ARTIGO DE REVISÃO

REVISTA PORTUGUESA DE CIÊNCIAS VETERINÁRIAS

Reproductive biotechnologies applied to the conservation of endangered ruminant - past, present and future

Biotecnologias reprodutivas aplicadas à conservação de ruminantes ameaçados de extinção – passado, presente e futuro

Joanna M. G. de Souza*, Ribrio I. T. P. Batista, Luciana M. Melo, Vicente José de F. Freitas

Laboratory of Physiology and Control of Reproduction, Veterinary Faculty, Ceará State University (UECE)
Universidade Estadual do Ceará, Av. Dedé Brasil, 700, CEP: 60740-093, Fortaleza, CE, Brazil

Summary: Intensely concerns about the future of the environment have grown intense in recent years. The possibility of extinction of various species by unnatural causes, instead of the slow action of the evolutionary process has become a reality. Thus, the objective of preservation can be understood as a guarantee of the survival and evolution of species and animal populations in their native habitat. Simplifying, biological diversity is the key to the sustainment of life as we know it. Therefore, all actions that aim to preserve species and breeds are important so that their genetic material is not permanently lost. Fortunately, the magnitude of preserving planet's biodiversity has been widely recognized and various conservation strategies to maintain the global ecosystem have been proposed by conservationists. The possibility of creating genetic resource banks (*ex situ* conservation) has been suggested as one way of achieving this goal and is considered an essential complementary activity to *in situ* conservation of genetic resources of farm animals. This measure would tend to encourage the growth of individual species and endangered breeds, affecting positively their population. The aim of this review is to report the work that is being done to conserve the genome and individual genes through the use of various assisted reproductive technologies in ruminants.

Keywords: Assisted reproduction, biodiversity, risk of extinction, threatened

Resumo: As preocupações com o futuro do meio ambiente têm crescido de forma intensa nos últimos anos. Dentre elas, destaca-se a possibilidade de extinção de várias espécies por causas não naturais, ao invés da ação lenta do processo evolutivo. Ecossistemas funcionais, que dependem da biodiversidade, são importantes para a manutenção da vida no planeta. Deste modo, o objetivo da preservação pode ser entendido como a garantia da sobrevivência e evolução da espécie e da população animal em seu habitat nativo. De uma forma simplificada, a diversidade biológica é a chave para manter a vida como nós a conhecemos. Desta forma, ações que objetivem a preservação de espécies e raças são importantes para que o material genético destes animais não seja perdido de forma definitiva. Felizmente, a importância de se preservar a biodiversidade do planeta tem sido amplamente reconhecida e diversas estratégias de conservação para manutenção do ecossistema global propostas por conservacionistas. A possibilidade de criação de bancos de recursos genéticos (conservação *ex situ*) tem sido sugerida como uma forma de atingir esse objetivo e pode ser também considerada uma atividade complementar para a conservação *in situ* de recursos genéticos de animais de produção. Esta medida pode promover o incremento do número de indivíduos cujas espécies e raças encontram-se ameaçadas, elevando o tamanho da população efetiva. Assim, o objetivo deste estudo é descrever as diversas tentativas que estão sendo realizadas para conservar genomas e genes individuais através do uso da tecnologia reprodutiva assistida em ruminantes.

Palavras-chave: Ameaçados de extinção, biodiversidade, reprodução assistida, risco de extinção

Introduction

In recent decades, the planet has experienced a significant reduction of genetic diversity due to the alteration or destruction of habitats, mainly as a result of anthropogenic forces. The importance of this phenomenon is illustrated by the IUCN "red list", that is widely recognized as the most comprehensive and global objective approach for evaluating the conservation status of animal species. Once the number of the population is determined, the species is given a term to describe its status, such as "endangered" or "threatened", that is sometimes misunderstood. Endangered are those species that are in danger of extinction throughout all or a portion of their range, whereas threatened are those likely to become endangered within the near future. The loss of animal genetic resources is occurring at alarming rates across the globe. According to FAO, at least one livestock breed has become extinct per month over the past several years, resulting in its genetic characteristics being lost forever. For an unpredictable future, traditions, cultural values and safeguarding diversity are all driving forces in support of genetic conservation, which is a human

