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Topic of General Interest

Maintenance of Brazilian Biodiversity by germplasm bank[1]

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ABSTRACT.- Machado L.C., Oliveira V.C., Paraventi M.D., Cardoso R.N.R., Martins D.S. & Ambrósio C.E. 2016. **Maintenance of Brazilian Biodiversity by germplasm bank.** *Pesquisa Veterinária Brasileira 36(1):62-66.* Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Av. Duque de Caxias Norte 225, Pirassununga, SP13635-900, Brazil. E-mail: lucianabiologa@usp.br

Currently the importance of using alternative strategies for biodiversity conservation is emphasized and since the establishment of germplasm bank is an alternative to the conservation of endangered species. This is a technique of great importance for the maintenance of Brazilian fauna. Since the early70'sthere was a growing concern about the need to preserve essential genetic resources for food and agriculture, mainly for conservation of genetic material from farm animals. Thus was created the Brasilia Zoo, in July 2010, the first Germplasm Bank of Wild Animals in Latin America, as an alternative strategy for the conservation of threatened or endangered species, using both gametes and somatic cells and stem cells. Then we argue to create new banks or research networks among different regions with aimed to tissue preservation.

INDEX TERMS: Preservation, germoplasm bank, threatened species, cryopreservation, biotecnology.

RESUMO.- [**Manutenção da Biodiversidade Brasileira através de bancos de germoplasma.**] Atualmente, a importância do uso de estratégias alternativas para a preservação da biodiversidade é ressaltada e, visto que a criação de bancos de germoplasma é uma alternativa para a conservação de espécies ameaçadas, esta é uma técnica de suma importância para a manutenção da fauna brasileira. Desde o começo da década de 70 houve uma crescente preocupação sobre a necessidade de se preservar recursos genéticos essenciais para alimentação e agricultura, voltados principalmente, para a conservação de material genético de animais de produção. Deste modo, foi criado pelo Jardim Zoológico de Brasília, em julho de 2010, o primeiro Banco de Germoplasma de Animais Selvagens da América Latina, como uma estratégia alternativa para a conservação de espécies ameaçadas ou em perigo de extinção, utilizando tanto gametas como células somáticas e células-tronco. Com isto ponderamos na criação de novos bancos ou redes de pesquisa inter-regionais que foquem nesta preservação tecidual.

TERMOS DE INDEXAÇÃO: Preservação, banco de germoplasma, espécies em extinção, criopreservação, biotecnologia.

INTRODUCTION

The biodiversity present in Brazil is one of the most significant in the world and its wealth, both animals and vegetables are the subject of great interest in various sectors, among which stands out the scientific community and there are interest in the preservation of the present species. (Costa & Martins 2008).

According to Houghton et al. 2011, it is estimated that in a few years endangered species will become extinct and others endangered because of the interference of both climate and vegetation, which can cause fragmentation of the habitats and impede the species migration, and these date are current. Although some initiatives to prevent the loss of biodiversity, such as the expansion of protected areas, cannot be avoided, because of referred them areas do not protect all animals or species of interest (Silva et al. 2012).

Preservation of the species depends of the minimum number of viable individuals. The loss of the populations

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is an unprecedented and may irreversible fate. To ensure a minimum of biodiversity, maintenance and perpetuation of the species, which cannot be sure secured, it is necessary the proper management of populations (Costa & Martins 2008).

Genetic resources are comprised by the genetic variability organized in a number of different materials among themselves, called germplasm. Each germplasm unit is formed by the genetic material of living organisms of current or potential interest. Consequently, the germplasm is the element of genetic resources operated and used in research in general, especially for genetic improvement programs (Ministério do Meio Ambiente 1998).

Currently, the importance of using alternative strategies for biodiversity conservation is emphasized and the establishment of germplasm bank is an alternative for the endangered species conservation; this is a short technical importance for the maintenance of the Brazilian fauna. The introduction of DNA collections can be used in numerous conservation studies as discrimination of endangered species, reproductive management strategies, selecting a preservation of a species status and currently these banks assist in monitoring the biodiversity protection laws as, for example, in identifying whale species from samples collected in the meat trade (Santos et al. 2002).

