The Biological Resource Banks as a Secondary Tool for Wildlife Conservation

Onkar Bagaria
Department of Management
Vivekananda Global University, Jaipur
Email ID: bagaria.omkar@vgu.ac.in

ABSTRACT: ***This work presents a Biological Resource Bank created to support the reproduction and in situ and ex situ conservation of the Iberian lynx as a complementary tool. The protection of the full existing genetic and biological diversity of the population and the harmless selection of the samples were prioritized in its design. We have processed and cryopreserved germ cells and tissues from dead animals, 7 males and 6 females, as well as somatic cells and tissues from 69 different individuals to provide potential reproductive opportunities via any possible technique. This reserve of somatic cells represents a very significant fraction of the biodiversity of the population, which will also allow the creation of a wide range of studies that can be easily extrapolated to most of the population. To isolate cells with stem-cell-like properties, we have established a new non-destructive process. Such cells may allow therapeutic applications if considered convenient in the future, and after proper study, and perhaps be a good source to be used in somatic cell nuclear transfer. There were also preserved samples of whole blood and its derivatives, hair, urine and faeces from several different people. In order to allow epidemiological studies to be carried out for the testing of different etiological hypotheses or, in general, to establish any bio-sanitary research to improve conservation strategies within the natural habitat, proper storage of such samples is required. The key aspects of the realistic implementation of the Iberian Lynx Biological Resource Bank as a model that could be useful for the creation of similar banks for other endangered species are mentioned in this work.***

KEYWORDS: ***Assisted Reproductive Techniques (ART), Biological Resource Banks (BRBs), Endangered Species, Somatic Cell Nuclear Transfer (SCNT).***

INTRODUCTION

Animal protection is meant to conserve and, if possible, improve biodiversity. By protecting the natural environment, the optimal way is to achieve this aim. In situ conservation methods, however, are often ineffective for the distribution of small populations and for the preservation of sufficient genetic diversity [1]. The above strategy should then be reinforced with ex situ programmes aimed at maintaining a viable population through captive breeding and the cryopreservation of the genetic resources of animals. Biological Resource Banks (BRBs) are collections of gathered, processed and stored biological materials, also referred to as genome or genetic resource banks. Their usage is being promoted extensively in the management and protection of endangered species [2]. The Frozen Ark Consortium and the Wildlife BRB of Southern Africa are some recent examples. These reserves have the ability to maintain the present genetic diversity of populations if used properly, as well as to provide future reproductive opportunities through various techniques.

The majority of cryobanks previously mentioned in the literature are specialized mostly or solely in gametes and embryos. Therefore, such deposits are germplasm banks, mainly containing samples of semen. Their basic aim is to obtain offspring by assisted reproductive techniques (ART), including, but not limited to, artificial insemination, in vitro fertilization and embryo transfer. These techniques have been successfully applied to domestic species and, after Kraemer et.al ground-breaking work; they also appear as a promising method for endangered species conservation [3]. While there is still a need for extensive study of the reproductive biology of wild animals, effective cryopreservation of spermatozoa of different non-domestic species has been carried out. As a basic component of any Biological Resource Bank, these sample forms must then be included. Nevertheless, at present, semen banks of wild endangered animals are poorly established, possibly due to the difficulty of collecting semen samples and, subsequently, the lack of basic information on species-specific procedures for cryopreservation. For oocyte cryopreservation, the situation is much more complicated, the selection of which is only feasible from recently dead or captive females. Consequently, it is very difficult to ban a genetic pool indicative of the biodiversity of the population if only gametes from endangered species are retained [4].

We also considered that Biological Resource Banks must also include other biological sources, such as somatic tissues and cells, in addition to gametes and embryos. During biopsies and necropsies, certain samples can be readily and harmlessly obtained. Therefore, we will achieve the greatest possible genetic biodiversity of both the male and the female population by their selection and processing [5]. Tissues and somatic cells have wide uses, including, among others, genetic, toxicological or epidemiological studies. Another possible utility of somatic cells is their use as donors of human genetic inheritance in somatic cell nuclear transfer (SCNT), popularly known as cloning. There are records of 16 successfully cloned mammalian species giving birth to healthy offspring. Sheep, calves, rats, goats, pigs, cats, rabbits, mules, horses, and endangered species of mammals such as bovids, mouflons, felids, or wolves are among these. Using intra- and inter-species variations of this technique, these children were born [6]. As a potentially integral part of wildlife management efforts, somatic cell nuclear transfer has thus been proposed. This system, however, has a very low efficiency rate at present, so further studies and approaches are required.