*Correspondência: : joannavet@gmail.com
Tel: +(55) 85 3101 9861; Fax: +(55) 85 3101 9840

responsibility. Nowadays it's clear that the various forms of life on Earth only exist because of its integrated complex system between living species and even between physical environmental components such as water, atmosphere, rocks and soil. As human change the physical environment, the environment also changes human life. The larger the number of living organisms the more stable is the ecosystem. Air and water purification, food provision, stabilization of the Earth`s climate, erosion control, disease control and nutrient recycling are some important conditions that depend on biodiversity conservation (Holt *et al*., 1996b).

For the reasons presented above it is imperative to conserve and maintain animal genetic resources to ensure preserving biodiversity and keeping alternative and potentially useful genes available in the gene pool. *In situ* and *ex situ* methods have been proposed to conserve genetic resources. The first one is ideal as the conservation of live species occurs in their natural habitats. The disadvantage of this method is that it requires land and people which are limited in some regions. *Ex situ* conservation, by the other hand, deals with protection of biological diversity components outside their natural habitats (Glowka *et al*., 1994). It covers widely applied conservation techniques such as the establishment of genome banks, referring captive breeding of animals far removed from their indigenous environment. It is noteworthy that *ex situ* and *in situ* conservation are not mutually exclusive. Frozen animal genetic resources or captive live populations can play an important role in the support of *in situ* program (Holt *et al*., 1996b). However, turning this idea into reality is a rather complex process, requiring interdisciplinary collaboration and clearly defined goals. Therefore, the aim of this review is to report the work that is being done to conserve genome and individual genes through the use of modern reproductive biotechnologies in endangered ruminants around the world.

Situation of domestic ruminants in the World and in Brazil

Of all ruminant livestock, cattle are the most numerous, followed by sheep, goats and buffalo. It is known that many ruminant breeds have already become extinct in the world: 3% of goat, 12% of sheep and 17% of cattle breeds. Nevertheless, goats have the largest percentage of breeds (28%) with a global unreported/unknown population size (Figure 1), hinting towards the possibility of that the number could be underestimated (Galal, 2005).

When Europeans discovered Brazil, about 500 years ago, the first ruminant specimens were brought by Portuguese settlers. Throughout the years, these animals resulted in the formation of "criollo", "local" or "naturalized" breeds that for centuries were responsible for livestock production in the country. Over the years, natural selection occurred and these breeds developed morphological and physiological characteristics adapted to specific Brazilian environmental conditions. Nevertheless, from the early 20th century, commercial breeds that have been imported have been responsible to promote the gradual replacement of naturalized breeds to such an extent that the latter are in danger of extinction. To avoid further loss of this important genetic material, in 1983, the National Research Center for Genetic Resources and Biotechnology (Cenargen) of the Brazilian Agricultural Research Corporation (Embrapa) decided to include among its priorities the conservation of animal genetic resources (Mariante and Cavalcante, 2006; Mariante *et al*., 2009). The *in situ* conservation of cattle, buffaloes, donkeys, goats and sheep is being carried out by Conservation Nuclei, located in the animal's original habitat. *Ex situ* conservation is centered at the Brazilian Animal Germplasm Bank (AGB) that is responsible for the storage of semen and embryos of various breeds of threatened domestic animals. Presently the AGB has almost 60,000 doses of semen and more than 250 embryos, as well as over 7000 DNA samples (Mariante *et al*., 2009).

Reproductive biotechnologies applied to *ex situ* conservation of ruminants

Modern reproductive technologies have allowed a large number of progeny to be produced from a single individual, and the transport has enabled the distribution of germplasm around the world rapidly and efficiently. The successful use of assisted reproductive technologies (ART) in domestic livestock suggests that they can also be used for the conservation of species and breeds in danger of extinction (Solti *et al*., 2000).