The germplasm banks are a fundamental role in the study of biodiversity and conservation. The possibilities facing a genomic collection are essential, when discussing the management and recovery of several species and may be noted endangered species and even extinct. Today it is possible to apply genetic knowledge in biotechnology research, evolutionary, comparative, taxonomic and ecological studies, saying the scientific and technological potential of genomic reserves (Santos et al. 2002).

LITERATURE REVIEW

Events in Brazil linked to concern of preservation of the species

Since the beginning of the 70 decade there was an increasing concern about the need to preserve essential genetic resources for food and agriculture. In 1974 was created Embrapa - Brazilian Agricultural Research Corporation - a research center whose mission was to coordinate appropriate resources to manage the genetic resources of the country and still responsible for it nowadays. The main activities involve enrichment, by collecting germplasm introduction, exchange and quarantine conservation: in situ (or in nature or on the farm) and ex situ (seeds in cold storage; explants in vitro culture, the micro-organisms; cryopreservation of semen, embryos and oocytes); characterization: phenotypic, genetic and information (Mariante et al. 2009).

Biological diversity is the key for life maintenance and there is no doubt that habitat destruction is the main factor responsible for the reduction of biodiversity or the total number of species that exist on the planet. Several attempts have been made to halt the population of various species decline rates. One of the suggested programs concerns the conservation of genetic material, specifically sperm, and embryos oocytes of endangered species knew as "genetic resource banks" or "genome bank". It is used as repositories germplasm, which can be use in artificial insemination or embryo transfer when necessary, and a storage interface programs ex situ and in situ. Genetic reserves of banks differ from one bank of stored cells and tissues available only as a resource for genetic research. The objective of the program is simple and directly applicable because of the effectively increase of the "genetic life" individuals value, and may continue as part of reproductive programs, even after its death. Since the objective of the reproductive programs for endangered species is the maintenance of maximum genetic diversity, it is clear that banks of genetic resources directly contribute to conservation objective (Loskutoff 1998; Holt & Pickard 1999).

Germplasm Bank in Brazil

The United Nations established the Convention on Biological Diversity (CDB), which included Brazil from Decree No. 2519 of March 16, 1998, and provided the access and technology transfer for the conservation, genetic heritage and protection, access to associated traditional knowledge, benefit sharing and access to genetic heritage existing in the country. Thereby, the CDB has implemented laws which aim protect the genetic heritage of a country and ensures the exchange with other countries, allowing for example, Brazil to have access and benefit from advances in genetic resources obtained internationally (Silva et al. 2012).

Banks of genetic resources are repositories of germplasm (gametes, embryos, blood products, tissues and DNA) to defined conservation programs, which are directly linked with biotechnology for the implementation of the ultimate goal, which is the animal reproduction. One possibility to preserve the wild germplasm of animals is the isolation, culture and cryopreservation of somatic cells such as: testicular, follicle and fibroblasts, and it can be used for molecular characterization and by nuclear transfer technique (cloning) might multiply the number of animals that are extinct or endangered (Martins et al. 2007).

In July 2011, the Workshop Germplasm of Brazil was organized by the Paulista Agency of Agrobussines Technology (APTA), from Agriculture and Supply Secretary (SAA) and the Brazilian Agricultural Research Corporation (Embrapa) of the Ministry of Agriculture and Livestock (MAPA) approved the creation of a national system curatorial germplasm. The proposal aimed to encourage other countries to create their systems of germplasm bank of curatorial and biological collections, and encourage the government to invest on this conservation in order to combat piracy, through the formation of a Brazilian network that includes public institutions research (Embrapa, Ministry of Agriculture and National Board of Agricultural Research of State Systems). In Brazil, the germplasm banks coordinated by Embrapa Genetic Resources and Biotechnology are meant primarily for the conservation of genetic material of producing animal.

