage resource use efficiency*.
  Such research goes beyond simply understanding how animals work as biological entities and embraces collateral understanding of the resource costs of managing animals for efficient biological performance. Within this would be induced the management of feed supplies and pastures, greenhouse gas emissions, control systems for disease reduction or eradication, housing and land use management, energy costs of systems of management etc.
- *Research on systems of land and supply chains that include farm animals.*
  This would include all aspects of land management systems of which farm animals are a part. Consequences for land use, water quality, biodiversity, communities etc. would be included.
- *Research that uses animals as biological models for other species, including man.*
  As a consequence of indisputable ethical considerations in humans, the animal models are of critical importance for biomedical research and especially in the field of perinatal. Many scientific progresses past, present and future in human medicine would have been and still are impossible to obtain without the use of animal models. For animal genetics, physiology, health and welfare, the main objectives are to gain basic knowledge of animal nutrition, physiological background for digestion and metabolism of nutrients, and also basic knowledge of animal immunology with the aim of improving disease resistant and health.

There are new fields existed in animal experiments, such as systemic biology and synthetic biology. High phenotypic rate of animal traits should also be organised and shared to optimise the development and interpretation of genomic data.

Science–based innovation support requires excellence in knowledge generation and procurement (research), knowledge transfer (dissemination activity) and knowledge absorption (education and training). This is particularly relevant to the future needs for research on animals in view of ongoing developments in specialised techniques and methodologies.

**Key words:** animals; sustainability; research; challenges; science innovation; needs

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## TRANSITION PROBLEM OF DAIRY COWS

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During the last decades, modern dairy farming has been marked by a trend towards intensification. While the total numbers of herds and cows have steadily declined, the average number of cows per herd and the average milk yield per cow have increased. The increasing production level per cow is the result of intensive genetic selection as well as improvements in dairy cow nutrition, housing and management. However, the economic advantage of higher yield is often overshadowed by metabolic (rumen acidosis, milk fever, liver lipidosis, ketosis, oxidative stress, displacement of abomasi etc.), inflammatory (laminitis, mastitis, metritis etc.) and reproductive (retained placenta, decreasing conception rate, extension of the calving interval etc.) disorders. The majority of all diseases occurs during 3 weeks before to 3 weeks after parturition (transition or peripartal period). The onset of lactation is generally characterised by a negative energy balance, due to a drastic increase in energy requirements for milk yield and a simultaneous depression in dry mater intake. Priorization of milk yield over maternal body functions is an universal biological strategy in all lactating mammals to buffer

the newborns nutrition from fluctuations in the dam´s energetic status. In case of an energy deficiency, the dam will mobilize fat and protein reserves in order to safeguard milk yield. Chronically elevated concentrations of non-esterified fatty acids and ketone bodies affect multiple organ systems including the immune system, reproductive axis and liver.

**Key words:** dairy cows; transition problem

## ISG12 IS A CRITICAL MODULATOR OF INNATE IMMUNE RESPONSES IN MURINE MODELS OF SEPSIS

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Sepsis is still a major burden for our society with high incidence of morbidity and mortality each year. Molecular mechanisms underlying the systemic inflammatory response syndrome (SIRS) associated with sepsis are still poor defined and most therapies developed to target the acute inflammatory component of the disease are insufficient. Recently the role of nuclear receptors (NRs) became a major topic of interest in transcriptional regulation of inflammatory processes. Nuclear receptors, such as the peroxisome proliferators-activated receptors (PPARs), have been demonstrated to exert anti-inflammatory properties by interfering with the NFκB pathway.

In our previous work (Papac-Milicevic *et al*., 2012) we identified the nuclear envelope protein, interferon stimulated gene 12 (ISG12), which directly interacts with NRs. ISG12 is a co-factor stimulating nuclear export of NRs, thereby reducing the anti-inflammatory potential of NRs such as NR4A1. Applying two models of vascular injury we could show that ISG12-deficient mice are protected from neo intimae formation.

To examine the role of ISG12 in acute inflammatory processes we used these recently generated ISG12-deficient mice and analyzed the effects of ISG12-deficiency in a model of endotoxemia and of experimental sepsis. We can clearly demonstrate that lack of ISG12 prolongs survival in endotoxemia and experimental sepsis. Furthermore, we can show that several acute inflammatory parameters, such as systemic IL6 cytokine levels, are downregulated in endotoxemic and in septic ISG12−/− animals. Consistently, similar results were obtained in *in vitro* experiments with peritoneal macrophages derived from ISG12-deficient mice. In contrast, mice deficient for the nuclear receptor NR4A1 exhibited an exacerbated innate immune response, and showed a significantly higher mortality after lethal endotoxemic challenge. This dramatic phenotype could be restored in ISG12/NR4A1 double deficient mice. We conclude from our *in vitro* and *in vivo* data that ISG12 is a novel modulator of innate immune responses regulating anti-inflammatory nuclear receptors such as NR4A1.

**Key words:** ISG12; innate immunity; sepsis

## EFFECT OF AN EXTENDER ON RESISTANCE OF BOVINE SEMEN AGAINST COLD SHOCK AND ITS SURVIVABILITY

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The aim of the study was to determine the effect of an extender on resistance of spermatozoa to cold shock and on short-term survivability during the cold test. The hypothesis was the assumption that the composition of different extenders, especially the presence of egg yolk, or LDL (low-density lipoprotein) cholesterol may positively affect the sperm resistance to the cold shock and ensures a higher survivability of spermatozoa during the short-term cold survival test.

Four bulls of the same breed and age and from the same A.I. centre were used. A total of 8 semen samples were examined. Each ejaculate was divided into 12 aliquots (3 controls and 9 experimental samples). Three commercially available extenders (AndroMed®, Bioxcell®, and Triladyl®) were used, each in standard or LDL enriched variants. In case of AndroMed® or Bioxcell®, 4 %, 6 % and/or 8 % of LDL were added. In case of Triladyl®, 6 %, 8 % and/or 10 % of LDL replaced the standard egg yolk component. Resistance of spermatozoa against cold shock (0 °C, 10 minutes) was evaluated by the percentage rate of live sperm using eosin-nigrosine staining immediately and 2 hours after the heat incubation (37 °C).

The results revealed the individual influence of a bull as an important factor. Among extenders tested, it is possible to recommend AndroMed® and Bioxcell® supplied with LDL cholesterol at 8 % concentration, since these extenders showed significantly ($P < 0.05 – 0.01$) lower decline of live sperm proportion during the cold shock test, when compared to Triladyl® with 8 % of LDL cholesterol (-13.24 %, respectively -8.79 %).

**Key words:** semen; extender; cold shock; sperm survival

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## PREIMPLANTATION MATERNAL RESTRAINT STRESS AND ITS NEGATIVE EFFECT ON MOUSE DEVELOPMENT

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The aim of the present study was to evaluate impact of maternal restraint stress on mouse embryo development and offspring behavior.

We exposed spontaneously ovulating and naturally fertilized female mice to restraint stress for 30 min three times a day during the preimplantation period of embryo development. Maternal corticosterone was measured using a commercially available EIA kit, according to the manufacturer's instructions (Corticosterone EIA kit, Enzo Life Sciences). Blastocysts isolated on day 4 of pregnancy from control and stressed dams were differentially stained using rabbit anti-mouse CDX2 polyclonal antibody, Texas Red-X goat anti-rabbit IgG and Hoechst 33342. Offspring were scanned with EchoMRI (Whole Body Composition Analyzer) for evaluation of the exact amount of body fat deposits. Behavior of delivered mice was tested in the open field (60 x 45 x 36 cm) for 5 min. Trajectories and actions of animals were recorded by CCD camera and evaluated by Ethovision XT 7.0.

Our results showed that maternal restraint stress significantly decreased maternal body weight ($P<0.001$), increased corticosterone concentration in blood plasma ($P<0.001$), slowed down transition of embryos from the oviduct to the uterus ($P<0.01$), negatively influenced stage-specific distribution of isolated embryos ($P<0.001$) and decreased the mean cell number in blastocysts ($P<0.001$). Furthermore, reduction of the cell number in the TE was observed, resulting in increased ICM: TE ratio, when compared to control ($P<0.05$). Restraint stress reduced number of implantation sites in uteri ($P<0.05$), significantly delayed eyes opening in delivered mice ($P<0.001$) and altered their behavior in two parameters (scratching on the base of apparatus, $P<0.01$; time spent in central zone, $P<0.05$) as well. Moreover, prenatally stressed offspring had significantly lower body weight (females: $P<0.001$; males: $P<0.05$), and fat deposits in stressed female was significantly lower ($P<0.05$).

Our results indicate that stress exposure during very early pregnancy can negatively influence the developmental capacity of preimplantation embryos, decrease the ability to conceive, and even influence maturation of nervous system and alter offspring behavior. Thus, the impact of exposure to psychical stress during very early pregnancy should be regarded as a potential risk factor in animal and human reproduction.

**Key words:** maternal restraint stress; corticosterone; blastocyst; open field

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## THE EFFECT OF MATERNAL BODY CONDITION ON *IN VIVO* PRODUCTION OF OOCYTES AND EMBRYOS IN MICE

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The study investigates the effect of altered maternal body condition on oocyte quality and zygote and blastocyst production.

A two-generation experimental model, based on overfeeding of outbred mice during intrauterine and early postnatal development, was used to produce four types of females: with physiological (7-8 %), slightly elevated (8-11 %), highly elevated (> 11 %) and low (< 7 %) amount of body fat. At 5 weeks of age, spontaneously ovulating females from all groups were mated with males and randomly selected for oocyte/zygote isolation, embryo isolation or delivery of pups.

There were no significant differences in the majority of evaluated parameters between normal controls and experimental mice with slightly elevated amount of the body fat. Moderately obese dams showed slightly increased number of ovulated oocytes *per* mice; however, the average number of delivered pups remained unaffected. Obesity-like phenotype in dams with highly elevated amount of body fat was accompanied predominantly with negative effects: increased number of isolated immature oocytes and degenerates, significantly lowered deposits of neutral lipids in the cytoplasm of normal isolates (Nile Red staining), later onset of active demethylation in male pronuclei of zygotes (5-methylcytosine IHC staining), increased incidence of apoptosis in blastocysts (TUNEL assay) and lowered weight of newborns (EchoMRI). The majority of evaluated parameters were impaired also in group of animals spontaneously displaying lean body condition: in dams with decreased amount of the body fat lower fertilization index, later onset of active demethylation in male pronuclei of zygotes, increased incidence of apoptosis in blastocysts and lower weight of newborns were recorded.

We might conclude that alterations in maternal body condition might affect reproductive process at several steps, including the period of ovulation, fertilization and early embryo development *in vivo*. The effect of obesity-like phenotype is dependent on its level (i.e. amount of maternal body fat deposits) and it might impact particular reproductive parameters in opposite manner. Nevertheless, generally, negative effect dominates.

**Key words:** obesity; undernutrition; oocyte quality; fertilization; embryo quality

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## THE RESPONSE TO THE SUPEROVULATION IN COWS WITH DIFFERENT INBREEDING LEVEL

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The aim of this study was evaluation of inbreeding effect on the superovulation response - number of corpora lutea (CL) in Holstein cows. The data included observation of 39 cows, from which 13 were inbred and 26 non-inbred. The study was performed on the farm in the Czech Republic during the year 2011. The oestrus of the cows was synchronized by injection of a PGF2-alfa analogue – Oestrophan (Bioveta a.s., Ivanovice na Hane, Czech Republic). The cows were superovulated by application of porcine pituitary gonadotropin

(Pluset®- FSHp-LHp, Laboratorios Callier, Barcelona, Spain) twice daily for 5 days at 8.00 and 20.00 hours given in a decreasing dosage rate: starting with doses of 150 IU FSH +150 IU LH in the morning on day 11 to 50 IU FSH+50 IU LH in the evening on day 15 of the oestrous cycle. This study involved only the cows with a complete pedigree to the fifth generation. The cows were divided into 2 groups according to $F_X$ level (first group, non-inbred $F_X = 0$; second group, inbred cows $F_X \geq 1.56$ %). The level of inbreeding – (inbreeding coefficient $F_X$) was calculated according to Wright (1922) and was in the range of $F_X = 1.56$-6.25 %. Average value of $F_X$ coefficient in inbred cows was 3.15 %. The number of CL was evaluated in monitored superovulated cows and basic statistic was calculated. The following results were obtained comparing non-inbred vs. inbred cows (in pcs.): average number of CL 7.5 vs. 5.46; standard deviation 4.36 vs. 4.56; minimum pcs. 0 vs. 0; maximum pcs. 17 vs. 13. Differences at $p < 0.01$ were considered as statistically significant. Results of this study show that inbred cows ($F_X \geq 1.56$ %) manifested worse superovulation response and, thus, had lower count of corpora lutea compared to the non-inbred cows ($F_X = 0$).