For biodiversity protection purposes, the preservation of other biomaterials is often useful. As these materials can be transformed into serum, plasma, blood cells, DNA and tissue/cell cultures, the processing and storage of blood and tissues was already considered by Wildt et al. For the study of genetic variation, phylogeny, paternity, gene flow and/or preference, these biomaterials may be used. Fecal or hair samples are widely used as genetic identification tools that are not invasive. Fecal samples are often used to classify gastrointestinal parasites and, through the measurement of various metabolites, to monitor reproductive activities. In epidemiological research, blood and urine are used and are often regularly taken for general sanitary checkups. However, to develop precise research, such biomaterials are usually collected separately and are rarely retained. We consider it beneficial to routinely collect process and conserve this form of biomaterial in endangered animals in order to facilitate any research in favor of in situ conservation, if possible [7].

In 2002, we founded a Biological Resource Bank for the endangered wildlife of Spain, including mammals, fish and many avian species, in order to provide a funding tool for reproduction and conservation programmes. This bank started as a reserve of cryopreserved tissues and somatic cells and was later expanded to include other biological samples in the case of the most endangered species. This work focuses on the Iberian Lynx Biological Resource Bank, developed by our group in partnership with the Regional Government of Andalusia's Environmental Council and with representatives of the Captive Breeding Programme. The Iberian lynx is perhaps the world's most vulnerable felid, with less than 170 individuals likely to be found in the south of Spain in only two meta-populations separated from each other. It is officially regarded by the World Conservation Union as critically endangered. We also found the protection of the full current genetic and biological diversity of the population as a main priority in the creation of such a biological reserve. To this end, all collected samples were harmlessly collected from as many people as possible. A broad range of sample types were selected, processed and stored with these conservation approaches to: (a) allow any potential bio-sanitary study addressed to enhance the conservation of existing individuals within their natural habitat; (b) provide future reproductive opportunities through all practicable techniques, taking into account both gametes and somatic cells. Under this latter goal, a new method has been implemented to try to increase the efficiency of the nuclear transfer of somatic cells. We searched for cells in cell cultures that have two key stem cell properties, a high rate of proliferation and the ability to form spherical colonies called embryoid bodies. The involvement of stem cells in adult tissues has recently been seen in several studies. Stem cells undergo more replication processes and have higher plasticity than somatic cells that are fully differentiated. Our hypothesis is that such characteristics could favor the nuclear re-programming that must take place during the nuclear transfer technique of somatic cells and we are therefore looking for cells with stem-cell-like properties [8][9].

As a supporting tool for reproduction and global conservation, both in situ and ex situ, this complementary conservation strategy is currently being established. In this article, we describe the methodology used during the 2003-2006 periods for the treatment of each portion of the Iberian lynx BRB created by our community, attending to the above objectives and conservation approaches. Our objective is to identify, as a model that could be useful for the creation of similar banks for other endangered species, the key aspects of its functional implementation.

DISCUSSION

In this work, we presented a BRB created by our group for the period 2003-2006 for the Iberian lynx. This complementary conservation strategy is being established as a supporting instrument, both in situ and ex situ, for reproduction and global conservation. We also found the protection of the full current genetic and biological diversity of the population as a main priority in the creation of such a biological reserve [10]. To that end, as many individuals as possible have harmlessly obtained all banked samples (germinal and somatic cells, as well as the rest of the biomaterials). We chose a wide variety of sample forms taken from necropsies or from live animals for this conservation method, in the latter case often benefiting for other purposes from animal manipulation. The key objectives were to prevent the irreversible loss of biodiversity from each dead organism and to achieve full representation of biodiversity from the population.

CONCLUSION

The Iberian Lynx Biological Resource Bank presented in this paper is a valuable tool in order to prevent the permanent loss of biodiversity from each dead person and to ensure full representation of the biodiversity of the population. The purpose of this article was to identify, as a model that could be useful for the creation of similar banks for other endangered species, the key aspects involved in its realistic implementation. We have processed and cryopreserved germinal and somatic cells and tissues, collected exclusively using non-invasive methods from dead animals and biopsies, to provide potential reproductive opportunities across all practicable techniques. The reserve of somatic cells represents a relevant fraction of the biodiversity of the population, which would also allow the findings of any study to be extrapolated to the remainder of the population. To isolate cells with stem-cell-like properties, we have established a new non-destructive process. In order to be used in the Somatic Cell Nuclear Transfer if it is deemed convenient in the future, these cells were cryopreserved as a theoretically better cell source than completely differentiated somatic cells. In order to enhance the survival of the Iberian lynx within its habitat, samples of whole blood and derivatives, pulled fur, urine and faeces were also routinely processed to produce any analysis at any time. At present, we are continuing to work on the Iberian Lynx Biological Resource Bank, with the same conservation priorities and approaches set out in this study.

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