A good example of ART application was reported by Ptak *et al*. (2002), that used the European mouflon (*Ovis orientalis musimon*) to demonstrate for the first time the potential of establishing an integrated package of modern reproductive biotechnologies to rescue an endangered species with substantial yields of oocytes, embryos and pregnancy, independent of age, breeding season and donor treatment. *Ex situ in vitro* conservation programs of endangered ruminant genetic resources have focused efforts on cryopreservation of gametes, embryos and somatic cells as well as testis and ovarian tissues, effectively lengthening the genetic lifespan of animals in a breeding program even after the death. Although significant progress has been made in both semen and embryo cryopreservation of several domestic species there still remains many difficulties in a few steps that will be detailed shortly.

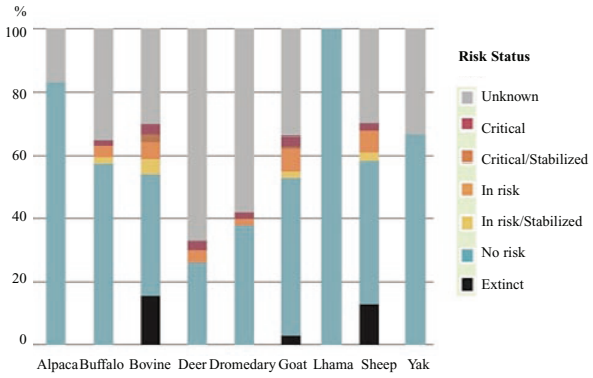

Figure 1 - Risk status (%) of ruminant species in January, 2006 (Commission on Genetic Resources - FAO, 2007).

Animal genetic resource bank (GRB)

Conservationists are looking for additional means to secure some portion of the biodiversity that are at risk of being lost. The establishment of biomaterial to constitute a GRB is rather complex and expensive, but it is essential to preserve genetic diversity through *ex situ* conservation in the variety of semen, oocytes, embryos, tissues or DNA of endangered animals (Andrabi and Mawxell, 2007). A disadvantage of this is that breed restoration may be extremely costly and time consuming. However, as a complementary conservation approach, cryopreservation provides a long-term insurance system to *in situ* conservation, since when combined with artificial insemination or embryo transfer ensures genetic variability (Holt and Pickard, 1999).

Otherwise, the formation of a GRB generates the possibility of re-establishing a particular population after disasters or epidemics episodes. Currently, there are two more realistic options, either cryopreserve embryos or gametes (i.e., spermatozoa and oocytes), which have advantages and disadvantages. A bovine breed could be saved with 1,000 sperm doses collected on 25 different males or 300 embryos (non-sexed) from 90 donors (Comizzoli *et al*., 2000). Boettcher *et al*. (2005) simulated a creation of a GRB for reconstruction of an extinct breed using different strategies: embryos-only, embryos in combination with semen, and semen-only. The strategy embryos-only required the shortest time to reach reconstruction, in the strategy embryos + semen the time increased with decreasing proportions of embryos, whereas in the semen-only, reconstruction time varied from 2 to 21 years. The risk of extinction was extremely high when a very small proportion of embryos (< 20%) was used. Decreasing the percentage of embryos further diminished costs. The authors emphasized that the combination of both embryos and semen would be more indicated as it would reduce costs allowing the conservation of more breeds. Similarly, Amstislavsky and Trukshin (2010) reported that embryo cryopreservation seems reliable and most simple, whereas the use of gametes requires the use of subsequent techniques. The authors also described that in recent years the number of cryobanks increased worldwide, what may be considered as response to this need.