**Key words:** cow; inbreeding; superovulation; corpus luteum

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## USE THE MICRONUCLEUS ASSAY FOR EVALUATION OF WHITE MISTLETOE TOXICITY IN MOUSE BONE MARROW CELLS

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*Viscum album* L. (further *V. album*) is a semiparasitic plant, widely used for the treatment of many disease states in traditional and complementary therapy. The scoring of micronuclei (MN) is often used as a surrogate biomarker of chromosomal damage inflicted by clastogenic agents or by aneugens. In this study, we evaluated the frequency of MN in bone marrow erythrocytes of the ICR strain of female white mice *Mus musculus var. album*, treated with white mistletoe *V. album* extracts. Experimental animals were admistered with the 0.25 %, 0,5 %, and 1 % leaves extracts of V. album via the subcutaneous route (s.c.) and kept at a temperature of 8 °C. The mice were observed for 24 hours. Acute effects of *V. album* leaves at the concentration 0.25 %/24 h/mouse caused a significant increase in the MN frequency with polychromatic erythrocytes 18.60 ± 6.35/ 1000 cells in comparison to the control group of animals 11.20 ± 3.19/1000 cells ($P = 0.048$, Anova test; $P < 0.05$ Tukey test). The frequency of MN with normochromatic erythrocytes in the experimental group was not statistically significant ($P = 0.384$; Anova- test) at given concentration, when compared to the control group of animals. The effects of 0.5 %/24 h/mouse were genotoxic in mice, because induced an increase in frequency of micronucleated polychromatic erythrocytes (18.80 ± 5.02 against control 11.20 ± 3.19 micronuclei/1000 cells; $P = 0.021$, Anova-test; $P < 0.05$, Tukey test). After dosing the *V. album* at a concentration of 1.0 % extracts at s.c. administration and at temperature of 8 °C the mice were alive for the maximum of 470 minutes. This experimental study shows the genotoxic effect of *V. album* extracts at the certain concentrations.

**Key words:** *Viscum album*; mice; micronuclei; bone marrow cells; genotoxicity

## BACTERIOCIN SUBSTANCE - DURANCIN ED26E7 AND ITS EFFECT IN RABBITS

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Bacteriocins are natural proteinaceous substances dominantly possessing antimicrobial effect. As it is known from our previous studies, they can also beneficially influence the unspecific immunity by the increase of phagocytic activity (PA) or weight gain of food-derived animals. Among those, rabbits are frequently bred because of the nutritional aspect of their meat. Health status of the animals can be positively influenced by natural substances. Basing on our previous work with the beneficial use of bacteriocins-enterocins produced by the strains of *Enterococcus faecium*, this study was focused on durancin-like ED26E7 produced by *E. durans* ED26E7 from sheep cheese. The rabbits of meat line M91, maternal albino tic line (crossbreed New Zealand white, Buskatt rabbit, French silver) and paternal acromalictin line (crossbreed Nitras rabbit, Californian rabbit, Big light silver) were used. Rabbits (n = 48, housed individually in cages) were divided into 2 groups; experimental (E) and control (C), 24 rabbits per each. Common breeding regimen was a cycle of 16 h of light, 8 h of dark; temperature 16 ± 4 ºC, humidity 70 ± 5 %. The animals fed commercial mixture (Vráble-Nitra a.s.) *ad libitum* with free access to water. Durancin-like ED26E7 (50µl/animal/day) was applied into water of E animals for 21 days. The sampling was done at the start of the experiment, on day 21 and 42 (the end of the experiment). The animals were slaughtered and caeca and appendix were sampled similarly to blood. The counts of controlled bacteria in faeces were not affected. In caecum and appendix, the reduction of coliforms on day 21 was found with difference 2.67 log cycle and 1.80 in E comparing with C. In appendix also clostridia were reduced (1.33 log cycle, 0.42). PA was significantly increased ($P<0.001$) on day 21 and 42 ($P<0.01$). GPx values and biochemical parameters or weight gain were not affected. *Eimeria* sp. also were not influenced. In E no *Passarulus ambiguis* (nematoda) was found, whilst in C it was 50.0 ± 7.07.

**Key words:** rabbits; bacteriocin-durancin; benefity; effect

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## EFFECT OF DIETARY MILK THISTLE (*SILYBUM MARIANUM*) EXTRACT ON FATTENING PERFORMANCE AND HEALTH STATUS IN BROILER RABBITS

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Milk thistle (*Silybum marianum*) extract called silymarin consists of flavolignans - silybin (the most bioactive component - 70-80 % silymarin), silychristin, silydianin, and isosilybin. The increase in the productive and reproductive performance and the health status improvement of livestock were noted with the silymarin diet supplementation. The proportion of silymarin components depends on the source, extraction and processing procedures. The aim of this study was to compare the influence of the different concentrations and processing methods (non-fermented vs. fermented) of *Silybum marianum* fruit extract on the fattening performance in broiler rabbits and their health status. There were 120 HYLA broiler rabbits divided into four groups. Animals were fed by a standard diet for rabbit fattening without any supplementation (group I), and with the *Silybum marianum* fruit extract supplementation at 0.2 % for group II (non-fermented), 1 % for group III (non-fermented) and 0.5 % for group IV (fermented). The experiment started at 42 days of rabbit age with average live weight 1330.6 g and finished by the achievement of 2600 g of live weight. At average, the fattening period of rabbits lasted 32 days. There were not any statistical significant differences found between the experimental and control groups in both parameters - the growth performance and the carcass yield. The morbidity and the mortality were lower in groups with *Silybum marianum* fruit extract supplementation, with the best results for group III and IV. These results demonstrated, that *Silybum marianum* fruit extract supplemented in the amount of 1 % could be a suitable substitution for chemical drugs commonly used in broiler rabbit fattening, and the process of fruit fermentation increases the availability of milk thistle active substances.

**Key words:** *Silybum marianum*; broiler rabbits; growth performance; health status

## ASSESSMENT OF THE EFFECTS OF AMYGDALIN ON REPRODUCTIVE FUNCTIONS

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Amygdalin is a controversial anti-tumor natural substance occurring in the seeds of apricots, bitter almonds, apples and other rosaceous plants. Its anticancer, anti-inflammatory activity and other medicinal benefits have been known for many years. The aim of the presented *in vivo* study was to observe the effect of amygdalin in two different forms (as a pure powder and as a bioactive compound of apricot seeds) on steroidogenesis of rabbits, as a model organism. Adult experimental females (n = 40, age: 150 days) were divided into 5 groups: control without amygdalin addition, two experimental groups were injected intramuscularly (IM) by pure amygdalin doses (0.6 and 3.0 mg/kg b.w.) during 4 weeks period and other two experimental groups were fed by apricot seeds (at dose of 60 and 300 mg/kg b.w.) added to the feed during the same period. After 4 week period the blood was collected and steroid hormones were assayed by ELISA. Obtained results showed no significant ($P \geq 0.05$) changes between control and experimental groups in progesterone plasma levels after 4 weeks of amygdalin exposure. Similarly, after 4 weeks of amygdalin addition no statistical differences in plasma levels of 17-β-estradiol were observed. In conclusion, amygdalin, as an anti-tumor compound, did not cause any changes in plasma levels of the selected steroid hormones.

**Key words:** amygdalin; apricot seeds; rabbits; steroid hormones

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## *ENTEROCOCCUS FAECIUM* CCM7420, BACTERIOCIN-PRODUCING AND PROBIOTIC STRAIN AND ITS BENEFIT IN RABBITS

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The influence of bacteriocin-producing strain with probiotic character *E. faecium* CCM 7420 (EF 2019 – our isolate is deposed to CCM) on colonization, faecal microbiota, *Eimeria* sp. occurence, phagocytic activity of leucocytes and morphometry (a parameter of the intestinal integrity and stability in the jejunum) of rabbits were tested. CCM7420 strain ($10^9$ cfu/ml; 500 µl/animal/day) was applied to drinking water for 21 days. Rabbits were in good health condition throughout the experiment. CCM7420 reached the value 1.00 ± 0.20 log10 cfu/g (day 21, 42) which was stable through the whole experiment. Enterococci were detected at higher counts, whilst the occurrence of *Clostridium*-like bacteria was not influenced. The stimulation of non-specific immunity (higher phagocytic activity of leucocytes, $p<0.001$) by CCM7420 and the anticoccidial effect (decreased levels of *Eimeria* sp. oocysts) of CCM7420 strain were also confirmed. The increase in resorption surface and functionality of intestinal mucosa was recorded by the increase in villus height:crypt depth ratio in rabbits administering CCM7420 (3.83) compared to control samples (3.73). CCM7420 strain showed beneficial effect on health and microflora of rabbits; mainly on the non-specific immunity and morphometry of jejunum in rabbits. Further studies to expand knowledge about mucosal stability and immunity are in process.

**Key words:** rabbit; probiotic; bacteriocin; application; microorganisms; morphometry; phagocytosis

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## THE EFFECT OF YUCCA SCHIDIGERA PLANT EXTRACT ON RABBIT EMBRYO DEVELOPMENT *IN VITRO*

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The aim of this study was to investigate the effect of *Yucca schidigera* plant extract added at different concentrations (0.1 mg/ml - E1 group; 0.01 mg/ml - E2 group; 0.001 mg/ml -E3 group) to the culture medium on the rabbit embryo development *in vitro* (percentage of morula, expanded blastocysts and total cell number per embryo). All embryos were flushed from the rabbit oviducts at the one-cell stage and cultured under *in vitro* conditions (38.5 °C, 5 % $CO_2$) in k-DMEM medium supplemented with 10 % of fetal bovine serum. No significant differences were found in the developmental potency of embryos when compared to control (C; 94.0 ± 24.0) and experimental (E1 - 59.4 ± 22.0; E2 - 81.4 ± 32.8; E3 - 76.2 ± 23.9) groups. However, slightly higher proportion of blastocyst stage embryos was found in the E2 group (50.0 %) compared to control (41.7 %). In conclusion, no significant effect of the addition of *Yucca schidigera* extract into culture media on rabbit embryo development *in vitro* was observed. Nevertheless, lower concentrations of *Yucca schidigera* extract (0.01, 0.001 mg/ml) seem to be more effective than higher concentration, as the more embryos reached blastocyst stage in these groups.

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

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## DETECTION OF RABBIT SPERMIOPHAGES

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Macrophages, engaging in sperm phagocytosis (spermiophages), are occasionally reported, though their relevance in the evaluation of rabbit semen is unknown. It has been suggested that spermiophages may be present under normal physiological conditions to allow the phagocytic elimination of dead or dying sperm. On the other hand, they have the ability to damage sperm via the generation of reactive oxygen species and the induction of apoptosis. This preliminary study attempts to establish a method to evaluate spermiophages in rabbit semen and to examine the effect of macrophages on semen quality parameters. In our experiments 15 New Zealand White rabbit males were used. Semen samples from rabbit males were collected using an artificial vagina. We investigated the possibility of detecting spermiophages in the rabbit semen. The ultrastructural analysis provided qualitative characteristics, whilst sensitive quantitative tests should better define the association between semen macrophages and semen parameters. We compared the detection of macrophages using either the fluorescent dye Alexa-AcLDL (Acetylated Low Density Lipoprotein) or the monoclonal antibody CD14 (TUK4). When the LDL has been acetylated, the LDL complex no longer binds to the LDL receptor, but rather is taken up by macrophages that possess “scavenger” receptors specific for modified LDL. The fluorescence output by AcLDL of spermiophages was detected under a Leica fluorescent microscope (Leica Microsystem, Germany). Macrophages were also identified by the CD14 antibody using flow cytometry. Flow cytometry was used also for the Yo-Pro-1/PI analysis to count the percentage of apoptotic and necrotic sperm. Flow cytometry using monoclonal antibodies seems be a simple, reproducible method that enables detection of macrophages in rabbit semen and also is a tool that may be used to monitor many markers of sperm function.