There was created by the Zoo of Brasília, in July of 2010, the first Bank of Germplasm Bank of Wild Animals of Latin America, as an alternative strategy for the conservation of threatened species or in extinction danger, using as much gametes as somatic cells and stem cells. In 2007, the La-

boratory of Germplasm Conservation, was create in the University Federal Rural of the Semi-arid - UFERSA, accomplishing researches destined to the collection and cryopreservation of gametes of different wild species (Silva et al. 2012).

In 1999, the Institute of Biological Sciences from University Federal of Minas Gerais created a Bank of DNA of the Brazilian fauna species, as birds and mammals. Also the National Research Institute of Amazonia (INPA) has a collection of plants, animals and microorganisms species from Amazonia, many preserved cryogenically living with collections of tissue and DNA samples of species of fish and small mammals (Santos et al . 2002). Fortunately, the institutions have been increasing for creating germplasm bank facing the Brazilian wild species, to create alternative strategies for the preservation of endangered species or in extinction risk.

The Ideal Germplasm Bank

The cryopreservation allows, theoretically, the indefinite conservation of biological material without deterioration over the time. Genetic germplasm banks have as the main focus the recovery of extinct populations, or species with unique biological importance characteristics to be preserved, or species locally adapted or in extinction risk (Silva et al. 2012).

The success and safety of semen cryopreservation, oocyte and embryo requires care, and some points should be considered, such as: the screening of the donor avoiding infectious diseases and the achievement of good health practices and procedures to ensure appropriate and less risk of contamination during collection, transport and storage, as well as higher rates of post thawing survival (Bielanski & Vajta 2009). It is recommended and proposed that the germplasm banks contain a wide variety of genetic material, from future populations which appear to leave or can suffer genetic limitations (Costa & Martins 2008).

Cryopreservation of tissue and cells

The reproductive system has the main organs responsible for the perpetuation of the species. The ovaries are paired organs and are located in sublumbar region caudal to the kidneys, this variable being located in different species because of a movement of these organs. It is the sexual hormone production and function, as well as release of germ cells as shown in Figure 1 (Liu et al. 2006, Horst & Hans 2011). In Figure 2 shows the primary male reproductive organ, the morphofunctional view distinguished into two compartments, the tubular compartment formed by the seminiferous tubules and where spermatogenesis occur (Castro et al. 1997).

The germ cells are responsible for originating the gametes lineage in mammals. The viability of the tissue from reproductive system after freezing can generate fertilized oocytes, as well as offspring of several species with cryopreservation of ovarian and testicular tissue, enabling to generate genebanks. With technological advances, these lineages can contribute to the generation of germplasm banks that is extremely important for the preservation of genetic material and perpetuation of the species at risk of extinction.

The cells from the skin are also sources of genetic information that can be cryopreserved in a simple and efficient manner. Skin tissues are used to perform more banks, as a source of somatic cells for different applications. The