Cryopreservation – Semen

This biotechnique has been the most widely used for germplasm preservation of endangered species, due to its abundant availability and ease of application. Thus, several attempts have been reported for its optimization. Coloma *et al*. (2010) compared different extenders and observed that the seminal plasma removal improved the response of freezing-thawing semen in Spanish ibex (*Capra pyrenaica*). The effects of cooling rates, glycerol concentrations and diverse extenders on the post-thawing sperm viability of camel sperm (*Camelus bactrianus*) were compared in Iran (Niasari-Naslaji *et al*., 2007). Cheng *et al*. (2004) reported that optimal extenders for semen were obtained when working with Formosan Sika (*Cervus nippon taiouan-ua*) and Formosan Sambar (*C. unicolor swinhoe*), both endangered. All freezing protocols tested were also useful to the post-thawing viability of semen obtained in the endangered Père David's deer (*Elaphurus davidianus*; Soler *et al*., 2003). In another trial, the performance of freezing the sperm-rich fraction showed better results than the whole ejaculate on the Iberian Red deer (*C. elaphus hispanicus*; Martínez-Pastor *et al*., 2009).

The use of epididymal sperm cells in ART on endangered animals could be considered a useful source. According to the sperm maturation process, sperm from the cauda epididymis are of good quality and potentially fertile (Bedford, 1978). In this approach, *post-mortem* semen cryopreservation from the cauda epididymis was successfully performed in five endangered gazelles (*Gazella gazella*, *G. dorcas* and *G. gazella acaiae*) in Israel. This result was specifically important for the last species since there were only 12 individuals left in the wild (Saragusty *et al*., 2006). However, the protocols currently used to conserve semen are still suboptimal and cannot be easily applied across species.

Cryopreservation – Embryos

Cryopreservation of embryos and their subsequent storage generates enormous potential for protecting the population integrity and heterozygosity. However, it is a more complex and costly procedure than semen cryopreservation and the successful use of this technology for wildlife is dictated by the singularity of the species (Pukazhenthi and Wildt, 2004). Protocols for freezing bovine embryos have been reasonably effective in studies that were conducted on non-domestic cattle such as gaur (*Bos gaurus*), eland

(*Taurotragus oryx*) and bongo (*Tragelaphus eurycerus*; Revised by Loskutoff *et al*., 1995). Similarly, Lopes Jr. *et al*. (2006) were also able to freeze embryos from Morada Nova (white variety), a domestic sheep breed that has a very small population in Brazil.

Traditional procedures to freeze embryos require much time and are quite onerous. This technique may be replaced by a relatively simpler one and less costly called vitrification. Vitrification is a physical phenomenon of amorphous solidification, achieved with extreme increase of cryoprotectants viscosity in temperatures below the melting point. The purpose is to prevent intracellular and extracellular ice crystals formation that is responsible for damage of membranes and cell organelles during the cryopreservation process (Vajta, 2000).

In this way, Thundathil *et al*. (2007) described for the first time that embryo production followed by vitrification in wood bison (*Bison bison athabascae*) could be able to recover its genetic material. Moreover, Bettencourt *et al*. (2009) conducted a study to compare the efficiency of three cryopreservation techniques: controlled slow freezing, conventional vitrification and open pulled straw (OPS) vitrification for the cryopreservation of *in vivo* produced Portuguese Black Merino ovine embryos. The authors concluded that all the three techniques were efficient in preserving and propagating genetic material. It is apparent that earlier and *in vivo* derived embryos can withstand cryopreservation better than later stage and *in vitro* produced embryos. Therefore, the current challenge is to develop a standardized protocol that can be applied to embryos of different species at various developmental stages.

Cryopreservation – Oocytes

Oocyte cryopreservation, despite its high impact on the preservation of genetic resources is has yet to be considered an established technology (Ledda *et al*., 2001). However, important progress has been made in recent years and successful oocyte cryopreservation has been obtained in several species (reviewed by Andrabi and Maxwell, 2007). These studies have provided substantial progress and can also be used for the conservation of endangered species.

Undoubtedly, oocyte freezing is the best way to preserve the genetic material of post-mortem females. Conversely, few gametes resist cryopreservation or develop when fertilized. Vitrification is considered a promising technique for cryopreservation of female gametes instead of classical slow freezing. However, vitrified prepubertal sheep oocytes showed a high sensitivity to both low temperature and cryoprotectants, leading to a low developmental competence after thawing (Succu *et al*., 2007). Therefore, new investigations should be made in order to improve their results.

