IN VITRO CONSERVATION AND PRILIMINARY MOLECULAR IDENTIFICATION OF SOME TURKISH DOMESTIC ANIMAL GENETIC RESOURCESI (TURKHAYGEN-I)

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INTRODUCTION:

Animal genetic resources are considered as a part of the present biodiversity. Unfortunately; our native animal genetic resources have not been appreciated as it should have been; some are already gone extinct and some are at the edge of extinction. Yet, animal genetic resources must be considered as the insurance of the future, since they may have an important potential to improve social and economic life.

Conservation of animal genetic resources is important; because, there might be a need of recovering the lost/decreased genetic variation and they might be possessing special genes or important genetic information. Conservation strategies must be planned (i) not to lose the genes or gene combinations that might be of use, (ii) to make use of the advantages of heterosis, (iii) to use the present resources as the insurance of the future, (iv) to preserve the cultural products, and (v) to use the present material in diverse research fields. In fact, animal genetic resources have been being conserved upon national conservation projects in economically developed countries (Hiemstra and FAO, 2003). Conservation of animal genetic resources includesinsituandex-situprograms (Ertuğrul M, 2005; Đhsan S., 2004; Hiemstra and FAO, 2003).

The animals are conserved alive at their native place in context ofinsituprogram.Ex-situconservation programs include both conserving the animals alive in a place different than their native place like zoo or breeding farm; and conserving the material (e.g. cryopreservation) taken from the animal like gamete, embryo, sperm, tissue or cell, and DNA. These conservation

methods have become more effective with the new advances in the modern biology, more specifically, in molecular genetics and biotechnology (Matsas et al, 2004;Shivaji et al, 2003; Mariante et al, 2002). Cryopreservation technique makes possible the long term storage of high number of samples in small volume containers. Moreover, rapidly developing technology enables to apply cryopreservation techniques to different species like cattle, sheep, keçi, at, swine, poultry (Piltti et al, 2004; Stachecki et al, 2004; Bağış et al, 2004; Aller et al, 2002; Leibo and Songsasen 2002; Dobrinsky JR 2002 and Dobrinsky JR 2001). There are important improvements in extracting embryonic stem cells and germinative cells, and their cryopreservation. It can be depicted that these topics will define the agenda in the future. Reports on the production of germ cells from embryonic stem cells shows that this technology can be used in ocnservation studies in the future (Zwaka and Thomson 2005; Wakamaya et al., 2005; Geijsen et al., 2004; Lacham and Kaplan 2004; Toyooka et al., 2003). In the future, using the cryopreserved sperm would only need the female individuals of the population in restoring the decreased genetic diversity in the population as well as the decreased population size. Moreover, cryopreserved embryo and sperm might enable us to bring back the individuals of a lost breed. 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; Stachechi et al, 2004).

Studies on resolving the phylogenetic relationships between breeds would be helpful in identifying the priorities in conservation of the animal genetic resources (ReistMartive et al, 2003). There are numerous DNA markers to analyze the genetic variation among breeds and among individuals within the breeds, for example, Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), mikrosatellite 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 (cytochrom b, cytochrom c oxidase 1, ND1,ND34, ND56 and Dloop), ribosomal subunit proteins and Major Histocompatibility Complex (MHC) loci sequences (Shivaji et al, 2003). These markers are employed in conservation studies aiming to identify the rare and important breeds, and the populations having high genetic diversity (Hall, 2004).

Embryo cloning techniques might also be employed in conservation of animal genetic resources

for different purposes. As the technology develops, there will be an increase in the application ways of these techniques. Recent advances in science more specifically in nuclear transfer (NT) technology are promising. There are successful studies on cloning of an adult individual using NT as well as on NT between closely related species, which indicated the possibility of restoring the populations having few members left and saving the species facing extinction (Chen et al., 2002; Loi et al., 2002; Kitiyanant et al, 2001; Loi et al., 2001; Arat et al., 2004, 2003, 2001). That is why, several US biotechnology companies, agricultural organisations and zoos have started to construct tissue and cell banks (Ryder et al, 2001).

