



## ANIMAL GENETIC RESOURCES IN SLOVAK REPUBLIC

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### ABSTRACT

Importance of preservation (cryopreservation) and use of the farm animals' gene pool emerge from biological, economic, landscaping and cultural needs of each country that are realized using national gene banks. Availability of the animal genetic resources has an impact on the present and also future life quality and important effect on the food safety. Ratification of the Convention on the Biodiversity obliges Slovak Republic to protect biodiversity, to guarantee sustainable use of its components and fair and equal access to benefit sharing from genetic resources.

**Keywords:** animal genetic resources, cryopreservation, gene bank

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### INTRODUCTION

Protection of the animal genetic resources emerge from the European Biodiversity Action Plan for Agriculture, The Global Strategy for the Management of Farm Animal Genetic Resources FAO OSN, Council Regulation (EC) No 870/2004 establishing a Community programme on the conservation, characterization, collection and utilization of

genetic resources in agriculture; Act No. 194/1998 Coll. on breeding and reproduction of the farm animals. In a cattle threats that within a few following years can monthly die out one breed what will resulting in a complete loss of this genetic potential. Therefore it is really necessary to conserve and maintain animal genetic resources as an insurance against climatic changes, occurrence of diseases, social changes, genetic problems, selective failures and unexpected catastrophic events that can markedly affect biodiversity (**Prentice and Anzar, 2011**).

NGB should be part of the national programme for protection and management of the animal genetic resources. It should guarantee monitoring, collection and preservation of samples from genetic resources (spermatozoa, oocytes, embryos, somatic cells, tissues, DNA etc.) in original condition, deeply frozen or lyophilized; their storage, molecular-genetic characterization of samples, research-comparison of the genetic biodiversity, management of information system about stored samples and their use for original breed restoration.

The present project will solve several logically follow-up phases:

- 1) Monitoring of the livestock population state, generation and operation of the national information system of the animal genetic resources biodiversity (<http://efabis-sk.cvzv.sk>) that is interconnected to supranational information systems (<http://efabis.tzv.fal.de>, <http://dad.fao.org>) and decentralized network of the national information systems (European countries). Construction of the deposit for genetic material samples of the spermatozoa and tissues and filling of the database about stored samples of genetic resources.
- 2) Working out and application of standard molecular-genetic methods for the population genetic variability evaluation of endangered farm animal breeds, determination of parentity and animal origin as well as selected genetic markers for production and reproduction features of the individual breeds.
- 3) Cryopreservations of spermatozoa, embryos, stem cells and DNA.

As a consequence of the farm animal breeding and intensive use of the relatively small breed number there is a progressive decrease in the genetic variability. The loss of genetic variability can lead to decrease in breed adaptative abilities, to worsening of the health state and in a final consequence to reduction of its farm use. Many of the original (autochthonous) farm animal breeds, which were substitute by more efficient breeds in the past, are now

endangered and preserved “*in situ*” as small populations in some regions. The possible extinction of these breeds would also mean irrecoverable loss of the genetic variability and so the loss of unique gene and allele combinations that would be very useful in the future e.g. for the generation of new farm animal genotypes. The Wallachian sheep (Valaška), Oravka hen and Slovak Pinzgau cattle belong to these endangered breeds in Slovak republic.

Therefore the global strategy for animal genetic resources management was created from the FAO initiative that uses the molecular methodology for breed characterization (**Bjornstad and Roed, 2001**). This strategy underlines the importance of molecular marker use for the genetic state evaluation of endangered breeds and appropriate methods of their protection. New information about genome structure of many farm animal species (cattle, pig, sheep, rabbit) as well as about different types of variability in the DNA are continuously discovered due to the technological progress in the area of sequencing methods and due to the development of animal genomics. This knowledge is intensively used for animal selection on the basis of different types of genetic markers so called MAS (marker-assisted selection) (**Goddard and Hayes, 2007**) or for the evaluation of genetic variability, parentity determination etc. (**FAO 2007, Boettcher et al., 2010**). At the present microsatellites appear to be the most suitable markers for the evaluation of important population parameters as they are present in the whole genome in a sufficient number, have a high degree of polymorphism and high allele number, are easy and reliably detected and show codominant heredity (**Bruford and Wayne, 1993**). Microsatellites also represent irreplaceable markers for genome mapping and construction of the linkage maps, diagnostics of the genetic diseases, origin verifying and monitoring of genetic variability in the population genetic studies. Evaluation of the population genetic variability using microsatellites is recommended also by the executive committee ISAG/FAO as a standard procedure mainly for the needs of genetic resources conservation. It is assumed that breeds with diversified genetic background may be in the future successfully used for the new QTL mapping and genotype breeding for the needs of production systems and market demands. Animal genetic resources preservation “*in vitro*” is carried out using gamete cryopreservation (spermatozoa, oocytes or embryo). Frozen biological material (ejaculate) represents one of the most practical ways for germ plasm preservation. Considering their availability and easy application (**FAO, 2007**), they may be used either immediately or after ten or hundred years for artificial insemination (AI) or IVF what represent the potential to preserve existed diversity and heterozygosis by minimization of live animal transport (**Andrabi et al., 2007**). Potential use is also for reconstruction of the

breed through back breeding series, however it is lengthy process, because 4-5 generations are needed for the original genotype reconstruction.

