Reproductive and molecular tools for animal genetic resources conservation and breeding

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SUMMARY

Considering the biodiversity and animal breeding national and international issues, the present paper aim to highlight the importance and the complexity of reproductive and advanced genetics methods involved in farm animal biodiversity conservation and animal breeding perspectives. Gene banking is enabled through the available and the potential of the reproductive technologies, cryopreservation and molecular genetics techniques, assuring animal genetic resources conservation. The limits and the relationship required by an efficient management system for animal resources concur to lay out putative alternatives for gene banking and animal breeding and improvement programmes.

Keywords**:** animal genetic resources, conservation, reproductive technologies, molecular techniques, gene banking

INTRODUCTION

Normal species extinction rate on a geological time scale is one species every 10,000 years. By 1950, however, the rate had increased to one species every 10 years and today it is estimated at one or more species per day. Global diversity in farm animals is considered to be under threat. Worldwide, a large number of domestic animal breeds is endangered, in a critical status or already extinct. The world's animal population is constantly being updated to meet the demands for high productivity. In global animal husbandry an accelerated rhythm is directing to high specialized breeds, the percent of extinct breeds and/or at risk of extinction having a fast, uncontrolled growing rhythm. Unfortunately, our native animal genetic resources have not been balanced properly; some are already extinct and some are at the edge of extinction. Many livestock breeds are vulnerable and could not be replaced in the case of

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natural decline or disaster. Animal genetic resources must be considered the insurance of the future, since they may have an important potential to improve social and economic life. A.N.A.R.Z. stands up for strategies that should focus on breeds that are of potential economic value and both, endangered and represent types with unique biological characteristics and programmes concerning the animal breeding sector, specifically animal improvement and reproduction area. The advanced reproductive and genetics technologies provide powerful tools for animal biodiversity conservation and breeding.

STATUS OF FARM ANIMAL LIVESTOCK GENETIC RESOURCES

A large number of domestic animal breeds are endangered worldwide, in a critical status or already extinct. According to FAO (2007), of the 6,379 domestic animal breed populations in Europe, 9% are in critical condition and 39% are endangered. Animal genetic resources are disappearing rapidly worldwide. Over the past 15 years, 300 out of 6,000 breeds identified by FAO have become extinct. Many breeds of local importance for food security are not being improved or utilized in a sustainable manner and are in danger of being lost or diluted by crossbreeding (Ruane and Sonnino, 2006).

The World Watch List for Domestic Animal Diversity (FAO, 2000) lists nearly 4,300 breeds of buffalo, cattle, goat, pig, and sheep and over 900 breeds of chicken, duck, goose and turkey. Nearly 1,300 breeds are listed as at risk and FAO (2000) Guidelines for Management of Small Populations at Risk recommend collection of frozen semen from at least 25 males per breed and use of semen from these males on an additional 25 females per breed to produce frozen embryos. For cattle, this would involve 300 endangered breeds and require cryopreservation of semen from 7,500 males and of approximately 100,000 embryos.

Conservation and development of local breeds is important due to the lower quality feed, higher resilience to climatic stress and local parasites and diseases, representing a unique source of genes, for improving health and performance traits of breeds. Breeds disappearance or drastic modification is caused by crossbreeding, absorption or replacement by other breeds (Ruane and Sonnino, 2006).

Considering the breeds at risk issue, the value of a breed is given through its genetic distinctiveness, adaptive traits, relative utility value for food and agriculture and historical and cultural use. An efficient management of animal genetic resources within a country compiles accurate and objective inventory and monitoring systems for strategic conservation and genetic improvement programmes. Livestock animal inventory involves identifying the status of breeds (population size and structure, geographic distribution of the breed), the risk status, breeds at risk and the value of breeds.

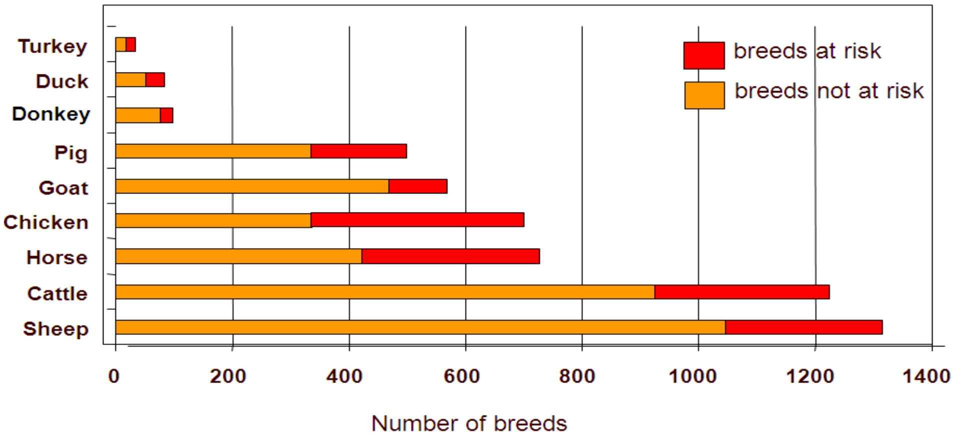

Figure 1. Breeds of farm animals at risk around the world (Hoffmann et al., 2004)