**Key words:** rabbit; spermiophages; flow cytometry; apoptosis

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**INTRAVAGINAL APPLICATION OF BIOLOGICALLY ACTIVE SUBSTANCES AND ITS EFFECT ON FERTILITY OF RABBITS**

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The aim of the study was to examine intravaginal (IV) application of biologically active substances, included into the rabbit insemination dose, on selected reproduction parameters. This study was performed in two experiments – *in vivo* and *in vitro*. In the *in vivo* experiment we observed intravaginal application of biologically active substances on kindling rate and number of total born kits. The experiments *in vivo* were carried out on adult female rabbits. All females were artificially inseminated (A.I.) by fresh heterospermic semen doses (0.5 ml per one female). Each female was applied with 25 I.U. PMSG i.m. 48 hours before A.I. All insemination doses (I.D.) were diluted by insemination extender for rabbit sperm with antibiotic (MiniTüb, Germany), which was diluted in apyrogenic water (MiniTüb, Germany). Concentration of spermatozoa varied from 24.0 to 95.2 x $10^6$ per insemination dose.
The animals were divided into four groups: group C (control) with intramuscular application (2.5µg per doe) of GnRH - Lecirelin (Supergestran, Czech Republic) immediately after the A.I., group H with intravaginal application of heparin (0.06 µl per doe) and intramuscular application of GnRH (2.5 µg per doe), the third group G with intravaginal application of GnRH – Lecirelin (7.5 µg per doe) and finally group D with intravaginal application of 1.75M DMSO (dimethylsulfoxide, Applichem, Germany) and intramuscular application of GnRH (2.5 µg per doe).
In the second experiment (*in vitro*) we analyzed the influence of intravaginal application of GnRH and heparin on total number of oocytes and zygotes, number of zygotes with two pronuclei and number of unfertilized oocytes. Significance of differences among groups was determined by t-test and $\chi 2$ test. The highest kindling rate (89.29 %) and number of total born kits (10.64 ± 3.41) were obtained in group H. IV application of GnRH and heparin positively influenced the number of zygotes with two pronuclei and decreased the number of unfertilized oocytes. However, these differences were not significant compared with group C. IV application of GnRH affected the number of zygotes with 2-blastomere stage at 18-20 h after artificial insemination, too. This is experimental evidence for the earlier interaction of sperm with the egg after IV application with Lecirelin.

**Key words:** rabbit; artificial insemination; DMSO; lecirelin; heparin; oocytes

**TETRASPANIN CD9 IN BOVINE REPRODUCTION SYSTEM**

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CD9 molecule, 24-27 kDa cell-surface protein, is a member of the tetraspanin superfamily. CD9 molecule was detected in the ooplasm of oocytes (mouse, pig, and cattle), blastocysts (mouse) and epithelial cells of endometrium (mouse, human and cattle). The sperm CD9 was detected only in mouse and in pigs on sperm within the reproduction tract.
The essential role of the oocyte CD9 tetraspanin in the gamete fusion was demonstrated in mice by the inability of CD9 knock-out mouse oocytes to fuse with the sperm and by the ability of anti-CD9 antibodies to inhibit the fusion in normal mouse. Reduction of sperm binding and fusion after the antibody treatment of the oocyte was also reported in pig and cattle.
In this work we focused on the expression of CD9 in the tissues of male and female reproductive system and sperm from individual segments of the epididymis using SDS-PAGE, Western-blotting technique and immunoperoxidase test.
Using anti-CD9 monoclonal antibody IVA-50, CD9 molecule was detected in the tissues of both male and female reproductive system and by Western-blotting also in the sperm from different parts of the epididymis. Molecular weight of detected protein in reproductive tissues and sperm from various parts of the epididymis is about 24-26 kDa. Histochemical assay showed the high expression of CD9 in the collagenous and smooth muscle tissues. The presence of CD9 in the secreta of the epididymis and the strong tissue staining in the range of epithelial microvilli of the epididymis suggests the secretion of CD9 by the epididymal epithelium.

**Key words:** CD9; reproduction tract

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**EXPRESSION OF THE IMMUNE-RELATED GENES IN CHICKEN B LYMPHOCYTES STIMULATED *IN VITRO* WITH KLH, LTA AND LPS ANTIGENS**

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The immune response in chicken genome is encoded polygenically by a number of genes with additive effects. On the basic of the association study between SNP markers and the level of the specific immune response against KLH and non-specific immune responses against LTA and LPS antigens, the panel of genes was selected that harbour QTNs. KLH (Keyhole lympet haemocyanin) is immunostimulatory, non-pathogenic antigen extracted from the marine mollusk *Megathura crenulata*. LTA (lipoteichoic acid) is derived from cell walls of G(+) bateria, while LPS (lipopolysaccharide) is located in the walls of G(-) bacteria and they reflect environmental antigens. The aim of the study was

to determine of expression of the panel of selected genes at the mRNA level, using an *in vitro* model, which is based on the stimulation of chicken B-cell line DT-40. The stimulation was carried out for 3, 6, 9 and 24 hours at two doses of KLH and LTA (5µg/ml and 50 µg/ml), and a single dose of LPS (5µg/ml). RNA was isolated using Universal RNA Purification Kit (EURx, Gdańsk, Poland). Gene expression analysis was performed using the RT-qPCR method. Primers were designed based on the sequence of the exons. For the relative analysis of the gene expression an ubiquitin was used as a reference gene. Results confirmed that the selected by association gene panel takes part in metabolic pathways of the immune response against KLH, LTA and LPS antigens. KLH antigen has the strongest impact on gene expression, especially at the high dose at all time ponits. In the case of LTA, the highest change of the gene expression was induced by a low dose of antigen at the initial and final hour of stimulation. LPS regulated the gene expression after 6 hours of stimulation. The highest activity was observed for *FOXJ1*, *ITGB4*, *MAPK8IP3*, *CARD11*, *KLHL6* and *MAP2K3* genes. KLH stimulation induced expression of *FOXJ1* and *ITGB4* genes after 9 and 24 hours, *MAPK8IP3*, *CARD11* and *MAP2K3* after 24 hours. LTA stimulated expression of *FOXJ1*, *ITGB4* and *CARD11* after 24 hours, and *MAP2K3* after 3 hours. LPS antigen resulted in an increase of expression of *ITGB4* and *KLHL6* after 6 and 9 hours.

**Key words:** antigen; immune response; gene expression; mRNA level

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## IMMUNOMODULATORY EFFECT OF PRE /SYNBIOTICS DELIVERED *IN OVO* ON CHICKEN MICROBIOME

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Supplementation of chickens with the prebiotics, probiotics, or their synergistic combinations (synbiotics) is essential for shaping the microbiome composition and stimulating the both digestive and immune traits of the host organism.

The aim of this study was to evaluate the immune-related gene expression signatures in the growing meat-type chickens that had underwent *in ovo* injection with pre- or synbiotic.

The fertilized chicken eggs (Ross 308) were injected at the 12th day of incubation with either: prebiotic 1 (inulin), prebiotic 2 (Bi2tos), synbiotic 1 (inulin + *Lactococcus lactis* subsp. lactis 2955) synbiotic 2 (Bitos + *Lactococcus lactis* subsp. cremoris 477) or saline (control). At the 1st, 2nd and 35th day after hatch specific organs were sampled (caecal tonsils, spleen and large intestine, n = 5). RNA was isolated using Trizol. RNA quantity was measured spectrophotometrically (NanoDrop), quality evaluation was checked on agarose gel, and integrity of 10 % of the samples was analyzed with Bioanalyzer. Selection of the most informative time point was based on the panel of various cytokines: IFNβ, IFNγ, IL-18 (Th1 type of immune response), IL-4 (Th2 type of immune response), IL-6, IL-12 (pro inflammatory) cytokines and IL-8 (chemokine). Subsequently, analysis of the whole transcriptome has been performed using Gene 1.1 ST Array (Affymetrix). Statistical analyses were performed in the R programm (http://www.rproject.org/) using the limma algorithm (Linear Models for Microarray Data), which is a part of the BioConductor package (http://www.bioconductor.org/). Preliminary results obtained with Transcriptome Analysis Console 1.0 (Affymetrix) show that the administration of pre- or synbiotics in ovo induces changes (gene expression up- and down-regulation) in the chicken transcriptome. Most genes related to the immune response pathways were down-regulated, what may indicate increased food tolerance or silencing of the genes that are not involved in metabolism or growth.

**Key words:** gene expression; immune response; cytokines

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## NITRA AND ZOBOR RABBITS SEMEN CRYOPRESERVATION

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In this study, the rabbit semen from four Nitra´ rabbit bucks (N1; N2; N3; N4) and four Zobor´ rabbit bucks (Z1; Z2; Z3; Z4) was cryopreserved to examine the potential differences in semen post-thaw quality between the two national breeds of rabbit as well as the differences among males within the same breed. Motility and damage of plasma membrane of fresh and frozen/thawed semen were evaluated *in vitro* using CASA system and fluorescence labelling (PNA-Alexa Fluor). Results obtained *in vitro* showed that the average motility of fresh semen from different males was similar between these two breeds. On the other hand, higher number ($P<0.05$) of frozen/thawed motile sperm was detected in Nitra´ when compared to Zobor´ rabbit breed (37. 21 % vs. 32.75 %), respectively. On the other hand, frozen-thawed Nitra´ rabbit semen showed higher number ($P<0.05$) of sperm with damaged plasma membrane than Zobor´ rabbit sperm (21.97 % vs. 16.74 %), respectively. Nevertheless, both rabbit breeds reached fertilization rate higher than 65 %, when the best frozen-thawed samples were selected for AI. The selection might be necessary as the differences between males and within the same male are noticeable. Relatively high deviations were found in the post-thaw motility of the semen collected from the same buck and frozen on different days during three months. For some bucks, the motility of frozen-thawed sperm varied by 20 %. Differences were also noticed in a percentage of preserved initial motility and progressive movement of frozen-thawed sperm between bucks within the same breed (Z1 vs. Z2: 47.72 and 38.16 vs. 30.85 and 21.89; N1 vs. N3: 51.08 and 41.55 vs. 44.46 and

32.40), respectively. In conclusion, the presence of DMSO, sucrose and ficoll as cryoprotective agents and short-term equilibration in liquid nitrogen vapours followed by direct plunging into $LN_2$ is beneficial for rabbit sperm. However, individual analysis of each fresh and frozen-thawed semen sample used is necessary, as the possible differences between males might occur. Moreover, differences within the same male and different season are noticeable.

**Key words:** rabbit; cryopreservation; sperm; motility; membrane integrity

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## CHARACTERIZATION OF RABBIT MESENCHYMAL STEM CELLS BY FLUORESCENT ANALYSES

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The objective of this preliminary study was to assess the viability of rabbit mesenchymal stem cells (rMSCs) using fluorescent staining. Rabbit mononuclear cells were isolated from the bone marrow (BMMCs) of humanely sacrificed New Zealand White line rabbit. BMMCs were collected from rabbit femurs under sterile conditions and cultured according to the original protocol for culturing of human MSCs. Rabbit MSCs were then isolated from the culture by plastic adherence. For the analysis, 3rd passage of cultured rabbit MSCs containing approximately two million cells was used. The viability and apoptosis of rMSCs were evaluated using fluorescent dyes: Yo-Pro-1, propidium iodide (both from Molecular Probes, Lucerne, Switzerland), Vectashield anti-fade medium containing DAPI fluorescent dye (Vector Laboratories, Burlingame, CA, USA) and Annexin-V-FLUOS Staining Kit (Roche Slovakia, Slovak Republic). Staining of rMSCs with propidium iodide (PI) revealed 1.24 % of dead cells. The apoptosis rate using Yo-Pro-1 that binds to nucleic acids and detects late apoptosis was 12.23 %. On the other hand, we detected early apoptosis rate of 4.96 % using Annexin-V-FLUOS, that labels phosphatidylserine-related destabilization of the cell membrane. The results have shown that rabbit MSCs are suitable for other manipulations, like cryopreservation, and subsequent experiments due to great viability and low apoptosis rates.