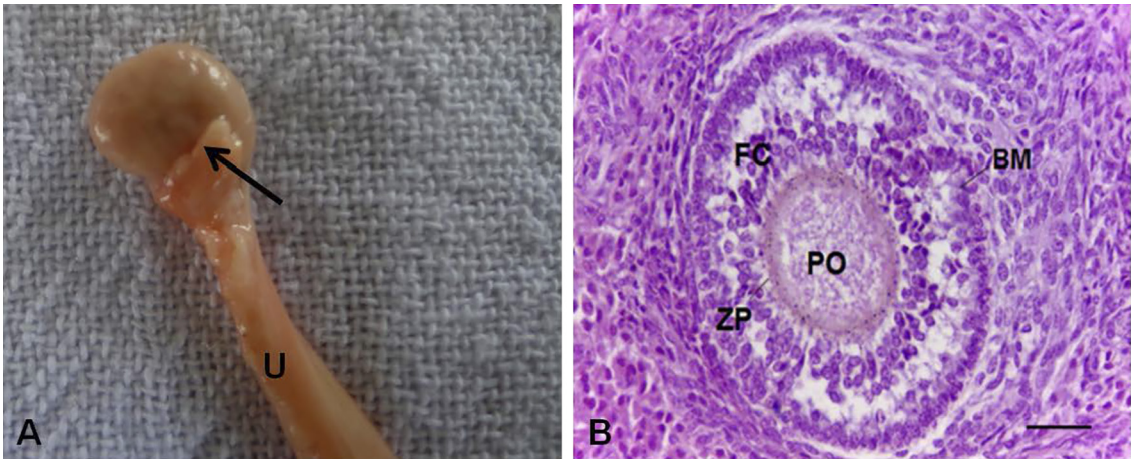

Fig.1. Ovary of the crab-eating fox (*Cerdocyon thous).* (**A**) Ovary (arrow) and uterine horn (U). (**B**) The pelucide zone (ZP), primary oocytes (PO), basal membrane (BM) and folicular cell (FC). HE, bar = 20µm.

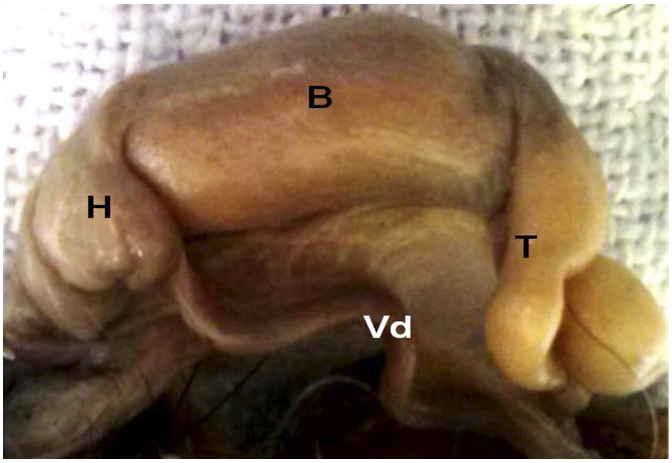

Fig.2.Testicle of the porcupine clerk (*Coendou prehensílis*). Epididymis with head (H), Body (B), Tail (T) and Vas deferens (Vd).

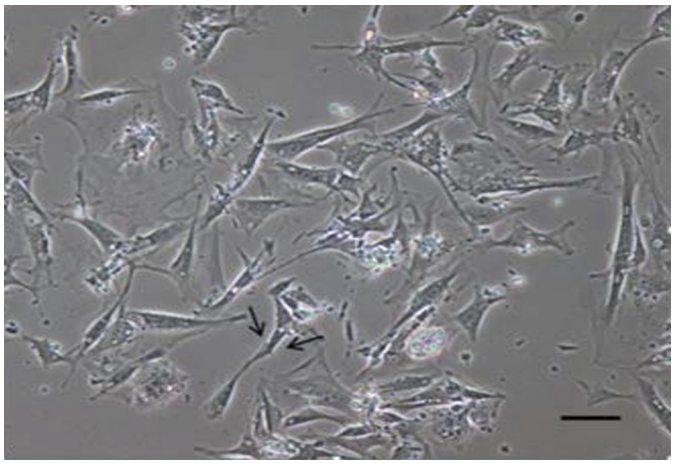

Fig.3. Fibroblast culture of Blue-and -yellow Macaw (*Ara ararauna*).

Figure 3 shows the fibroblast line are the most commonly used, because they are differentiated adult cells and considered a good source for IPSC (Induced Pluripotent Stem Cells), drug testing, molecular studies in other applications. To realize the cryopreservation, is used as a cryoprotectant glycerol or dimethylsulfoxide (DMSO) in suspension and storage at - 80°C (Hiemstra et al. 2005). After having conditioned in germplasm banks, the somatic cells can be used for cloning, for example (Silva et al. 2012).

Cryopreservation of spermatozoids

Among the ex situ techniques, the main is the cryopreservation of male gametes. The collection techniques are mainly eletroejaculation, rectal massage and artificial vagina, and the main cryoprotectants based on glycerol, with effective results in wild species such as impalas, giraffes, and buffaloes, among others. Testicular biopsy is an elective methodology for the collection of material for physiology and reproductive pathologies study in wildlife, providing enough material for histological analysis (Lopate et al. 1989, Threlfall & Lopate 1993, Attia et al. 2000, Crosta et al. 2003, Azevedoet al. 2006, Mascarenhaset al. 2006, Guião-Leiteet al. 2006, Bittencourtet al. 2007).