Cryopreservation - Somatic Cells

The cryopreservation of blood and its products, tissues and DNA, allows them to be stored and used at a later time. Moreover, these cells provide rich material for the development of basic and applied research. The establishment of fibroblast banks has also been proposed as a practical approach to endangered species conservation. This technique associated with nuclear somatic cell transfer allows the restoration of extinct or endangered species with greater genetic diversity. Therefore, some studies have reported success in constituting fibroblasts banks from ruminant threatened species such as the Jining Black Goat (Li *et al*., 2009).

Artificial Insemination (AI)

According to Durrant (2009), AI is the ART that is less complex, invasive and costly and is therefore the first logical choice for companion animals or non-domestic endangered animal species. One of the most interesting AI application in conservation is to avoid the genetic depression caused by group fragmentation into free species. Thus, in situations where a given species live in small groups, females can be captured for a short period of time, be inseminated using sperm from animals from zoos and then be released back into their habitat. It still may be possible to capture males living freely and to collect their semen to inseminate females in captivity (Pukazhenthi and Wildt, 2004).

There are a small amount of reports applying AI that produced endangered ruminants such as the Blackbuck (*Antilopre cervicapra*; Holt *et al*., 1988) and the oryx (*Oryx dammah*; Morrow *et al*., 2000) antelopes and the eland (Bartels *et al*., 2001). Moreover, conceptions from AI were also reported in Adras gazelles (*Gazella dama mhor*; Holt *et al*., 1996a) and Gazelle giraffe (*Litocranius walleri*; Penfold *et al*., 2005). Santiago-Moreno *et al*. (2006) reported for the first time the birth of a Spanish ibex (*C. pyrenaica hispanica*) after AI. This species inhabits the mountains in Spain and is in risk of extinction due mainly to inbreeding.

Multiple Ovulation and Embryo Transfer (MOET)

The use of the MOET technique is not yet widespread in wildlife populations. A key conservation strategy has been the interspecies MOET biotechnique, i.e., embryo transfer from endangered species to non-threatened recipients. Some examples of success are a gaur born to a Holstein cow (Stover *et al*., 1981), an Armenian Red sheep (*Ovis orientalis*) born to a domestic sheep

(Coonrod *et al*., 1994) and a Spanish ibex born to domestic goats (Fernandez-Arias *et al*., 1996).

Regarding traditional MOET, Othen *et al*. (1999) proposed an experiment to refine estrous synchronization and superovulation steps following the administration of different hormones in bison. This study showed that the synchronization treatment commonly used in domestic cattle may be successfully applied to the bison, but new superovulatory protocols are required for an effective response in this species. In wildlife, scant knowledge exists about the kinetics of embryo development and maternal-fetal recognition. Thus, Demmers *et al*. (2000) administered recombinant interferon-_ in cervid recipients and observed a significant reduction in embryo loss after asynchronous transfer that had as an objective the reduction of asynchrony between transferred embryos and recipients.

Chagas e Silva *et al*. (2003) conducted a study to evaluate different treatments for estrous synchronization and superovulation in Saloia sheep, an endangered breed native of Portugal. The authors emphasized that the semilaparoscopic transfer allowed high rates of embryo survival and hence the birth of lambs. Recently, the MOET program was achieved in endangered Portuguese Black Merino ewes irrespective of the season of the year with a better response when using ovine FSH instead of porcine (Bettencourt *et al*., 2008).

In Vitro embryo Production (IVP)

In vitro embryo production (IVP) involves collection and *in vitro* maturation (IVM) of the oocytes, *in vitro* fertilization (IVF) of matured oocytes and *in vitro* culture (IVC) of probable embryos obtained up to a stage that is compatible with its transfer to the recipient uterus (Freitas and Melo, 2010). Its application has been proposed as a valuable strategy for the conservation of endangered species (Comizzoli *et al*., 2000).