**Key words:** rabbit; MSC; stem cells; viability

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## ACROSOMAL STATUS OF BOVINE SPERM CAN BE EVALUATED BY A MONOCLONAL ANTIBODY IVA-520

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Mammalian reproduction is a unique event depending upon successful binding and fusion of the spermatozoon and the oocyte plasma membrane. The acrosome reaction of sperm is a prerequisite step for penetration of the *zona pelucida* by the spermatozoa and fertilization of the oocyte. Only undamaged gametes participating in fertilization can successfully undergo this essential process. For evaluating the sperm acrosome status various lectins - *Pisum sativum agglutinin* (PSA); *Arachis hypogaea*- [peanut] *agglutinine* (PNA); *Triticum vulgare* [Wheat germ] *agglutinine* (WGA) - are commonly used. The purpose of the present study was to compare reaction pattern of these lectins and the monoclonal antibody (mAb) IVA-520 for identification of acrosomal changes. IVA-520 was obtained after immunization of BALB/c mice with a whole bull sperm, and was fully characterized as a mAb against bovine CD46 (cell surface protein) molecule. Our previous results suggest a localization of CD46 on the outer acrosomal membrane of bovine sperm and, therefore, could be used in evaluation of sperm acrosomal status. By triple immunofluorescent staining the sperm before and after acrosome reaction the patterns of three lectins and IVA-520 were compared. The term “acrosome reacted” refers to the loss of the acrosomal cap identified by the disappearance of fluorescence. The reaction pattern of IVA-520 is consistent with PSA, PNA and WGA lectins. It can be concluded, that IVA-520 - bovine anti-CD46 monoclonal antibody is a suitable marker for monitoring the integrity as well as the acrosomal status of frozen-thawed bovine sperm.

**Key words:** spermatozoa; acrosome reaction; monoclonal antibody; lectins

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## *IN VITRO* STUDY ON EFFECTS OF MYCOTOXINS ON SUPEROXID DISMUTASE ACTIVITY IN PORCINE OVARIAN GRANULOSA CELLS

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Mycotoxins are known as fungal secondary metabolites, found in food crops due to the widespread nature of fungi in the environment. Deoxyvalenol (DON) is cytotoxic and genotoxic to mammalians cells. Zearalenone (ZEA) is non-steroidal mycoestrogen that activates estrogen receptors where it acts as an agonist and partial antagonist to estradiol. There are some studies suggesting oxidative stress-induced

changes in animal cells after mycotoxin exposure. Antioxidant enzymes in cells of the body are the major defence system to prevent organ injuries from the excessive quantity of reactive oxygen species that cause cellular lipid peroxidation. The aim of present study was to determine the activity of superoxide dismutase (SOD) in porcine ovarian granulosa cells after DON and ZEA exposure *in vitro*. The cells were incubated with DON/ZEA administrations as follows: group A (10/10 ng.ml$^{-1}$), group B (100/100 ng.ml$^{-1}$), group C (1000/1000 ng.ml$^{-1}$), and the control group without any additions for 48 h. Activity of SOD was analysed by spectrophotometer Genesys 10. In the present study mycotoxins developed stress reaction of porcine ovarian granulosa cells what resulted in increasing ($P<0.05$) activities of SOD. Our results contribute towards the understanding of the response of cells to cellular stress.

**Key words:** porcine ovarian granulosa cells; superoxide dismutase; mycotoxins

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## INFLUENCE OF AMYGDALIN SUBSTANCE ON CHROMOSOMAL PLOIDY IN RABBIT

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The aim of this study was to determine possible effect of amygdalin supplement to rabbit diet on chromosomal ploidy level. A total of 15 adult females of the New Zealand White rabbit line were used in the experiment and were bred at the farm of the Research Institute for Animal Production, NAFC Nitra. The animals were divided into four experimental groups P1-P4 and one control group. Each group consisted of three individuals. Groups P1 and P2 were treated by intramuscular application of isolated amygdalin substance (P1: 0.6 mg.kg$^{-1}$ of live weight, P2: 3 mg.kg$^{-1}$ of live weight); P3 and P4 were fed by a mixture of commercial diet and apricot seeds (P3: 60 mg.kg$^{-1}$ of live weight, P4: 300 mg.kg$^{-1}$ of live weight). The assessment of the chromosomal number was performed on peripheral blood lymphocytes arrested at the metaphase stage by colcemid solution at final concentration 0.6µg.ml$^{-1}$. The occurrence of diploid cells was recorded in experimental rabbit groups as following: P1 - 75.6 %; P2 - 71.67 %; P3 - 82.33 %; P4 - 70 % and control - 68.87 %. Aneuploidy manifested by hypodiploid cells showed the following average values: P1 - 22.73 %; P2 - 28.33 %; P3 - 17.67 %; P4 - 30 % and control - 25.57 %. Polyploid cells were recorded with low average values in P1 (1.67 %) and control (5.56 %) rabbit groups. The results were statistically processed using Chi-square t est. In conclusion, no significant differences were found between individual experimental groups and control rabbits.

**Key words:** amygdalin; rabbit; aneuploidy; chromosome; number

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## S-ALLYL CYSTEINE INFLUENCES MEIOTIC MATURATION OF PORCINE OOCYTES

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Garlic has long been considered a food with many health benefits. However, only little is known about garlic effects on reproductive system. The aim of this study was to reveal the effects of important garlic sulfur compound, S-allyl cysteine, on porcine oocyte meiotic maturation. Porcine cumulus-oocyte complexes (COCs) were aspirated from 3-5 mm follicles in pre-pubertal gilt ovaries. Oocytes were matured by 24 hour incubation in modified M199 at 39 °C in an air mixture with 5 % $CO_2$. The oocytes of the experimental group were matured with S allyl cysteine (SAC) at concentrations of 0.1 mM, 0.5 mM and 1 mM. Oocyte meiotic maturation was evaluated after orcein staining under a phase contrast microscope. Samples from COCs, collected for the determination of the hyaluronic acid (HA) production, were subjected to digestion by lyase and evaluated using spectrophotometry (216 nm). Onset of early apoptosis was evaluated by immunocytochemistry using measurement of cleaved caspase-3 expression. The data were analyzed by General Linear Model (SAS 9.0). The results are relatively expressed to control and given as mean ± standard deviation.

The culture of oocytes with SAC had no effect on nuclear maturation. Nevertheless, SAC enhanced HA production after 24 hours of culture (1.0 for control group vs. 1.91 ± 0.69 for 0.1 mM SAC and 2.13 ± 0.81 for 1 mM SAC). SAC also suppressed expression of cleaved caspase-3 after 24 hours of culture (1.0 for control group vs. 0.74 ± 0.23 for 0.1 mM SAC and 0.56 ± 0.12 for 1 mM SAC). SAC influenced meiotic maturation through enhanced HA production in cumulus-oocyte complexes. This effect of SAC can be mediated by its antioxidative action, as it suppressed expression of early apoptosis indicator, cleaved caspase-3.

**Key words:** garlic; meiotic maturation; oxidative stress; pig; S-allyl cysteine

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## THE EFFECT OF 4 WEEKS APPLICATION OF STRAWBERRY LEAVES AND PATULIN ON MACRO- AND TRACE ELEMENT CONCENTRATION IN RABBIT MEAT

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The goals of the present study was to determine the effect of 4 weeks application of strawberry leaves at various doses and acute dose of patulin on mineral profile of rabbit‘s meat. Animals (adult male rabbits, body weight 4 ± 0.5 kg) were divided into eight groups: two control groups C (n = 3), EP (n = 3) and six experimental groups E1- E6 (n = 4 per each group). Animals were fed *ad libitum* using KKV1 feeding mixture (FM) with strawberry leaves at various doses (0,5 %, 1,0 %, 1,5 %) and some groups received patulin intramuscularly in injectable form at 10 μg.kg$^{-1}$ for 28 days twice a week. The samples of *musculus longisimus dorsi* were collected for mineral profile analysis after four week exposure. There is a great variability in trace element content of rabbit meat among different studies. The content of chemical elements (copper, zinc, iron, potassium, sodium, magnesium, manganese, calcium) was analysed by atomic absorption spectrophotometer (iCE 3000 Series Atomic Absorption Spectrometers). The phosphorus content was determined by spectrophotometry at a 430 μm (Camspec M 501 Single Beam Scanning UV / Visible Spectrophotometer). Addition of strawberry leaves either alone or in combination with patulin caused decrease in the calcium content. Significant decrease ($p < 0.05$) in the calcium content was observed in the experimental groups E2 (10μg.kg$^{-1}$ patulin, 0.5 % leaves), E3 (0μg.kg$^{-1}$ patulin, 1.0 % leaves), E4 (10μg.kg$^{-1}$ patulin, 1.0 % leaves), E5 (0μg.kg$^{-1}$ patulin, 1.5 % leaves) and E6 (10μg.kg$^{-1}$ patulin, leaves 1.5 %) compared to the E1 group (0μg.kg$^{-1}$ patulin, 0.5 % leaves). Final decrease ($p < 0.05$) was determined in the E2, E3, E4, E5 and E6, compared to the EP group. Rabbits have a unique calcium metabolism and they absorb nearly all of the dietary calcium ingested and excrete the excess through the kidneys. Other minerals were not significantly ($p > 0.05$) influenced by dietary treatments. This fact could be related to the mineral and trace element distribution in the carcass and rabbit meat, therefore further studies are needed in order to evaluate this distribution.

**Key words:** macro and trace element; strawberry leaves; patulin; rabbit meat

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## CHANGES IN OVARIAN HORMONAL SECRETION INDUCED BY AMYGDALIN APPLICATION *IN VITRO*

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Amygdalin, plant nitriloside, is natural cyanide-containing substance abundant in the seeds of apricots, almonds, peaches and other plants. It is a controversial anti-tumor compound that has been used as an alternative cancer drug for many years. Amygdalin is composed of two molecules of glucose, one of benzaldehyde, which induces an analgesic action, and one of hydrocyanic acid, which is an anti-neoplastic compound. The aim of the presented study was to investigate the effect of natural substance amygdalin at selected doses (1, 10, 100, 1000, 10 000 μg/mL) on the progesterone and 17-β-estradiol release by porcine granulosa cells (GCs) from non-cyclic ovaries. The release of both steroid hormones by GCs was evaluated by ELISA. Results from this investigation showed that amygdalin administration did not cause significant ($P \geq 0.05$) differences in progesterone release by GCs from non-cyclic ovaries. However, the release of 17-β-estradiol by ovarian GCs was significantly ($P \leq 0.05$) increased only in the experimental group with the highest dose of amygdalin (10 000 μg/mL) compared to the control group without amygdalin addition. In conclusion, our findings indicate possible dose-dependent effect of amygdalin on secretion activity of porcine ovarian granulosa cells and its possible involvement in the processes of steroidogenesis.

**Keywords:** amygdalin; secretory activity; granulosa cells; steroid hormones

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## EFFECT OF ROOSTER ON EGG WEIGHT OF ORAVKA BREED CHICKEN

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The egg weight of Oravka breed was measured in two lines (OR2 and OR3) during the two laying seasons. The *in situ* conservation flock kept in National Agriculture and Food Centre - Research Institute of Animal Production (RIAP) Nitra was included in the experiment. For the experiment, the breeding males were purchased from different farmers, then the breeding females were born after the mating between females raised in the RIAP Nitra flock and these purchased breeding males. The eggs were weighted each month weekly during the laying seasons. The housing system was the same in both seasons. The animals were fed the same feed during the whole experiment (in both seasons).

