

## EX-SITU CONSERVATION OF ANIMAL GENETIC RESOURCES IN SLOVENIA

Drago KOMPAN, Andrej ŠALEHAR, Angela CIVIDINI, Mojca SIMČIČ, Peter DOVČ

University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Groblje 3, SI-1230 Domžale, Slovenia

### ABSTRACT

*Ex situ (in vitro)* conservation has numerous purposes. The population of live animals can become extinct because of different reasons. Slovenian legislation for the preservation of animal genetic resources include the Agricultural Act (2000), the Livestock Breeding Act (2002) and the Regulation on Conservation of Farm Animal Genetic Resources (2004). Gene bank (*ex situ* conservation) in vitro was established for the specific indigenous and other breeds classified as critical or endangered pursuant to the provisions of the Regulation Concerning Genetic Reserves. According to the "Regulation on Conservation of Farm Animal Genetic Resources" for the continuous monitoring of biodiversity in animal husbandry in Slovenia, a Register of Breeds with a Zootechnical Assessment was prepared. The aim of *ex-situ* gene conservation is the preservation of gene information in the form of isolated DNA or the frozen tissues which could be used for the isolation of DNA and for the preparation of genome groups (libraries). For this purpose we established and build a collection of population samples that enables gene variability of populations and the assessment of gene distances between populations. Our depository comprises of cattle, horse, sheep, goat, and pig tissue samples. Mostly they are blood and skin tissue samples. Very often they are already supplemented with the isolated DNA. The selection of AnGR preservation methods with cryopreservation greatly depends on the final purpose which is used for the performance of such programmes. Genetic material can be collected in the form of semen, eggs, embryos and in the form of somatic tissues. In Slovenia we have quite some of genetic material already stored. Mostly, it is bull semen preserved in the insemination centres. Slovenia has a long tradition in the conservation of farm animal genetic resources (FAnGR). The Lipizzan horse is one of the oldest cultivated horse breeds in the world. Its name is closely related to Lipica (Slovenia), the place of origin of the Lipizzaner breed, situated in the Slovene Karst (slovenski Kras) region, founded in 1580. In the last two decades Slovenia established, with the financial help of the government, some programmes for the research and to support the programme for the conservation of local breeds.

### INTRODUCTION

*Ex situ (in vitro)* conservation has numerous purposes. The populations of live animals can become extinct because of different reasons (contagious diseases). The stores of conserved genetic material make it possible for breeds to survive. *Ex situ* conservation is important also from the point of conservation of genetic structure in breed population which is not endangered. Selection and gene input from other populations effects the rearing economy. Both can also cause the individually positive traits to get lost, which could be even more important in the future. However, the small animal populations are effected by random gene flow and can also change the genetic structure of the population (Kastelic and Terčič, 2007).

The Regulation on conservation of FAnGR has been established gradually. The first part of Slovenian legislation can be found in the Agricultural Act (2000), which demands the conservation of agricultural biodiversity (agricultural plants and animals). It states, that the conservation on agricultural biodiversity programs was done by public services.

Livestock Breeding Act, approved in 2002, comprises of chapter 6 titled: Conservation of Genetic Variability and Genetic Reserves of Farm Animals.

The following articles are earmarked for that purpose:

- Article 66: Conservation of genetic variability
- Article 67: Biotic diversity in animal husbandry
- Article 68: Indigenous breeds
- Article 69: Monitoring and analysing of biotic diversity in animal husbandry
- Article 70: Special protection of the indigenous breeds of *Apis Mellifera*.

Regulation on conservation of farm animal genetic resources, approved in 2004, comprises the following chapters:

- general provisions,
- livestock biodiversity,
- genetic variability,
- recognition of new breeds and lines of farm animals,
- indigenous breeds of farm animals,
- traditional breeds,
- gene bank of agricultural animal gene resources.

Gene bank (ex situ conservation) *in vivo* (farms outside the original environment, especially in the protected areas and national parks, educational and research farms) shall be established for specific autochthonous and other breeds, when the number of breeding animals in gene banks *in situ* becomes lower than the number of pure-bred breeding dams. The number and the ratio between dams and sires shall enable mating of non - relatives, or, if this is not possible, ensure the least level of mating between relatives. All autochthonous breeds referred to in the Act and the breeds recognised pursuant to the Act are included in the gene bank. The same provisions as established in the previous paragraph shall apply to traditional breeds classified as critical and endangered (Regulation..., 2004).

Gene bank (ex situ conservation) *in vitro* was established for specific autochthonous and other breeds classified as critical or endangered pursuant to the provisions of the Regulation concerning genetic reserves (Regulation..., 2004).