Turkey, which has a great number of endemic species, has an important share in the genetic resources of the earth. Therefore, Turkey must conserve its biodiversity and make use of it where necessary, which is accepted as the indicative of economic and genetic richness, and is proved to be useful in medicine, agriculture and industry. In addition, biologic diversity is connected with populations' ecologic, cultural and spiritual richness and with its past. However, the increase in the population size as well as the economic pressure speed up the changes in traditional farming techniques and causes the decrease in biodiversity.

Animal genetic resources are considered within the context of biodiversity components, and they also meet the world's demands in food and agriculture. The production traits of native breeds have been affected by the ecological, sociological and economical features of populations and in time the breeds having high productivity traits are preferred over low productive indigenous breeds.

As in other countries, the breeding and hybridization practices are being conducted and highly productive animals are being imported to increase the productivity of local breeds of Turkey. Consequently, this brought about the decrease in biodiversity of farm animal genetic resources and endangered their existance. Thus, the importance of preserving the balance between breeding studies and conservation of the animal genetic resources was revealed.

The archeological and genetic data indicates that domestication centers for cattle, sheep, goat, and probably swine are in the southwest Asia including a part of Anatolia (Bruford et al, 2003).

Our native breeds, being the closest relatives of the first domesticates, must be given priority in conservation (Bruford et al, 2003). Molecular genetics studies have shown that the European cattle, sheep and goat breeds have originated and spread from Anatolia ((Loftus et al., 1999; Troy et al., 2001, Bruford and Towsend, 2004, Lenstra et al., 2005). The information lost during this stage might still be present in Turkish native breeds. Yet, the 47% sharp decrease in the last two decades (Oskam et al, 2004), hybridizations with non native breeds and preferring non native highly productive breeds over native breeds are the important threats in losing of the genetic information present in our native breeds. It must be stated that employing molecular genetics in management strategies may also lead to loss of some genetic information. For example, using molecular genetics in breeding and management of livestock species in context of food safety as in eradication of diseases like BSA and scrapie (European Food Safety Authority Report, 2003), may cause losing some of the alleles. The management strategies must be constructed carefully and accordingly.

The importance of our native genetics resources is widely accepted andexsituconservation studies must be started immediately. Moreover, genetic studies must be carried out and their economic value must be estimated, the priority of the populations and/or breeds must be identified forin-situconservation. It has been shown that the gene pool of the populations in breeding farms can easily differentiate from the breed's gene pool (Koban, 2004) due to genetic drift and wrong management strategies. Therefore, the results of the proposed project will guide breeding farms in both choosing the individuals to conserve and constructing proper management strategies.

The results of the projects, which are either completed or ongoing, on Turkish native breeds will guide the proposed project. These studies include the linkage analysis between some production traits and blood and milk protein polymorphisms. Following the new advances in DNA technology, DNA markers have also been employed in Turkey in studying genetic variation with and among breeds, in locating the domestication centers, in pedigree analysis and in studying the production traits.

Conservation of the biological richness and diversity is one of the main concerns in most of the countries' agenda. Several different establishments in the world have been carrying out studies on

the conservation of the endangered species (Ryder, 2001; Millennium Seed Bank; Wildlife Conservation and Monitoring Center; Conservation on Biological Diversity; Japanese Ministry of Agriculture; Mariante et al., 2002; Matsas et al., 2004; Hiemstra and FAO, 2003). In Turkey, the establishment of a central bank or an institute with affiliated banks for conservation, thus enabling the sharing of the samples and knowledge among the country has been emphasized within the priority subjects in context of TÜBĐTAK (Turkish Scientific and Technological Research Council)'s Vision2023 Science and Technology Prevision Project. In addition, "Biodiversity Conservation Program" within the area of "Biotechnology and Genetic Technologies" was listed among the priority action topics by the Turkish Academy of Sciences (TÜBA) Molecular Life Sciences and Technology Foresight Project (20032023). Moreover, the importance of conservation of animal genetic resources was emphasized in the 1st Comission Report of the II. Agriculture Assembledge (2004). Furthermore, Turkey took part as contractor in the international agreements on conservation of the biodiversity and genetic resources. One of these agreements is the UN Convention on Biological Diversity. In the meantime, a commission to work on conservation of animal genetic resources was formed by FAO (UN Food and Agricultural Organisation) and started its studies. The proposed project will contribute to fulfil the commitments resulted from these agreements.