The average weights of eggs were compared. In the season

2011/2012 the average egg weight of line OR3 was higher in each month when compared to line OR2. On the contrary, in the following season (2012/2013) the average egg weight of line OR2 was higher in each month when compared to line OR3. The lowest average egg weight was found in the beginning of the laying seasons. In the season 2011/2012 the highest egg weight was found in females of OR2 line in June (56.797 ± 0.622 g). In the season 2012/2013 the highest egg weight was found in females of OR3 line in March (55.078 ± 0.599 g). Mostly, significant differences (t-test) were found in respective months between egg weights during the two laying seasons. We can conclude, that differences in the egg weight are caused by the rooster effect.

**Key words:** Oravka; genetic resources; egg weight

## THE ROLE OF CALCINEURIN IN OOGENESIS OF PORCINE OOCYTES

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The oogenesis includes intracellular changes which are controlled by many proteins. Phosphatase 2B, calcineurin could be one of them. Calcineurin has very conservative structure composed of 2 subunits – the catalytic subunit A and the regulatory subunit B. Calcineurin was detected in many tissues, not only in mammals, and probably has a number of physiological functions. In somatic cells calcineurin is involved in calcium signals and cell signal pathways or regulation of ion channels and changes in gene transcription. However, there is a lack of information about the role of calcineurin in oogenesis. The expression of calcineurin phosphatase was confirmed in oocytes of invertebrates (*Drosophila* spp.) and amphibians (*Xenopus* spp.) where it regulates meiotic maturation.

In our previous studies we used inhibitors of calcineurin and documented the influence of calcineurin on meiotic competence and maturation of porcine oocytes. We also revealed changes in the expression and localization of calcineurin in oocytes during meiotic maturation *in vitro*. Based on these results, we hypothesize that protein phosphatase calcineurin plays also an important role in the regulation mechanism and course of meiotic maturation of porcine oocytes. The objective of this study was to find out possible influence of calcineurin on the activity of two major kinases which regulate oocyte meiotic maturation – M-phase Promoting Factor (MPF) and Mitogen-Activated Protein Kinase (MAPK).

The activity of MPF and MAPK in oocytes was measured using histone H1 kinase and Myelin Basic Protein (MBP) kinase assays, respectively. Activity of MPF and MAPK was analyzed in oocytes at three stages of meiotic maturation – germinal vesicle stage (GV) and at the stage of first and second meiotic metaphase (MI and MII). For inhibition of calcineurin activity specific and effective inhibitor (cyclosporine A) was selected.

Our preliminary results from kinase assays do not demonstrate the involvement of calcineurin in activation or inactivation of MPF or MAPK in porcine oocytes. It seems that inhibition of calcineurin does not have an influence on activity of these kinases. We can assume that calcineurin interferes with regulation mechanism of meiotic maturation of oocytes in distinct way. Further studies for understanding calcineurin mode of action are needed.

**Key words:** calcineurin; phosphatase 2B; oocyte; meiotic maturation; pig

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## EFFICIENT DETECTION OF THE ASIP MUTATIONS IN THE DOMESTIC DOG

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Agouti Signalling protein (ASIP) controls the distribution of red and black pigment in many mammals through interactions with products of MC1R (Newton *et al.*, 2000) and CBD103 (Candille *et al.*, 2007) genes. The wild type allele $a^w$ produces a wolf-sable color, the allele $a^y$ (fawn) consist of by two mutations A82S and R83H and the recessive solid black allele a is caused by the mutation R96C. These mutations are localized in exon 4 of the ASIP gene. For the discrimination between $a^w$ (the wolf-sable) and $a^t$ (black and tan) alleles variable SINE (a short interspersed nuclear element) insertion in intron 1 can be used (Dreger *et al.*, 2011). Two different SINE sequences are located in intron 1 of the ASIP gene – the first has a forward orientation and it is present within all alleles, second SINE has a reverse orientation and is present only on $a^t$ and *a* alleles. The functional effect of the reverse SINE is altering of properties of ASIP transcripts due to the 3´splice acceptor site (Wang and Kirkness, 2005).

DNA from our DNA bank of dog samples was used for the method validation. DNA was isolated from buccal swabs with Nucleospin Blood kit protocol (Macherey–Nagel). Primer sequences for detection of three mutations A82S, R83H and R96C in one reaction and reverse SINE were designed by reference sequence AY052751 from UCSC. Segments were amplified by polymerase chain reaction in 10 µl consisting of 1 µl of extracted DNA (~40 ng), 0,5 M F and R primers and 1X Thermo-Start PCR Master Mix (Thermo Scientific). PCR products were cycle sequenced by BigDye 1.1 and detected mutations on 3100 Avant sequencer (Applied Biosystems). Sequences were aligned using the Geneious software (Biomatters). For detection of the specific SINE insertion we used routine fragment analysis using 3100 Avant (Applied Biosystems) with 6-FAM labeled F primer.

In summary, we have developed an efficient strategy for rapid identification of three mutations A82S, R83H and R96C in one tube reaction in the dog genome by sequencing targeted DNA of the ASIP gene. Targeted amplification of reverse SINE regions successfully identified the differentiation of the allele $a^w$ and allele $a^t$ by the fragment analysis. This method reduces time and costs and helps

the breeders with artificial selection for the popular black and tan phenotype.

**Key words:** dog genome; mutation; DNA; fragment analysis

## UBIQUITOUS DISTRIBUTION OF CD46 IN NON-REPRODUCTIVE BODY SYSTEMS IN CATTLE

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Membrane cofactor protein - CD46/MCP is a transmembraneous type I glycoprotein belonging to the complement regulatory proteins family. This protein has been studied on human somatic tissues rather than on the other species. In cattle, Maurer *et al*. (2004) described bovine homolog of CD46 firstly as a cellular receptor for bovine viral diarrhoea virus. Antalíková *et al*. (2007) proved the complement regulation activity of erythrocyte CD46. In our study, the distribution of CD46 on bovine tissues has been analysed. The cells or samples of non-reproductive body system organs and fluids have been examined by immunohistochemistry and ELISA test. Presence of CD46 antigen, as well as molecular weight of particular CD46 isoforms, was determined by Western-blotting. Consistently to human, our results showed a wide expression of CD46 in different non-reproductive organs, including blood cells and body fluids in cattle and in pig. Ubiquitous expression of CD46 is a necessary to prevent self-complement activation on cell surfaces.

**Key words:** CD 46; MCP; complement; bovine; boar

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## INFLUENCE OF MATERNAL STRESS ON IMPLANTATION RATE IN MICE

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Maternal stress activates the hypothalamic–pituitary–adrenal axis (HPA) and the sympathetic –adrenal–medullar system (SAM) and leads to increase in glucocorticoid (cortisol and corticosterone) levels. Excretion of steroid hormones has negative effect on female reproductive system and impairs the regulation of the hypothalamic–pituitary–gonadal axis (HPG). The aim of this study was to assess the influence of maternal stress experimentally induced during the period of preimplantation embryo development on implantation rate. In model experiment spontaneously ovulating and naturally fertilized ICR mice dams were used. Mice were divided into two groups – control group (untreated) and stressed group in which the immobilization „ restraint“ stress was applied for 30 min/3 times a day (at 8.00 am, 12.00 am and 4.00 pm) from ED1 to ED4 (ED1 means the first day of pregnancy). On ED6 a portion of dams was slaughtered and assessed for implantation rate during dissection. The rest of dams were left to deliver offspring in natural way. Our results showed that the maternal stress caused decrease in the number of implantation sites. Unfortunately, we did not find significant differences in litter size or weight of newborns between stress and control groups of dams. On the other hand, the preimplantation maternal stress slowed down postnatal development of offspring (evaluated by “eye opening” test). In conclusion, we confirmed the hypothesis that stress at early stages of pregnancy might have negative influence on some parameters of reproductive process.

**Key words:** preimplantation embryo; restraint stress; implantation rate

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## EVALUATION OF LIVER ENZYMES ACTIVITIES AFTER APPLICATION OF QUERCETIN IN RABBITS

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The aim of the present study was to determine the effect of intramuscular application of quercetin at various doses on the activity of liver enzymes - aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in rabbit‘s blood. Adult male rabbits (n = 20) were divided into four groups (n = 5 in each group), one control group (C) and three experimental groups (E1, E2 and E3). Experimental groups received quercetin through intramuscular injection (E1–10 $\mu g.kg^{-1}$, E2–100 $\mu g.kg^{-1}$ , E3–1000 $\mu g.kg^{-1}$) three times a week. Blood was collected on 30$^{th}$ and 60$^{th}$ day of the experiment. Automatic analyzer Microlab 300 (Merck, Darmstadt, Germany) was used to measure the samples according to manufacturer conditions.

Significantly higher values ($P<0.05$) of ALT were observed in experimental group E2 ($0.77 \pm 0.05$ $\mu kat.l^{-1}$ ) in comparison to E1 ($0.47 \pm 0.18$ $\mu kat.l^{-1}$ ) on 30$^{th}$ day of the experiment. The difference between control group and experimental groups was not significant ($P>0.05$). The activity of AST enzyme in experimental groups E1, E2 and E3 was higher when compared with control group on 30$^{th}$ day of the experiment and differences among the groups were insignificant ($P>0.05$). The values of AST in experimental groups (E1, E2 and E3) were similar in comparison with control group on 60$^{th}$ day of the experiment. However, further examination is needed in order to raise the final statement about the effects of quercetin.

**Key words:** rabbits; quercetin; AST; ALT

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## THE INTERACTION BETWEEN COPPER AND BOVINE SERUM ALBUMIN IN THEIR ACTION ON THE SPERMATOZOA MOTILITY *IN VITRO*

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The target of this *in vitro* study was to analyze the influence of copper on the spermatozoa motility in the presence of bovine serum albumin (BSA) as a component of the culture medium and to provide additional information on the interaction between the action of serum albumin and copper ions. The motility was determined after the sperm exposure to $CuSO_4.5H_2O$ (3.9; 7.8; 15.6; 31.2; 62.5; 125; 250; 500; 1000 µmol/L) using the Sperm Vision™ CASA (Computer Assisted Semen Analyzer) system during different time periods (0, 1, 2 and 24 h). The culture medium, containing 20 % BSA, triladyl, 5 % glucose and redistilled water, maintained the overall percentage of spermatozoa motility in all experimental groups during short-term periods (0 or 1 h). Evaluation of the percentage of motile spermatozoa (MOT; > 5.0 $\mu$m/s) showed significant ($P<0.001$) decrease at high concentrations (≥ 500 $\mu$mol/L) of $CuSO_4.5H_2O$ after 2 h or 24 h of the exposure to $CuSO_4.5H_2O$ at doses of ≥ 125 $\mu$mol/L when compared to control group (medium without $CuSO_4.5H_2O$). Similar value was measured for the percentage of progressively motile spermatozoa (PROG; > 20.0 $\mu$m/s) during all time periods. The results suggest that the addition of energy and protein substrate to the incubation medium increases the spermatozoa motility despite the presence of $CuSO_4.5H_2O$ at high doses during short-term periods. Concurrently, BSA maintained the overall percentage of spermatozoa motility (≤ 62.50 $\mu$mol/L of $CuSO_4.5H_2O$) during the long-term (24 h) incubation, which confirms the protective effect of albumin binding to the copper ions. These findings demonstrate the importance of metal-protein interactions.