Monitoring is necessary to evaluate progress in the implementation of action plans and to identify new priorities, issues and opportunities (Kantanen, 2011). An objective status of farm animal breeds allow the set up efficient in vivo and in vitro conservation programmes and also genetic improvement programmes tended to pure/straight breeding and cross-breeding.

The objective number of animal resources in Romania will be revealed at the end of the agricultural census 2010, when data collected will be processed. All these information come to back up and to fundament an objective strategic management of animal genetic resources for our country. The number of animals in Romania is following up to decrease, which is really concerning as much as the pure breeds are disappearing or are under the threat of crossbreeding. Şonea et al. (2010) and Rosu et al. (2010) contend such assertion based on the studies performed. Thereby, Romania has to overdraw immediately the basic animal genetic threats, including: the failure to match genetic resources to the production environment, development of an adequate infrastructure for animal genetic resources improvement, development of valuable *ex situ* collections and raising the efficiency of institutional capacity to manage such programmes.

GENETIC RESOURCES CONSERVATION USING REPRODUCTIVE TECHNOLOGIES AND CRYOPRESERVATION

Conservation of animal genetic resources is important mainly for recovering the lost/decreased genetic variation and the specific genes or important genetic information that could be not yet discovered. Conservation strategies must be designed to maintain the genes or gene combinations that might be of use, the advantages of heterosis, to use the present resources as the insurance of the future and for research purposes. The reproductive technologies applied are artificial insemination, embryo transfer, reproductive cloning and germplasm cryopreservation; artificial insemination is now the most used reproductive technique in our country. Transplantation of ovarian tissue and germ cells (even spermatogonial stem cells) are emerging technologies with potential for future use in conservation programmes (Ruane and Sonnino, 2006), that may enable production of gametes or offspring of rare or extinct breeds by abundantly available individuals of related common breeds (Hiemstra and Woelders, 2007).

Rapidly developing technology enables to apply cryopreservation techniques to different farm animal species like cattle, sheep, swine, poultry (Kocaeli, 2005). Regarding semen cryopreservation there is a good survival rate after thawing for most species, but also a variability in freezability among species, breeds/lines and males; the most important is that mitochondrial genes are not conserved and also back-crossing is required, at least for 6 generations (to recover the elite genotype) (Hiemstra and Woelders, 2007).

Considerable progress with oocyte cryopreservation has been achieved in last 10 years; showing good results in oocytes viability after thawing in great number of species, but the efficiency of in vitro embryo production still require further studies (Hiemstra and Woelders, 2007). There are important improvements in extracting embryonic stem cells and germinative cells, and their cryopreservation. Reports on the production of germ cells from embryonic stem cells shows that such technology can be used in conservation studies in the future (Kocaeli, 2005). Cryopreservation of embryos/embryonic cells is adequate for mammalian livestock species (highly successful in cattle, recent good results in pigs); but non-surgical embryo transfer procedures are available in cattle, horses and developing in pig; the major advantages consist in full complement of chromosomal and mitochondrial genes and no backcrossing needed (Hiemstra and Woelders, 2007).

Cryopreservation of somatic cells is available for different cell types (fibroblast), using relatively simple cryopreservation techniques, but the use of somatic cells to generate offspring requires reproductive cloning. Somatic cells could be an alternative for semen/embryos as: in mammals, live offspring was reported in sheep, cattle, mice, pigs, goats, horses, rabbits and cats (no successful cloning in poultry), the proof of full complement of chromosomal, but no mitochondrial genes, after transfer of nuclei. Still reproductive cloning is not yet efficient and not safe (<4% transferred embryos develop into viable offspring) (abortions and malformed young) (Hiemstra and Woelders, 2007).

Cryopreserving healthy germ cells and embryo may help to eradicate animal health problems that might emerge in the future, as well (Piltti et al., 2004).

GENETIC RESOURCES CONSERVATION USING MOLECULAR TECHNIQUES

Molecular markers are a powerful tool to study genetic diversity and germplasm sampling for gene banks; the most widespread use of molecular markers being to assess diversity within and between breeds. Microsatellites are used in 90 % of all diversity studies (Baumung et al., 2004). A standard set of microsatellite markers is recommended to be used for neutral genetic variability in the genome of major farm animal species, which is constantly reviewed and extended to a larger number of species (Hoffman *et al.*, 2004).