The differences in the effectiveness of the cryopreservation technique seem to be due the particularities of each species and have been described with success in the cryopreservation of gametes of wild cat, jaguar, leopard, cathetus and monkeys. A new technique still in development and has been getting the attention, it consists in isolate the spermatozoids from the epididymis from cryopreservation. Nowadays the most common form is to obtain the gametes from epididymis of dead animals, leaving the testicle and cooling before the extraction this new technology can an alternative (Costa & Martins 2008).

Cryopreservation of embryos

The cryopreservation of embryos is the most successful instrument in the preservation of genetic resources of wild animals. This technique depends of factors such as the origin of the embryo (in vivo or in vitro), the stage of development at the moment of freezing and the specie (Silva et al. 2012), more recently appeared as an alternative to conventional cryopreservation process of vitrification of the oocytes and embryos. This technique uses less equipment and has high applicability in the field, and the direct transition from solid to liquid phase, which results in no formation of ice crystals which damage the cell, preserving the embryo with more effectiveness (Costa & Martins 2008). The cryoprotectant elected continues, however, the ethylene glycol, due to its low toxicity and high permeability (Silva et al. 2012).

Cryopreservation of oocytes

The cryopreservation of female gametes is extremely important, which may be carried out storage of ovarian tissue, the isolated follicles or oocytes from mature or immature (Silva et al. 2012). Currently, there is significant progress with fast protocols in cryopreservation of oocytes for recovering viable oocytes after freezing and thawing. (Costa & Martins 2008).

The gametes can be obtained easily by follicular puncture, ovarian tissue biopsy, ovariectomy or ovarian harvest immediately post mortem. For conservation, the most frequently used solution is coconut water in refrigerated powder by a maximum of 36 hours and cryopreservation slow freezing, which can be used in low concentrations of cryoprotectants. This technique is the gradual temperature decrease, controlled by a programmable freezer (Silva et al. 2012).

DISCUSSION AND CONCLUSION

The biological diversity is the key for sustaining life as we know it and there is no doubt that the habitat destruction is the main factor responsible for the reduction of biodiversity or the total number of species that exist on the planet (Loskutoff 1998). The genetic material of any animal can be lost at any time per animal death, a reduction in their genetic heritage (gene pool) and possibly a loss of important genes for the species. In this case, efforts can be made through the use of assisted reproduction techniques to prevent total loss of genetic material of importance (Martins et al. 2007).

Regardless of the risk of extinction, conservation must be carried out not only by the law of the animals have to exist, but also for possible applications in humanitarian activities. Species of plants and animals that may be useful in the future are being lost before such usefulness is tested for humans (Wilson 1997).

Extinction is a slow and natural process that should keep balance in the number of speciation, mutations and changes to the frequencies of the alleles that generate new species. The excessive operating environment causes the reduction of biodiversity, since the extinction ratio becomes greater than speciation. The current loss of species is unprecedented and may be irreversible (Primack & Rodrigues 2001).

Therefore, technological advances in the conservation area have contributed to the generation of germplasm banks, and have an exceptionally important for the perpetuation of the species at risk of extinction. Due to significant biodiversity both, animal and vegetable, in Brazil, the

germplasm banks have been the subject of great interest in the preservation and maintenance of the genetic patrimony.

To ensure a minimum biodiversity and the maintenance and perpetuation of the species, it is necessary the appropriate manipulation of the populations, with that it is used alternative strategies for the preservation of the biodiversity, where the creation of germplasm banks is stood out as an alternative for the conservation of threatened species. The technique has been assuming as an addition importance for the maintenance and preservation of the Brazilian fauna, contemplating the cryopreservation of somatic cells as testicular cells, follicular and fibroblasts, long-term in order to conserve their genetic resources as an important material for further research. Several banks appeared in the last years with the intention of preservation of the Brazilian fauna, but they are still many necessary studies in that area and investments for a fauna warranty. It is stood out although the best form of maintenance of the fauna continues being the preservation of the natural atmospheres of those animals.

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