**Key words:** copper; bovine serum albumin; bull spermatozoa; motility

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## IDENTIFICATION OF GLUCOCORTICOID RECEPTOR PROTEIN IN MURINE AND BOVINE BLASTOCYSTS

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The aim of this study was to examine expression of glucocorticoid receptor protein (GR) in murine and bovine blastocysts. Using the antibodies specific to GR, the expression of GR protein was examined by immunohistochemistry (IHC) in *in vitro* derived bovine and *in vivo* derived murine preimplantation embryos. Embryos were fixed in 4 % paraformaldehyde, washed and permeabilized. Non-specific immunoreactions were blocked with 5 % normal goat serum. Blastocysts then were incubated with two different primary antibodies raised against glucocorticoid receptor. Specific secondary antibody coupled with Alexa Fluor 488 was used to visualize the binding sites of primary antibodies. Cell nuclei were counterstained with Hoechst 33342. Finally, embryos were observed using an epifluorescent microscope. Negative control groups of embryos were incubated without the primary antibody only or without both the primary and secondary antibody, or with the primary antibody pre-adsorbed with an excess (10 times by weight) of the corresponding immunizing peptide.
The immunohistochemical study showed the presence of glucocorticoid receptor in *in vitro* derived bovine and in *in vivo* derived murine blastocysts. The specificity of the signal was confirmed using several negative controls: the intensity of immunostaining signal was significantly reduced in controls incubated with the primary antibody preadsorbed with the corresponding immunizing peptide, and in controls incubated without the primary antibody or without both the primary and the secondary antibody.

**Key words:** glucocorticoid receptor; preimplantation embryo; immunohistochemistry

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## POLYMORPHISM OF THE BOLA-DQA1 GENE IN HOLSTEIN-FRIESIAN CATTLE

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The major histocompatibility complex (MHC) in cattle (BoLA) plays a key role in the immunity response of organisms to intracellular and extracellular infections. There are three classes of MHC molecules, located in different cells, which perform precisely defined functions. Many BoLA genes are highly polymorphic. The greatest genetic variability in the BoLA class IIa was found for BoLA-DRB3 loci (130 alleles), BoLA-DQA (60 alleles) and Bola-DQB (82 alleles). The aim of this study was to analyse polymorphism in the BoLA DQA1 locus in Polish Holstein-Friesian (black and white variety) cows in one of the farm in south-western Poland. The research material consisted of milk samples collected from 64 cows and semen from bulls used for inseminations. The genotype of animals in particular BoLA loci was determined by sequencing PCR products

using a MiSeq (Illumina) sequencer. In the studied cattle population we identified 14 alleles, of which 3 were determined for the first time. At locus BoLA-DQA1 the highest frequency was found for the BoLA-DQA*10011 and BoLA- DQA*0101 alleles. Studies carried out by many researchers demonstrated that alleles BoLA-DQA1*10011 and *0101 are the most frequent in the Holstein cattle. The results obtained in this study indicate that the BoLA-DQA1 locus is highly polymorphic and new alleles are still identified.

**Key words:** polymorfism; BoLA genes; Holstein-Friesian cows

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## ENTEROCIN GENES AND BACTERIOCIN ACTIVITY AMONG ENTEROCOCCI FROM EUROPEAN HARE

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European brown hare (*Lepus europeus* Pallas 1778) is a species of hare naturally occurring in the landscapes of south-west and south-east Slovakia. There is still lack of knowledge concerning the microflora of this wild living animal. Our group has focused on the several year studies of Firmicutes and especially on the genus Enterococcus. In this study, the main concern has been done on the occurrence of genes for enterocin (Ents) production among enterococci isolated from rectal content (faeces) of hares. *Ent* genes in enterococci or their bacteriocin activity among enterococci from different sources have been detected by us previously. To expand this knowledge, enterococci isolated from E. hare were examined. A total of 32 E. hares were shot in six districts of south-west Slovakia during the winter season of 2007/2008 years. Recta (faeces) were transported to our laboratory. Samples were processed using the standard microbiological method (ISO), diluted in Ringer solution; the dilutions were plated on M-Enterococcus agar (Difco). Randomly picked colonies were controlled for purity. On the basis of PCR, phenotypic characterization as well as Maldi-Tof identification, 7 isolates were allotted to 2 species *Enterococcus faecium* (4) and *E. faecalis* (3). The *Ent* A gene was detected in 3 strains *E. faecium*, EFZih2b was *Ent* A gene absent. *E. faecalis* strains did not possess *Ent* A gene. *Ents* P, B, L50B were not detected. Antibiotic-sensitive *E. faecium* Tr11b with *Ent* A gene showed inhibition against 12 of 30 strains (the zones up to 38 mm). Bacteriocin substance of Tr11b strain was partially purified and its more detailed study is in progress.

**Key words:** European hare; enterococci; bacteriocin; gene

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## CHARACTERIZATION OF THE GENETIC DIVERSITY IN SLOVAK GEESE BREEDS

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Characterization of genetic diversity can inform about within- and between- breeds variability. Also genetic diversity study is particularly important in the case of endangered breeds as well as for reconstructing the history of livestock. The genetic diversity and the structure were assessed in two national goose breeds, Suchovska and Slovak, and one extinct production crossbreed - Tesedik Goose in a total population of 102 birds (32 Suchovska, 20 Slovak and 50 Tesedik). A total of 40 alleles were found across 6 detected microsatellite loci with a mean number of 6.67 alleles per locus. The mean observed heterozygosity in total population was 0.40. The mean observed heterozygosity of Suchovska, Slovak and Tesedik was 0.33, 0.42 and 0.43, respectively. The degree of inbreeding of Suchovska, Slovak and Tesedik, calculated as a mean $F_{IS}$, was 0.15, 0.11 and 0.07, respectively. The populations were low differentiated, with a mean $F_{ST}$ value 0.075 in total population. The results of genetic diversity showed that Suchovska and Slovak Goose breeds meet criteria for endangered breeds.

**Key words:** genetic diversity; genetic structure; microsatellite; goose/geese

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## BIOACTIVE COMPOUNDS FROM POMEGRANATE AND THEIR EFFECTS ON RELEASE OF STEROID HORMONES BY RABBIT OVARIAN FRAGMENTS

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Pomegranate is rich for bioactive compounds, such as polyphenols, flavonoids, ellagitannins, proanthocyanidins and minerals, mainly potassium, nitrogen, calcium, phosphorus, magnesium and sodium. The most protective compound of pomegranate seems to be punicalagin and its metabolite- ellagic acid. Every substance should be responsible for effect on intracellular mechanism and should

have antioxidant effect, but higher concentration of these compounds may have opposite effect on viability of cells. The aim of our study was to examine effects of natural antioxidants from pomegranate (punicalagin) on the release of steroid hormones (progesterone and 17β-estradiol) by rabbit ovarian fragments. Fragments from non-cyclic ovaries of rabbits were cultured during 24 h in an incubator at 37 °C and 5 % $CO_2$ with various doses (0, 1 and 10 µg/mL) of bioactive compound – punicalagin. Steroid hormones of the female reproductive system – progesterone and 17β-estradiol were evaluated by ELISA. Progesterone release was not significantly ($P \geq 0.05$) influenced by punicalagin treatment at the doses of 1 or 10 µg/mL. On the other hand, 17β-estradiol was significantly ($P \leq 0.05$) decreased after punicalagin application at the dose of 10 µg/mL (but not at 1 µg/mL). In conclusion, our findings indicate possible dose-dependent effect of punicalagin on the secretion activity of rabbit ovarian fragments and its possible involvement in the processes of steroidogenesis.

**Key words:** punicalagin; secretory activity; ovary; steroid hormones.

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## THE IMPACT OF THE T2264A POLYMORPHISM OF FASN GENE ON THE FATTY ACID CONTENT IN POLISH RED COW'S MILK

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The content and composition of fatty acids in milk is currently the subject of many studies, for the sake of people awareness that milk and milk products are the primary source of about 500 different fatty acids, including many bioactive polyunsaturated fatty acids, which have directly a huge impact on people health. One of the major quantitative traits loci (QTL) for fat and fatty acids in milk has been mapped on *Bos taurus* chromosome 19 (BTA19). In this area there is a fatty acid synthase gene (FASN), which is suggested as candidate gene that may have a significant impact on the content of fatty acids in both dairy and meat cattle. Fatty acid synthase (FASN) is a multifunctional enzyme complex that catalyzes the *de novo* fatty acid synthesis in mammals. Several SNPs in the various exons of the FASN gene and the different functional domains of the FASN protein have been associated with the percentage of fat and medium and long chain fatty acids content of the cow's milk. One of SNP in the FASN gene is the g.17924A> G, which results in the substitution of threonine (ACC) for alanine (GCC) at 2264 (T2264A) amino acid position of the protein chain.
The aim of the present study was to analyze the polymorphism of the g.17924A> G in the FASN gene and the relationships between the FASN genotypes and percentage of milk components (fat, protein, lactose, total solids) and the fatty acids in milk fat of Polish Red cattle. Genotyping was carried out using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method.
All possible FASN genotypes were detected in the studied population of Polish Red Cattle breed, but the GG genotype was the most frequent (approximately 75 % of cows). We found the significant associations between the g.17924A> G mutation of FASN gene and the some fatty acids composition in the milk fat of Polish Red cows.

**Key words:** polymorphism; FASN gene; milk fat; Polish Red cattle

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## EFFECT OF T-2 TOXIN ON SERUM ALBUMIN AND BILIRUBIN LEVEL IN RABBIT BLOOD

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The aim of the present paper was to determine effect of application of single acute dose of T-2 toxin on the blood level of bilirubin (BR) and albumins in rabbits. Animals were divided into control group (C) and experimental group (E). T-2 toxin at dose of 0.08 mg per kg of body weight was applied 72 hours before the slaughter. Control group received injection of water (Imuna Pharm a.s. Šarišské Michaľany, Slovak Republic).
After T-2 toxin treatment statistical analysis showed insignificant ($P > 0.05$) lower values in the level of bilirubin in the experimental group (E) in comparison with the control group (C). Our results are consisted with results of other authors.
In case of albumins a slight but insignificant decrease in content of albumins in the experimental group vs. the control group was observed.
Based on the literature data mycotoxins could slightly modify the serum content of bilirubin and albumins and consequently influence antioxidant balance in the organism. To prove this idea, research on the T-2 toxin will be worthy of further investigation.

**Key words:** T-2 toxin; bilirubin; albumin; rabbits

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## IMPACT OF SELECTED METABOLIC PARAMETERS IN PROFITS AND QUALITY OF EMBRYOS IN MOUFLON FEMALES DONORS

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The aim of our reasearch was to study the effect of prevalence serum cholesterol value, urea and total protein before and after superovulation on the yield and quality of embryos in mouflon donors. Positive corelation was found between the level of cholesterol and superovulatory response (r = 0.68), production of embryos (r = 0.50) and transferable embryos (r = 0.48). Levels of urea were in negative corelation with the superovulatory response (r = -0.37), to flushed embryos (r = -0.56) and transferable embryos (r = -0.64). The influence of total proteins in blood serum of donor muflones on effectiveness of embryo transfer was not proved in our research.

**Key words:** blood; embryos; heat; mouflon females

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## EXPRESSION OF CD46 MOLECULE DURING THE DEVELOPMENT OF BOVINE GAMETES

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The membrane cofactor protein (MCP, CD46 molecule) belongs to the group of membrane-associated complement regulator molecules which could regulate the function of complement (immune) system by cleavage of complement cascade in discrete sites. Distribution and special function of CD46 in reproduction system has been studied in man, rat, guinea pig and mice. Several lines of evidence suggest that the CD46 molecule, even through the non-complement activities, may play a unique role in mammalian reproduction. It was found that the CD46 may be involved in the sperm–oocyte interaction at the level of the oocyte plasma membrane. The aim of this study was to identify (characterize) the CD46 molecule on bovine gametes and reproductive tissues during their development.

Tissues for the histochemical staining (bovine testis, epididymis, ovary and accessory glands) were been obtained at local slaughterhouses. Ejaculated spermatozoa and early bovine embryos were received from Slovak Breeding Services Inc. The embryos at different developmental stages were prepared by in vitro culture of ovarian eggs. The CD46 molecule was detected by indirect immunoperoxidase test using the anti-CD46 monoclonal antibody IVA-520.