Embryo freezing enables genetic material cryoconservation of males as well as females and represent a big opportunity for the preservation of population heterozygosis and integrity. However it is more complex and economic demanding procedure than the spermatozoa freezing. Moreover, in case of the complex population reconstruction big amount of embryos is required and it could not be assumed that the embryos may be obtained anytime for example from the donors of endangered species (**Boettcher et al., 2005**). Embryos almost from all mammals were successfully frozen, thawed and transferred in the past, but embryos of some animal species such as pigs or horses are more cryosensitive in comparison to the others (bovine, sheep). Different freezing sensitivity of embryos is subject not only to the animal species, but also to the embryo developmental stage. Earlier developmental stages of *in vivo* embryos survive the freezing better than the later stages and *in vitro* produced embryos. Therefore there is an effort to create standardized protocols that could be used for embryos from different animal species and in different developmental stages (**Pereira and Marques, 2008; FAO, 2007**).

Perspective chance how to preserve endangered animal species (breeds) appears to be the use of stem cells. Of course, before their use the cells have to be collect, cultivate *in vitro* for the purpose of their proliferation, evaluated in the terms of originality and quality for the subsequent freezing. Thawed stem cells could be used either for the reproductive cloning when new individual has the genetic information from the nucleus of thawed stem cell or for the genetic modification of thawed stem cells (iPSc) and desired differentiation for the purpose of gamete (spermatozoa or eggs) generation of endangered species or breed (**Ben-Nun et al., 2011**).

Stem cells (SC), mainly embryonic, but also of other origin are extraordinary, because they afford an opportunity to study developmental biology and potential source of cells useable for cell or tissue regeneration. Embryonic stem cells lineages were successfully obtained from more than 12 animal species including rabbits (**Fang et al., 2006; Wang et al., 2007; Catunda et al., 2008; Honda et al., 2008**), but still remain some unsolved questions, mainly about successful production of isolated and defined embryonic stem cell generations related to the differences between rabbit breeds.

In the time when Slovak agriculture and mainly the animal production permanently year by year achieve decrease in the farm animal number it is necessary to be aware of the fact that the farm animal breeding has not only production function, but also out of production

functions which supply culturally country cultivation, countryside development etc. In the last fifty years there was a significant decrease in the animal number in SR and in some species the origin, autochthonous breed completely disappeared (**Chrenek et al., 2011**).

Standard microsatellite panels will be used for the molecular-genetic characterization of the individuals and subpopulations included in the programme for animal genetic resources protection according to ISAG methodology, what enables to compare results in the international scale. Besides the standard panels also other microsatellite markers will be tested which together with chosen markers for agricultural valuable marks will provide new and complex knowledge about genotype of the tested animals.

Protocols for the genotyping of selected endangered or original farm animal breeds will be developed on the basis of markers (microsatellites, genes) and genetic characterization of the biological material meant for the cryoconservation will be carried out within the solved project. The collection of DNA samples in a required purity and amount will be created for the purpose of the long-term storage. Obtained results will be part of the archived documentation about genetic resources and will be open for the comparative studies, breeding programs within SR as well as for the foreign institutes involved in the programme for animal genetic resources protection, mainly national gene banks.

The protection of farm animal gene pool has no direct economic function. We can only assume that breeding of the Slovak Pinzgau cattle and sheep of Wallachian breed, representatives of the mountain breeds adapted to the pasture conditions on the mountain up to alpine meadows and pasturelands, will preserved a piece of tradition from the regions of north Slovakia. The milk of Pinzgau breed is rich in proteins and from them especially rennet type of casein (kapa-casein). Therefore this milk is more suitable for the cheese production.

Wallachian sheep belongs to the most primitive sheep breeds with the multiple efficiencies. Although it has coarse wool assortment, lower milk and meat yield, its outstanding feature is great walking ability and resistance against unfriendly environment conditions. It is very suitable for the farming in the alpine regions what helps to maintain cultural character of the country. Similarly also Oravka hen belongs to the breeds with multiple efficiencies. It was bred on the basis of the domestic fowl from the north regions of Orava. It is adapted for the extremely cold weather and so mainly by the crest type and high reaction ability against flying predators.

All mentioned breeds belong to the cultural heritage of the Slovak nation and are suitable for small farming mainly in the submontane and mountain regions of Slovakia. They fit in the production and sale scheme of agricultural products from the yard (milk, cheese,

eggs and meat), so they participate in the maintaining of countryside settlement and contribute to the nutrition of population. Changing of the consumer orientation on the domestic products purchase may help Slovakia not to be a market for not always the high-quality products of the developed economics. The whole added value remain in Slovakia by the purchase of the domestic product from the domestic manufacturer.

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