In conclusion, the reasons stated above and the "commonwill" on the conservation of native animal genetic resources resulted from the preliminary studies have lead the preparation of the proposed study. The project aims at conserving some of the native animal genetic resources and construction of banks to hand these on next generations.

METHOD A1 R&D Activities and Distribution of Data The Main Work Package 1 (WPI): The Preparation of Studying Material This project comprises to exsitu in vitro preservation methods. This WPI consists of assurance of living materials and identification of the breeds to be conserved in context of the project. Based on reports of national and international organizations and population records, the species and the breeds to be included in conservation studies were determined. Together with the onset of this project, it will be ensured that the genetic variation within populations chosen to be conserved will represent the genetic diversity of the breed. For each livestock species, 25 male and 25 female individuals will be chosen for the population. However, due to possible problems during the study, 12 male and 12 female individuals will also

be selected as back up. Thus, in total each population will have 74 individuals. During formations of those populations, non related individuals will be selected, only 13 animals will be bought in accordance with the flock's population size. Data (diseases about the region, vaccinations, sheltering and feeding) of the flock from which studied animals are bought will systematically be recorded to standard files. Location data will also be recorded by using GPS devices.

The Main Work Package 2 (WP2): Technological Development about Preservation of Live Material (normal and clone embryo, egg cell, somatic cell, embryonic cell)

In order to develop and ameliorate the technologies of live material preservation techniques, WP2 consists of several sub work packages. These sub packages employ techniques like tissue cryopreservation, using of alternative cell types and alternative auxiliary reproduction techniques, as well as embryo and gamete cryopreservation. Hence, in the context of WP2 while genetic sources of defined animals are protected by using routine technologies, development of some new technologies which would be oriented in future usages are planed.

The Main Work Package 3 (WP3): Preservation of Genetic Resources This work package consists of cryopreservation in order to protection of genetic resource of identified animals. Those resources are genomic DNA, somatic cell, tissue, embryo, germ cell.

Procedures taking part in the work package are going to be specified according to methods defined by international sources and international organizations such as FAO. The research staffs working on this package have sufficient experiences in these fields. At the same time, the researches of technology development group are going to ameliorate the accuracy of the procedures. For cryopreservation, 25 female and 25 male animals from each breed are going to be employed. However, due to difficulties in sample collection processes, the number of animals would be less than 25. For this reason, 24 backup individuals for sheep, goat, cattle and water buffalo species are going to be assigned in case of the possible problems. From each breed 300 dose sperm and 300 embryos should be cryopreserved. The ex situ and in vitro protected samples of those species and breeds will be kept in gene banks constructed in LHMAE/TAGEM and GMBE.

The Main Work Package 4 (WP4): Characterization of Species A comprehensive sample collection will be planned in the context of the proposed project. However, in genetic diversity and phylogenetic studies, since establishment of the necessary infrastructure and acquiring enough number of qualified staff will take remarkable time, most frequently used two molecular methods (microsatellite markers and mtDNA sequencing) are going to be employed. The Main Work Package 5 (WP5): Distribution and Standardization of Data WP5 consists of protocol standardization, distribution of data by constructing a database and a web page, getting in touch with several national and international organizations. Moreover, WP5 includes arrangement of seminars, congresses and media declarations to represent the project.

A2 Educational Activities

In the context of the project; (i), short and long term courses project's staff, and (i) short term courses for other researchers are going to be arranged.