The study demonstrated that the bovine MCP (CD46) is developmentally expressed in the testis. CD46 molecule formation is parallel with the development of an acrosome of bull spermatozoa. In the ejaculated spermatozoa the acrosome cup is intensively stained. Similarly to spermatozoa, the CD46 expression was found throughout the development of bovine oocyte and expression of CD46 molecule was persisted in early embryos (the blastocyst stage). This study should provide a basis for the further characterization of bovine CD46 molecule in the bovine reproductive tract and the function of CD46 molecule in reproduction processes.

**Key words:** animal reproduction; CD molecules; spermatozoa; oocyte

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## ADHERENCE OF VAGINAL AND INTESTINAL STRAINS OF LACTOBACILLI AND ENTEROCOCCI TO MUCUS COLLECTED FROM DIFFERENT PARTS OF REPRODUCTIVE TRACT OF HEIFERS AND COWS

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In the present study the ability of the vaginal and intestinal bacteria to adhere to mucus collected from different parts of the reproductive tract of heifers and cows was examined. Tested bacterial strains were isolated from vagina of healthy heifers and cows: *Lactobacillus mucosae* (Biocenol™ 7697, 9/K, 29S8), *Lactobacillus büchneri* (5/K, 24S8), *Enterococcus faecium* (ECHA2, EAC, 21); from piglets´ intestine: *Lactobacillus plantarum* L81, *Lactobacillus* spp. 71, *Lactobacillus* spp. 26, and from caecum of domestic fowl: *Lactobacillus fermentum* 2I3. Mucus was collected from the vagina, uterine cervix, uterine horns, and uterine bodies of three slaughtered heifers and seven slaughtered cows with healthy reproductive tracts. A microtiter plate binding assay was performed according to the methods of Štyriak and Ljungh (2003) with some modifications. The absorbance values ($A_{580\ nm}$) were determined using a Synergy™ 4 Multi-Mode Microplate Reader (BioTek Instruments Inc., Winooski, VT, USA). Bacteria were classified as strongly adherent ($A_{580\ nm} \geq 0.25$), weakly adherent ($A_{580\ nm}$ 0.15–0.24), or non-adherent ($A_{580\ nm} < 0.15$). Most of the vaginal strains adhered to mucus collected from different parts of the reproductive tract. *L. büchneri* 24S8 was non-adherent to vaginal and uterine horn mucus, and *L. mucosae* 9/K was non-adherent to uterine body mucus. Among the vaginal *Lactobacillus* strains, only *L. büchneri* 5/K was strongly adherent to all mucus collected from the reproductive tract. *L. mucosae* Biocenol™ 7697 was weakly adherent to uterine cervix mucus. The vaginal strains of *E. faecium* (E21, EAC, and ECHA2) and intestinal *Lactobacillus* strains were strongly adherent to all types of mucus collected from the reproductive tract. Compared to *Lactobacillus* strains, *E. faecium* showed the strongest adherence to all mucus types. The strains did not exhibit host specificity, but rather strain specificity. The capability to adhere to mucus was a particular property of each strain. To our knowledge, this is the first report regarding *in vitro* adherence of GRAS (Generally Regarded as Safe) lactobacilli isolated from different sources to mucus collected from different parts of the reproductive tract.

**Key words:** reproductive tract; cow; mucus; adherence; *Lactobacillus* spp.; *Enterococcus* spp.

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## ANNEXIN-V DETECTED ALTERATIONS IN BULL FRESH AND FROZEN-THAWED SEMEN

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The aim of this study was to localize and compare alterations in sperm membranes labelled by annexin-V binding in fresh (Fs) and frozen-thawed (FTs) bull sperm samples. Annexin-V conjugated to fluorescein isothiocyanate (FITC) serves as a sensitive probe that can be used for identification of sperm with loss of membrane asymmetry associated with membrane phosphatidylserine translocation (PST) from inner to the outer membrane leaflet. Semen cryopreservation and thawing processes may cause damage of sperm membrane structures, which can lead to lower viability and motility of post-thaw sperm. The sperm with PST exhibited green fluorescence under a fluorescent microscope and was regarded as sperm with membrane destabilization. Presence of annexin-V positivity in sperm was localized on the acrosomal part of the sperm head, mitochondrial segment, equatorial segment and on sperm membrane along the entire head. Freezing-thawing process significantly ($P < 0.01$) decreased sperm total motility (CASA analysis) and increased ($P < 0.05$) occurrence of sperm with membrane destabilization ($40.14 \pm 4.98$ % in FTs compare to $10.84 \pm 2.96$ % in Fs). In fresh sperm, annexin-V binding sites were localized mainly in the mitochondrial segment (44.77 %) and in equatorial segment (19.40 %) and only 16.42 % of sperm were marked in the acrosomal region of the head. It seems that cytomembrane PST of the mitochondrial (midpiece) region may be influenced by oxygen free radicals or cytochrome c released form mitochondria, which may lead to translocation by inducing peroxidation of membrane phospholipids, including PS (Kotwicka *et al*., 2011). In frozen-thawed sperm, localization of PS was changed and the most of sperm ($P < 0.001$) were marked on the acrosomal region (52.74 %). Significantly lower counts of sperm were marked in the equatorial ($P < 0.01$) and in the mitochondrial segment ($P < 0.001$) compared to fresh sperm. Freezing-thawing process significantly increased PST in bull sperm. PST is considered to be one of the early apoptotic markers and little is known about the circumstances responsible for PST in sperm. Since PST was observed both in morphologically normal and in abnormal cells, it seems doubtful to include this phenomenon in the physiological mechanisms of elimination of abnormal spermatozoa. In frozen semen, these results may indicate negative effect of frozen-thawing process on sperm membrane stability, mainly in the acrosomal part of the head, which can lead to decrease in fertilization ability of semen. In conclusion, our finding indicates that binding sites of annexin-V are substantially different in fresh and frozen-thawed sperm, and refer to different areas of disruption of membrane stability. Fresh sperm are the most susceptible to occurrence of membrane destabilization in the midpiece region, whilst frozen-thawed sperm in the acrosomal part of head. Sperm membrane destabilization detected by annexin-V binding can be used as an additional parameter for the assessment of fresh and frozen-thawed semen quality.

**Key words:** bull semen; phosphatidylserine translocation; membrane destabilization

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***Reference:***

KOTWICKA, M. – JENDRASZAK, M. – JEDRZEJCZAK, P. 2011. Phosphatidylserine membrane translocation in human spermatozoa: Topography in membrane domains and relation to cell vitality. *Journal of Membrane Biology*, vol. 240, 2011, p. 165-170.

## USE OF MICROSATELLITE MARKERS FOR DETERMINING THE ORIGIN OF BEES

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Microsatellite markers could be used for determining the origin of breeds, genetic characterization of the population structure and monitoring the genetic diversity. Carniolan honeybee (*A. mellifera carnica*) is endangered breed in Slovakia due to its illegal crossbreeding with allochtonous breeds, imported by individual beekeepers. A detailed genetic characterization of Slovak honeybee population has not been done yet. The aim of our study was the selection and testing of microsatellite markers to be suitable for genetic analysis of carniolan honeybee. Fifteen microsatellite markers running in two multiplex PCR reactions were tested on samples of 97 randomly selected workers of carniolan honeybee from several geographical regions of Slovakia. Markers were evaluated for PIC index, frequency of alleles, expected heterozygosity ($H_E$), observed heterozygosity ($H_O$), estimated frequency of null alleles and Hardy-Weinberg equilibrium (HWE) using Cervus 3.0.3. We identified a total of 153 alleles in the honeybee samples with the mean value of 10.2 alleles per locus. Eleven loci (A007, C1602, THE01, A024, THE03, A079, A107, A113, C1601, THE02, UN351) were highly informative ($PIC > 0.5$), three loci (A028, A014, THE04) were reasonably informative ($0.5 > PIC > 0.25$) and one locus (AP043) was only slightly

informative (PIC < 0.25). The mean value of $H_E$ for all loci was 0.672, the mean value of $H_O$ was 0.649. The frequencies of genotypes for seven markers were in HWE. The average value of null allele frequency was 0.029 per locus. Selected microsatellite markers, except AP043 marker, are suitable for genetic characterization of honeybee population.

**Key words:** carniolan honey bee; microsatellite markers; multiplex PCR

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## PRECISION ELECTRONIC SYSTEM FOR MEASURING MILK FLOW KINETIC DURING MILKING OF EWES

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In high producing dairy husbandries the precise technology allows us to better understand the physiological needs of animals to breeding environment and to recognize possible pathological disturbances in animal organism. The milk removal process represents very sensitive response of animals to tactile contact of machine to the udder during the milk ejection process. Milk ejection is caused by oxytocin release from the brain and relaxation of the tonus of vegetative nervous system that indicates complex physiological responses to milking conditions which could be easily disturbed. Disturbed milk ejection causes ineffective milking, lower milk yield and increased risk of udder health problems, especially mastitis. The aim of our work was to develop electronic system which could precisely monitor the intensity of milk flow from the teat to machine equipment. The developed system is based on the recording the milk flow from the udder to electronic collecting jar (2.5 L). Within the jar there is a two-wire compact magnetostrictive level transmitter (NIVOTRACK; NIVELCO Ipari Elektronika Rt, Budapest, Hungary) connected to the computer. Milk level in the jar is continuously measured by a transmitter recording the signals on the computer every second. Thus, collected data are further processed using adopted program to calculate and make a graphic image of milk flow kinetic and other parameters of milkability. Such electronic system is very important for further applied scientific work to improve farming systems based on the maintenance of good udder health, welfare of animals, and maximal production of high quality milk, on the one side, and to support the competitiveness of our farmers on the EU market, on the other side.

**Key words:** ewes; milking; milk flow kinetic; electronic recording

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## GENOTYPING OF THE SINGLE NUCLEOTIDE POLYMORPHISM ASSOCIATED WITH MEAT PRODUCTION IN PINZGAU HEIFERS

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In the pas, the Pinzgau breed was kept as dual purpose cattle. Due to low competitiveness of its milk production the breeders start to prefer the beef production. Because the breed is fully adapted to the Carpathian Mountains by its excellent walking and grazing ability and robustness (health and fertility), it is popular in low input systems of suckler cow production (cow–calf system). The aim of the study was to investigate the possibilities to improve the beef production of the Pinzgau breed kept under the extensive conditions. The genetic markers associated with the meat production were chosen and so far their frequencies were calculated. We tested the presence of the F94L mutation in the myostatin gene and two single nucleotide polymorphisms (SNP) in the leptin gene. In this study, 106 heifers were genotyped for F94L polymorphism and other 39 heifers were genotyped for leptin polymorphism. Only one carrier of the L allele for F94L mutation in the myostatin gene was found; other animals were carrying the original F allele. Genotypes AA and AT within the LEP Y7F polymorphism and CC, CT and TT within the LEP R25C polymorphism were identified. As the only one animal carrying the mutant L allele of the myostatin gene is available, further analysis of its effect is not possible. However, other animals carrying this allele are being searched. Further association studies will be performed in order to evaluate the effect of the leptin SNPs on meat production characteristics under the extensive conditions.

**Key words:** Pinzgau cattle; beef production; single nucleotide polymorphism; myostatin; leptin

## GENETIC DIVERSITY OF RABBIT GENETIC RESOURCES IN SLOVAKIA

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This study attempts to provide the first insight into genetic status of rabbit genetic resources in Slovakia. The rabbits of national breeds „Nitran´ rabbit“ and „Zobor rabbit“ are maintained under smallholder farming system. Genetic characterization of these breeds will serve as an essential prerequisite for their identification, effective management, utilization and conservation. Six polymorphic microsatellite loci (INRACCDDV0106, INRACCDDV0183, INRACCDDV0259, Sol08, Sol28, Sol33) were chosen from literature and used to elucidate the degree and pattern of genetic variability of two small populations of Zobor rabbit

and Nitran´ rabbit. The seventeen rabbits were genotyped using single multiplex PCR and every individual conformed to the breed standard. The number of alleles, frequency of alleles, heterozygosity and polymorphic information content were calculated using Cervus 3.0.3 software.
The number of alleles per locus was 3 alleles for INRACCDDV0183, Sol28 and Sol33, 4 alleles for INRACCDDV0106 and INRACCDDV0259, and 5 alleles for Sol08 microsatellite locus, PIC between 0.297 (Sol28) and 0.6 (INRACCDDV0259). The allele number 6 (159 bp) of INRACCDDV0183 locus with allele frequency of 0.45 in Nitran´ rabbit was not detected in Zobor rabbit animals.