ERFP (European Regional Focal Point), acknowledged by FAO as a global net for the conservation of animal genetic resources in Europe, published in the Guidelines for the Constitution National Cryopreservation Programmes for Farm Animals in 2003.

## THE STATE OF FARM AnGR IN SLOVENIA

According to the “Regulation on conservation of farm animal genetic resources” for the continuous monitoring of biodiversity in animal husbandry in Slovenia, a Register of breeds with a zootechnical assessment was prepared, also available on the web site: [http://www.bfro.uni-lj.si/Kat\\_center/genska\\_bank/Default.htm](http://www.bfro.uni-lj.si/Kat_center/genska_bank/Default.htm).

In 2006 the Register of breeds with a zootechnical assessment included 13 autochthonous breeds, 16 traditional breeds, 18 foreign breeds. With the exception of one breed all the other autochthonous breeds have the accepted and legally verified breeding programme. For the whole group of traditional breeds, breeding programmes have been accepted and legally verified, representing the basics for the preservation of these breeds. The most important zootechnically assessed breed groups, entered to the Register are seen in Table 1.

Table 1: Most important zootechnically assessed breed groups in the agriculture of Slovenia (Register of breeds with a zootechnical assessment, 2007)

| Breed group     | No. of breeds | Breeding programme | Basic zootechnical characterisation | Gene bank*   | Depository    | Breeding value | Zootechnical assessment and measures |
|-----------------|---------------|--------------------|-------------------------------------|--------------|---------------|----------------|--------------------------------------|
| Autochthonous** | 13            | 12<br>(92,3%)      | 13<br>(100%)                        | 9<br>(69,2%) | 11 (84,6%)    | 5<br>(38,5%)   | 13<br>(100%)                         |
| Traditional**   | 16            | 16<br>(100%)       | 16<br>(100%)                        | -            | 7<br>(43,8%)  | 9<br>(56,3%)   | 16<br>(100%)                         |
| Foreign**       | 18            | 13<br>(72,2%)      | 18<br>(100%)                        | -            | 8<br>(44,4%)  | 5<br>(27,8%)   | 17<br>(94,4%)                        |
| Total           | 47            | 41<br>(87,2%)      | 47<br>(100%)                        | 9<br>(19,1%) | 26<br>(55,3%) | 19<br>(40,4%)  | 46<br>(97,9%)                        |

\*number of female breeding animals *in situ*

\*\*definitions of the breed groups are available on

[http://www.mkgp.gov.si/fileadmin/mkgp.gov.si/pageuploads/saSSo/Microsoft\\_Word-Priloge PRP 2007 - 2013 26.1.07.pdf](http://www.mkgp.gov.si/fileadmin/mkgp.gov.si/pageuploads/saSSo/Microsoft_Word-Priloge_PRP_2007_-_2013_26.1.07.pdf)

The analysis of the most important zootechnically assessed breeds produced in Slovene agriculture, entered into the Register showed, that the state of biodiversity conservation in Slovenia can be considered as satisfactory. The basic zootechnical characterisation is known for all breeds. There are six breeds without the accepted and legally verified breeding programme. The usage of breeds and the implementation of breeding programmes are considered the most important terms under which the FAnGR conservation can be efficient. The *in situ* gene bank includes nine autochthonous breeds, and there are tissues of 26 breeds preserved in the tissue deposit bank. Breeding value is calculated for nineteen breeds (40.4 %), while the zootechnical measures are accepted for 46 breeds (96.9 %).

Expert consideration of the basic zootechnical definitions was accomplished in Slovenia in 2006, including definitions and classification of breeds and was published on DAD-NET on 5<sup>th</sup> December 2006.

## THE STATE OF EX SITU GENE CONSERVATION

### Depository

The aim of *ex-situ* gene conservation is the preservation of gene information in the form of isolated DNA or the frozen tissues which could be used for the isolation of DNA and for the preparation of genome groups (libraries). The material could later be used for the isolation of genome regions and transfer to the other genetic background, where the alleles diminished (very frequent example are genes for resistance). *Ex-situ* conservation is a rather cheap way for FAnGR preservation, however it does not allow the total reconstruction. It gives the possibility for later genome DNA analysis of a certain species, breed, selection line (i.e. purity testing).