QTL markers reflect the genetic potential of an animal for a given quantitative or qualitative trait. Farm animal research focuses on mapping QTLs and single genes so that such markers will be increasingly available in the future. Of special interest are markers linked to disease-resistance QTL (Hanotte et al., 2003).

DNA markers used to analyze the genetic variation among breeds and among individuals within the breeds are: Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), microsatellite or Short Tandem Repeats (STR), Sequence Tag Site (STS), Expressed Sequence Tag (EST), Restriction Fragment Length Polymorphism (RFLP), Single Nucleotide Polymorphism (SNP), mitochondrial DNA sequencing (maternal inheritance: cytochrom b, cytochrom c oxidase 1, ND1, ND3-4, ND5-6 and D-loop), Y chromosome-specific SNPs and microsatellites (paternal inheritance) and autosomal microsatellites (bi-parental inheritance) (Avise, 1994), ribosomal subunit proteins and Major Histocompatibility Complex (MHC) loci sequences (Shivaji et al, 2003).

Mitochondrial DNA markers are particularly useful for studying evolutionary relationship among various taxa. Among the nuclear markers, AFLP has greater differentiation power than RAPD, though RAPD is a comparatively more simple and least–expensive method. Both microsatellite and AFLP are highly powerful markers in determining the genetic diversity. Microsatellites are rarely used for high–level systematics however, but are the best for parentage and strain analysis (Arif and Khan, 2009).

These markers are employed in conservation studies aiming to identify the rare and important breeds, and the populations having high genetic diversity (Hall, 2004). Interestingly, while recent developments in cytogenetic technologies should facilitate the isolation of Y chromosomes specific markers, for most livestock species there are still few Y polymorphic markers. (Ruane and Sonnino, 2006). Studies on phylogenetic relationships between breeds concur in identifying the priorities in conservation of the animal genetic resources (Reist-Martive et al, 2003).

GENE BANKING

Gene banks storing animal germplasm and tissues are a relatively new construct to conserve animal genetic resources (Blackburn et. al., 2009). Farming methods are continually evolving, and there is an often a need to go back to bloodlines adapted to specific tasks. Developing gene bank germplasm collections for animal genetic resources requires establishing germplasm collection goals, that consider capturing the genetic diversity of the population in question and the amount of germplasm required for its reconstitution or other purposes, or both (Blackburn et. al., 2009). Repositories include the environment in which the genetic resource has developed, or is now normally found (*in situ*) or facilities elsewhere (*ex situ – in vivo* or *in vitro*). For *in vitro*, ex situ genome bank facilities, germplasm is stored in the form of one or more of the following: semen, oocytes, embryos or other samples.

The gene bank provides a comprehensive DNA archive for farm animal genetic resources; furthermore it helps to keep track of rare alleles. Gene banking in cryobanks alone certainly cannot keep a breed alive but it can be a valuable help in managing inbreeding in small populations, preventing loss of genetic variability by genetic drift or selection and preserving and promoting endangered lines (Berger and Fischerleitner, 2008). There are two levels for sampling donors for gene bank: breeds that must be included and sampling of individuals within selected breeds. Individual genotypes need to be identified to become part of the conservation scheme. A gene bank mainly aims to keep animal genetic material for long term storage; a part of the genetic resources could be allocated to approved conservation programmes such as contract mating, testing for viability, fertility and gene marking; it also could be available for the free use of people who donated them in the first instance or for general use, but with a limitation imposed for any purchaser, to be used only for pure breeding.

CONCLUSIONS

Embryos cryopreservation is the most powerful tool for breed reconstruction, followed by semen and somatic cells, where further studies are still required to eliminate inconvenient. Semen cryopreservation seems to be recommended in synthetic breed creation and gene introgression as an important source of genetic variation in livestock populations.

An imperative issue that must be considered and carried through is the implementation of molecular techniques concerning: DNA repositories for research and development, genetic distancing, identification of parentage and genetic relationships (DNA fingerprinting), marker assisted selection using DNA markers, gene mapping, molecular analysis of DNA to identify carriers of known genes and maybe new alleles.

Romania needs to exceed the present animal genetic resources threats through adequate, immediate and objective national conservation and genetic improvement programmes, and to continue to support the reproductive techniques. Only objective animal resources inventory and monitoring, reproductive, cryopreservation, molecular, database and gene banking activities corroborated and interrelated will stand up for the animal breeding sector in our country.

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