**Key words:** rabbit; microsatellites; genetic diversity

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## THE EFFECT OF PATERNAL BULL ON FATTY ACID COMPOSITION OF MILK FAT OF DAIRY COWS OF DIFFERENT BREEDS

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Intake of milk fat in human nutrition is important because of the presence of unsaturated and especially essential fatty acids (FAs), linoleic and α-linolenic acid, as well as conjugated linoleic acid (CLA), which are found only in meat and milk of ruminants. The objective of our study was to investigate the effect of paternal bull on fatty acid composition of milk fat of dairy cows of different breeds. The milk samples were taken from 360 cows placed on 11 dairy farms. We monitored the following breeds of cows: Pinzgau (P, n = 74), Holstein (H, n = 105), Slovak Pied (S, n = 61) and Red Holstein (R, n = 120). Individual milk samples were analyzed for fatty acids in milk fat using gas chromatography (apparatus GC Varian 3800, Techtron, USA) with FID detector in a capillary column Omegawax 530; 30 m. By the chromatography, 54 fatty acids including particular isomers were identified. Their relative proportions were expressed in percents (%). Among the studied breeds, the highest content of CLA was revealed in the breed P (0.67 %). Within this breed high variability was noted, and daughters of fathers LOH2, COS1, TOL1 and GOZ1 showed over-average values of CLA proportion (0.72 - 1.22 %). The breed S was on the second place with the CLA content of 0.55 %. All the bulls of this breed showed balanced values - 0.56 % (RSS1, EGE10) and 0.60 % (KOD3). Breeds H and R reached similar values (0.41 and 0.43 %, resp.). Even within these breeds there were some bulls whose daughters reached higher levels of CLA, namely 0.92 % (H - RUH13) and 0.64 % (R - PAT1). The lowest CLA value (0.15 %) was reached by the bull LET2 from the H breed. The highest content of essential FAs (EFAs) was reached by breeds S (3.33 %) and P (2.98 %). In the breed S, the highest value (3.46 %) was measured in the daughters of the DIK5 bull. Although the P breed had an average value of EFAs less than the S breed, some fathers of P breed had higher EFAs values (COS1 - 3.71 %, LOH2 - 4.67 %). Bulls of the P breed that had the lowest amount of CLA may worsen the content of EFAs. Breeds H and R achieved the similar EFAs levels (2.71 % and 2.73 %, resp.). Although there are fathers who have achieved higher average levels of EFAs (4.21 % - H - FOM17 and 3.91 % H- RUH13). In the breed R it was the bull MOR506 (3.80 %). The highest content of monounsaturated FAs (MUFAs) was found in the milk of dairy cows of the S breed (27.11 %). MUFAs content higher than the average was measured in daughters of fathers DIK5 (28.21 %), which had the highest content of EFAs and EGE10 (27.24 %), which had the best result in the CLA. On this basis both bulls had the lowest atherogenic index (AI, 2.61 % and 2.79 %, resp.). Similar tendencies were found also in the breed P, H and R.
The above described variability in the composition of milk fat of dairy cows and the subsequent relationships between these values suggest that the selection under fathers of dairy cows according to the fatty acid composition of milk fat may be considered.

**Key words:** cow’s milk; milk fat; fatty acids; breed; bulls; paternal effect

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## HYDROGEN SULFIDE DONOR ACCELERATES MEIOTIC MATURATION OF PORCINE OOCYTES BY REGULATION OF KINASE ACTIVITY

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Hydrogen sulfide (HS), a gasotransmitter, participates in the somatic cell cycle through the involvement in cAMP/PKA and PI3K/Akt signal pathways. The role of HS in the re-initiation of meiotic division of oocytes arrested in prophase I and its effect on key factors of meiosis is not clear yet. We hypothesize that an exogenous HS donor influences meiotic maturation of porcine oocytes *in vitro*. The porcine oocytes were cultured in a modified M199 medium with 0.3 mM $Na_2S.9H_2O$, a HS donor, at 39 °C and 5 % $CO_2$ for 12-48 hrs. The oocytes were fixed and stained during this period. The stages of meiotic division were determined after the acetic-alcohol fixation and staining by aceto-orcein. The MPF and MAPK activity was measured by the Histone H1 Kinase and Myelin Basic Protein Double Assay. We observed that the HS donor accelerates meiosis re-initiation and attainment of further meiotic stages. Similarly, meiosis I to II transition and the second meiotic

arrest in metaphase II were achieved more quickly. We found that accelerated meiosis is a result of increased MPF and MAPK activity after 16 hrs and 22 hrs of *in vitro* culture. These findings are in agreement with earlier observations of HS influence on the cell cycle. The mechanism by which HS increases MPF and MAPK activity in mammalian oocytes remains unclear. We presume that its effect is based on the regulation of MPF up-stream signal pathways, such as PI3K/Akt and/or direct protein modification of MPF and MAPK through a process called sulfhydration. In conclusion, hydrogen sulfide acts as a signal molecule in oocyte meiosis via the MPF and MAPK regulation.

**Key words:** hydrogen sulfide; meiosis; oocyte; M-phase/ Maturation Promoting Factor; Mitogen Activated Protein Kinase

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## ACTIVITY OF SUPEROXIDDISMUTASE AND GLUTATHIOPEROXIDASE AFTER EXPOSURE OF PORCINE BLOOD TO ZEARALENONE *IN VITRO*

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Contamination of food by mycotoxins is a serious problem investigated by many researchers. Zearalenone (ZEN) is produced mainly by *Fusarium graminearum* and related species, principally in wheat and maize but also in sorghum and barley. ZEN toxicity and oxidative stress play an etiological role in its toxic effects. In this study, activity of antioxidant enzymes (superoxiddismutase - SOD and glutathionperoxidase -GPx) in porcine blood after short-term exposure to ZEN *in vitro* was analysed. Blood samples were divided into 4 groups (one control group and 3 experimental groups). Group (n = 5 tubes) without ZEN exposure served as a control. Samples of blood were exposed to ZEN (E1-E3 groups; 5 tubes in each group) at concentrations of 10, 100 and 1000 $ng.ml^{-1}$ for 4 hours at 37 °C. After exposure the activity of GPx was analysed in a whole blood and the activity of SOD was measured in lysates from erythrocytes by spectrophotometer Genesys 10 (Thermo Fisher Scientific Inc., USA), using commercial assay kit (Randox, Bratislava). The activities of SOD and GPx were not influenced by ZEN exposure as the differences among the groups remained insignificant ($P>0.05$). Based on presented results the dose of ZEN was probably low, so that the cells were able to maintain the antioxidant defence and the activity of main antioxidant enzymes was unchanged. Further studies are needed to investigate which dose of this toxin is able to cause significant changes in antioxidant defence system of blood cells *in vitro*.

**Key words:** zearalenone; antioxidant enzymes; porcine blood

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## THE INFLUENCE OF BISPHENOL S ON MEIOTIC MATURATION OF PORCINE OOCYTES

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The meiotic cell cycle is controlled by a number of regulatory factors, changes in the activity of MPF and MAPK meiotic proteins are the most important. Some of regulatory mechanisms could be significantly affected and disturbed by the action of endocrine disruptors. Recent studies have included bisphenols in the group of endocrine disruptors which affect the regulation of physiological processes in mammals.

Bisphenols are an essential fundamental part of most plastics. It has been shown that the most widespread bisphenol - bisphenol A (BPA) - mimics the effects of natural estrogens, disrupts hormonal balance and has genomic, non-genomic and epigenetic effects on mammalian oocytes. BPA has usually been replaced by bisphenol S (BPS). However, scientific literature concerning the influence of BPS on mammalian oogenesis is still lack.

The objective of our work was to verify the hypothesis, that bisphenol S as an endocrine disruptor disturbs oogenesi, as well as to determine its role in porcine oocyte meiotic maturation process.

Cumulus-oocyte complexes (COC) were cultured in a modified M199 medium. Oocytes were cultured in 1 ml of culture medium at 39 °C and 5 % $CO_2$ for 24 or 48 hours to achieve the first or second meiotic metaphase stage, respectively. Effective concentrations of BPS were added to the medium before the COC culture. At the end of the culture, the oocytes were mechanically cleaned of the surrounding cumulus cells and fixed in a solution of acetic acid and alcohol for at least 24 hours. After that, the oocytes were stained with 1 % orcein. The stage of the oocyte meiotic maturation was evaluated under phase contrast microscope.

The results had shown that BPS could influence first meiotic metaphase (MI) and meiotic spindle development. Further experiments will be focused on the transition from germinal vesicle breakdown to MI stage, from MI to anaphase I stage, and resumption of meiotic maturation to the stage of second meiotic metaphase MII.

**Key words:** bisphenol; oocyte; meiotic maturation; pig

***Acknowledgement:*** This project was support by the grants NAZV QI101A166, MZeRO No. 0714, CIGA No. 20132035 and CIGA No. 20142049.

**GENETIC TRACEABILITY OF FOOD BASED ON ASSISTED MACHINE LEARNING**

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This paper demonstrates the utility of the data mining and machine learning algorithms for the traceability of deer meat. The aim of this work was to analyse the value of DNA markers to the traceability of meat with a focus on determining the origin of deer. The basis of the statistic model is automatic classification of unknown data according to the known reference data used for model learning. A wide range of supervised learning-based methods (Naive Bayes, Multilayer perceptron, K-NN, C4.5 etc.) were used for the traceability of meat products and identification at the individual level. Genetic diversity based on microsatellite data contains sufficient genetic information suitable for classification of animal into binomial groups (farm vs. wild, Slovak population vs. another). The genetic structure of several populations of deer was analysed, and it serves as a pattern for machine learning methodology. The results suggest an application of machine learning algorithms for fast traceability monitoring in food industry with classification error below 5 %.

**Key words:** traceability; assisted learnig; meat; population; logical group

**HYDROGEN SULFIDE: A SIGNAL MOLECULE IN OOCYTE MATURATION AND CUMULUS EXPANSION**

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Progress of reproductive biotechnologies depends on improved methodologies for successful meiotic maturation of oocytes *in vitro*. Oocyte maturation and acquisition of developmental competence are supported by surrounding cumulus cells and their expansion. Thus, the extent and quality of cumulus expansion may be a possible biomarker of oocyte quality. The cumulus expansion encompasses synthesis and accumulation of extracellular matrix rich in hyaluronic acid. These processes are regulated by a wide range of upstream signalling molecules. Hydrogen sulfide (HS) has been revealed to be an essential signalling molecule in reproductive tract. We hypothesized that exogenous HS influences meiotic maturation and cumulus expansion of porcine cumulus-oocyte complexes (COCs) and oocytectomized complexes (OOXs) after oocyte removal. Porcine COCs were matured *in vitro* in a modified M199 medium with 150-900 μM $Na_2S.9H_2O$, the HS donor. Concomitantly, OOXs were cultured under the same conditions. Proteolytic digestion of expanded cumuli and measurement of hyaluronic acid by Enzyme-linked ImmunoSorbent Assay (ELISA) were used for evaluation of cumulus expansion. Stage of oocyte maturation was evaluated after fixation in acetic-alcohol and orcein staining under a phase contrast microscope. The HS donor significantly ($P \leq 0.05$) accelerated oocyte maturation. Concurrently, the hyaluronic acid production by the expanded cumulus was significantly inhibited. The exogenous HS donor had no effect on oocytectomized complexes. Our results suggest that hydrogen sulfide signalling is involved in regulation of meiotic maturation and cumulus expansion. Presumably, its inhibitory effect on cumulus expansion is dependent on signalling cross-talk between oocyte and cumulus cells.

**Key words:** hydrogen sulfide; oocyte maturation; cumulus expansion

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