For this purpose we established and build a collection of population samples that enables gene variability of populations and the assessment of gene distances between populations. Our depository comprises of cattle, horse, sheep, goat, and pig tissue samples. Mostly they are blood and skin tissue samples very often they had already been supplemented with the isolated DNA. The depository also contains samples of other tissues that were taken in the frame of functional studies, representing a good source of RNA specialised tissues or special physiological states (mammary gland in lactation, fat tissue). The samples are registered in laboratory evidence. Its major part being in electronic form and thus enabling a quick overlook of depository content (Kunej et al., 2007). A list of different sample categories preserved in the depository is presented in Table 2.

Table 2: A list of samples preserved in the depository (Kunej et al., 2007)

| SPECIES | Autochthonous Breeds        | No. | Type of tissue | DNA |
|---------|-----------------------------|-----|----------------|-----|
| HORSE   | The Posavje horse           | 64  | B              |     |
|         | The Slovenian cold-blooded  | 204 | B              | √   |
|         | The Slovenian warm-blooded  | 28  | B              | √   |
|         | The lipizzan horse          | 896 | B              | √   |
| CATTLE  | The Cika cattle             | 9   | B              | √   |
| PIG     | The Krskopolje pig          | 121 | T              |     |
| SHEEP   | The Bovec sheep             | 45  | B              |     |
|         | Istrian pramenka sheep      | 46  | B              | √   |
|         | Jezersko-solcava sheep      | 55  | B              |     |
|         | Bela Krajina pramenka sheep | 6   | B              |     |
| GOAT    | The Dreznica goat           | 45  | B              |     |

(No.=Number of samples, B = blood, T = tissue sample , DNA = Deoxyribonuclein acid)

The selection of FAnGR preservation methods with cryopreservation greatly depends on the final purpose which is used for the performance of such programmes. Most demanding and most expensive among them are programmes that aim to embryo conservation in later, preimplantation phases of development (morules, blastocites). These could be very efficiently used for breed

reconstruction with relatively low costs. The principle here is following the motto: "pay now, use later". In such strategies the preimplantation foetus can also be used. Most often used material in cryopreservation programmes represent:

- semen
- oocyte
- foetuses in preimplantation phases
- somatic cells (granulosa of the cells, lymphocytes, fibroblasts)
- tissue samples (skin, ovaries, liver).

When mating the selection of species and breeds to be included to gene bank, the following points should be considered:

- success in conservation of genetic material in separate species of domestic animals
- present and future value of species and breed in certain country – the extent of participation in national agriculture is important
- available infrastructure
- available sources and expected costs
- the value of population which we intent to conserve
- the endangerment degree.

Genetic material can be collected in the form of semen, eggs, embryos and in the form of somatic tissues (Kunej et al., 2007).

Freezing of semen has been a relatively well known method for quite some time. With the exception of cattle, the frozen semen has a restricted usage in the production process. In case of the conservation of genetic resources the main point is not economic success, but the possibility to preserve genetic material.

In Slovenia we have quite some of genetic material already stored. Mostly, it is bull semen preserved in the insemination centres. At the moment the semen is not declared as gene bank, neither is it described in such manner. The technology for freezing of semen in other species except the semen of bulls, is not on the high enough level in Slovenia.

Gene bank in Slovenia is a government financed programme that has not as clearly stated activity structure yet, as prepared in other countries. The programme includes breeding organisations, so that its application actually presents the cooperation of the state, that private sector together with its organisations, as well as scientific institutions. So far the gene bank included mostly live animals, supported by the government through direct payments, therefore such loose organisation has been sufficient. Larger cryobank requires permanent financial sources and professional management, so a working scheme that will follow the ERF recommendations, would have to be worked out (Kastelic and Terčič, 2007).

### **Genetic reserves – bull semen**

From the zootechnical point of view the conservation and maintenance of genetic resources in the form of bull semen is not satisfactory solved. The approved insemination centres make semen banks for separate bulls, but this kind of work is not planned, nor is it systematic. The fact is that the Republic of Slovenia is obligated to preserve genetic reserves for different species, breeds and lines of domestic animals in the form of minimal number of domestic animals semen doses, egg cells or embryos (Article 66 of LBA) and to assure the systematic monitoring and analysing biodiversity in animal production (Article 66 of LBA). This obligation also stands for bull semen.

The approved insemination centres should, in accordance with the accepted breeding programmes, perform the provisions regarding the conservation of biodiversity in animal production and the preservation of gene variability (3<sup>rd</sup> paragraph of Art. 61, LBA). Therefore we are convinced that the field of gene variability conservation needs to be professionally and systematically arranged.

To assess the required genetic reserves – bull semen and at the same time respect the provisions listed in the Regulation on Conservation Farm Animal Genetic Resources. The global zootechnical assessment for each cattle breed needs to be worked out. It will serve as a base for the selection of sires that will have to be included in gene bank, as well as determine the necessary numbers of

semen doses for each sire. In the cooperation with the approved insemination centres we shall prepare the assessment of costs required for the conservation of biodiversity in cattle.