RESULTS

Changing environmental conditions (dramatic increase in human population, global warming, irregular settlement, environmental pollution) negatively and inexorably affect to the world fauna and flora. Even though the importance of sustainability of biodiversity is vital; regarding the animal resources only as food material can make someone realize the urgency and severity of the topic. In all over the world, there is an increasing tendency among livestock breeders for inbreeding and artificial selection to improve the productivity. These are called as "culture" breeds having high productivity traits, but, their resistance to harsh environmental conditions and diseases were not taken into account during selective breeding. Those negative factors seriously threaten the future of lots of species and breeds. On the other hand, animal breeds still having genetic variations, are insurances of the future since those might be regarded as gene resources. Turkey is an affluent country for this purpose. However, if we do not conserve our natural richness, they will eventually be lost. Within the context of TÜBITAK Vision2023 Scientific and Technological Prevision Project, the establishment of a central bank or an institute with affiliated banks for conservation and construction of an intra national network which will provide data and material flow were especially considered as priority areas. In addition, "Biodiversity Conservation Program" within the area of "Biotechnology and Genetic Technologies" was listed

among the priority action topics by the Turkish Academy of Sciences (TÜBA)' Molecular Life Sciences and Technology Prevision Project (20032023). Besides, the importance of the topic was also mentioned in the First Commission Report of the Second Agriculture Council (2004).

To provide an urgent solution to this concern the proposed project was prepared. The owner of the project is Turkish Ministry of Agriculture and Rural Affairs. The project is directed by TÜBITAKGEBI. Furthermore, there are 10 Turkish universities and TAGEM. The aims of the proposed project are;

. • Construction of DNA, cell, tissue, embryo and sperm banks for animals facing extinction.
. • Characterization of entire animal species within the context of the project.
. • The implementation of new scientific technologies and developments on animal biotechnology and animal genetics, into the researches carried out in Turkey and the ameliorations of extant technologies. Furthermore, educating qualified staffs on those fields.
. • Gathering and distribution data.
. • Coupling infrastructures and human resources for a specific purpose.

The project comprises 13 sheep, 6 cattle, 5 goat, Anatolian water buffalo and 5 horse breeds. The study populations are going to be formed by staff of Ministry of Agriculture and Rural Affairs and then they are going to be held in the farms of universities taking part in the project. While genetic characterization will be done, necessary materials will be provided for the embryo, sperm and cell banks. As a part of the project, one of the gene banks is going to be established within Genetic Engineering and Biotechnology Institute of TUBITAK's Marmara Research Center and the other is going to be established within Lalahan Livestock Central Research Institute. Cryopreserved DNA samples, sperms, cells and embryos from 1500 different individuals are going to be kept in those gene banks. Besides, by means of constructed database and designed web page, data are going to be gathered and distributed.

The most important result of this project is animal gene banks since it will be a pioneer in Turkey in conservation of most of native animal gene resources. Two different gene banks are going to be established in conformity with FAO proposals. Studied DNA samples and stored tissues and cells of conserved animal species as part of this project will also be sole resource both in Turkey and in the world. In genetic diversity and phylogentic studies, highly used molecular marker types (microsatellite markers and mtDNA sequencing) are going to be used. For the first time a

remarkable number of breeds are going to be genetically characterized in such extensive ways. Besides, generated data will contribute to registration of studied breeds.
Different cryopreservation conditions (DNA, somatic cell, germ cell, embryo) are going to be used at the same time. In Turkey, on that field this project is most comprehensive and well attended project with 2 governmental organizations and 10 universities.

By means of combining infrastructures and knowledge and gathering different dispersed researches, unnecessary expenditures would be prevented. In addition to that, present inactive infrastructures would be reused and performing similar projects in the same field would also be prevented. Owing to constructed databases, knowledge would easily be reached. Furthermore, these databases will also be open to new additions and developments.

In Turkey the number of qualified researchers is remarkably low and insufficient comparing to the other developed countries. Organizations taking part in the project are going to both train their own staff in advanced new technologies and grow new researchers especially in animal genetics and reproduction biotechnology areas. Thus, the funds otherwise allocated for researchers' education in abroad are going to be utilized within the country in context of the project. Because the project is highly comprehensive, the duration was determined as 4,5 years and budget of the project is : 9.126.532 YTL.

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