## CONCLUSIONS

Slovenia has a long tradition in the conservation of farm animal genetic resources (FAnGR). The Lipizzan horse is one of the oldest cultivated horse breeds in the world. Its name is closely related to Lipica (Slovenia), the place of origin of the Lipizzaner breed, situated in the Slovene Karst (slovenski Kras) region, founded in 1580. The conservation of FAnGR in the last century was sporadic and as a consequence many breeds have been lost in Slovenia. Some activities for the protection of breeds have been proposed by some institutions or some individuals since 1983, but in that time there has been no initiative from the government and politics. They even frequently disagree with the conservation of the old local breeds. In the last two decades Slovenia established, with the financial help of the government, some programmes for the research and to support the programme for the conservation of local breeds. The first multiyear (2001-2008) programme for the conservation of FAnGR became approved in 2001. The first regulation on this subject was accepted no more than 6 year ago in the Agricultural Act and especially in Livestock Breeding Act for local breeds in the year 2002. The special Regulation on Conservation of Farm Animal Genetic Resources was approved in 2004.

The recent needs and future expectations are adapted to the strategy in the preparation of the original cell cultures of the most endangered breeds (species) and their cryopreservation. This process could enable the multiplication of genome DNA and its typisation after the repeated planting of cell culture, which would at the same time represent a possibility to obtain nuclei for the nuclear transfer. This part of the programme is, due to the extremely low clone efficiency, questionable, although it represents the likely reasonable alternative to the frozen fetuses.

The small population of live animals can very rapidly become extinct due to a sudden outburst of contagious and other diseases. In such cases gene bank with its compulsory genetic reserves, should play an important part in the reconstruction of the lost breed. Additional reserves are essential in small breed populations, namely in the form of semen to take part in the blood refreshment, especially in cases of too great population unification or to refresh separate traits that diminished due to selection or other reasons. When the small populations are endangered, such reserves are financed by the government in Slovenia. Reserves used for the refreshment of lost traits would be relevant also for larger populations, where breeding organisations would have to be involved too.

## REFERENCE

1. Agricultural Act. 2000. Official Journal of the Republic of Slovenia, No. 54/2000.
2. ERF (European Regional Focal Point). 2003. Guidelines for the constitution of national cryopreservation programmes for farm animals. Publication No. 1 of the European Regional Focal Point on Animal Genetic Resources. Himestra S.J. (ed.): 54 p.
3. Kastelic, M., Terčič, D. 2007. Proučitev pogojev za vzpostavitev ex situ (in vitro) genske banke za avtohtone in tradicionalne pasme vseh pomembnejših vrst domačih živali (govedo, konji, drobnica, prašiči, kokoši) In: Ohranjanje biotske raznovrstnosti v slovenski živinoreji. Poročilo za leto 2006. Domžale, University of Ljubljana, Biotechnical Faculty, Zootechnical department, p.374-380.
4. Kunej, T., Sušnik, S., Frajman P., Snoj, A., Razpet, A., Hobor, S., Flisar, T., Božič, A., Malovrh, Š., Dovč, P. 2006. Molekularno genetske naloge In: Ohranjanje biotske raznovrstnosti v slovenski živinoreji. Poročilo za leto 2006. Domžale, University of Ljubljana, Biotechnical Faculty, Zootechnical department, p.475-490.
5. [http://www.bfro.uni-lj.si/Kat\\_center/genska\\_banka/Default.htm](http://www.bfro.uni-lj.si/Kat_center/genska_banka/Default.htm)
6. [http://www.mkgp.gov.si/fileadmin/mkgp.gov.si/pageuploads/saSSo/Microsoft\\_Word-Priloge\\_PRP\\_2007\\_-\\_2013\\_26.1.07.pdf](http://www.mkgp.gov.si/fileadmin/mkgp.gov.si/pageuploads/saSSo/Microsoft_Word-Priloge_PRP_2007_-_2013_26.1.07.pdf)
7. Livestock Breeding Act. 2002. Official Journal of the Republic of Slovenia, No. 18-716/2002.
8. Register of breeds with a zootechnical assessment. 2007. Ohranjanje biotske raznovrstnosti v slovenski živinoreji. Poročilo za leto 2006. Domžale, University of Ljubljana, Biotechnical Faculty, Zootechnical department, p. 2-153.
9. Regulation on conservation of farm animal genetic resources. 2004. Official Journal of the Republic of Slovenia, No. 90/2004.