The major studies involving IVP in threatened ruminants were performed in gaur (Johnston *et al*., 1994), Armenian Red sheep (Coonrod *et al*., 1994), Sika deer (*C. nippon*) (Comizzoli *et al*., 2001) and European mouflon (Ptak *et al*., 2002). Live births were possible only in some of them, probably due to the scarcity of the physiological knowledge of the species of interest.

Recently, Wirtu *et al*. (2009) demonstrated the feasibility of oocytes retrieval and IVM in eland and bongo (*T. eurycerus isaaci*) antelope. IVP was successfully achieved when oocytes were obtained from slaughterhouse ovaries from Red deer (*C. elaphus*) or after successive laparoscopic follicular aspiration in Sika deer. Some factors affecting IVP efficacy after both methods of collection were tested such as the effect of EGF vs. FSH and follicular fluid supplementation in IVM, sperm concentrations and incidence of polyspermy on IVF (Comizzoli *et al*., 2001). Locatelli *et al*. (2005) carried out an experiment with suggestions to improve IVM and IVF and studied a system of co-culture with sheep oviduct cells that allowed the production of 39% of viable blastocysts after IVC in Red deer. This viability was confirmed by pregnancies and births of normal offspring after transfer to recipients. Subsequently, the same laboratory was responsible for the first report of blastocyst in Sika deer produced after IVP (Locatelli *et al*., 2006).

Somatic Cell Nuclear Transfer (SCNT)

Cloning or SCNT is a process by which the nucleus (DNA) is removed from a donor cell to an enucleated recipient cell to create an exact genetic match of the donor (Andrabi and Maxwell, 2007). The report of the first mammal produced by SCNT (Wilmut *et al*., 1997) indicated that this technology could be adopted to increase the population size of threatened or extinct animals. Undoubtedly, the bucardo (*Capra pyrenaica pyrenaica*) is the best example of the use of SCNT for an extinct species recovery. The bucardo population was abundant in the Pyrenees, but decreased sharply due to hunting, leaving only three old females in 1989. By 1999, only one female about 12 years old remained alive. Skin samples from her were obtained, multiplied and cryopreserved. This female died in 2000 and the Spanish government declared the species extinct. Experiments were conducted and it became possible the birth of a normal morphologically bucardo female. However, the newborn died few minutes after birth due to physical defects in the lungs. This was the first animal born of an extinct subspecies and the first interspecies nuclear transfer (SCNTi) from adult somatic cell in Capra with success (Folch *et al*., 2009).

Currently, the use of SCNT for reconstruction of endangered species occurs mainly by SCNTi. Often, as the oocytes availability in endangered species is low, the possibility of using oocytes from other species as donor cytoplasts has been the proposed method of SCNTi. Also problematic such as the low availability of oocytes, the accessibility of recipients to promote the development of a cloned embryo is a real obstacle (Andrabi and Maxwell, 2007). To overcome this difficulty, the strategy commonly used is to transfer embryos obtained for the phylogenetically closest available species.

Future application opportunities

Embryo Sexing

Embryo sexing has been recognized to control effectively the sex of offspring in the embryo transfer industry and it could be a useful conservation tool.

Through a careful analysis about the population of a given species, this technique would allow us to achieve a balanced sex ratio of any population at risk. The accuracy of sex prediction was 100% in 58 bovine embryos when the blastomeres dissociated from a morula exceeds three (Zoheir & Allam, 2010). Mara *et al*. (2004) described a work with embryo sexing of *in vitro* produced sheep embryos by the duplex PCR that demonstrated the viability of transferring fresh sexed embryos on the same day of biopsy. However, when recipients are not accessible, sexed embryos could also be vitrified (Akiyama *et al*., 2010) after gender selection. An accuracy rate of 100% was reached for sex determination with a single blastomere at the blastula stage isolated from 43 goat embryos (Tsai *et al*., 2010). Thus, efforts to extend the sexing of embryos for endangered species should be studied in a near future, since the technique seems to be the same as for sexing bovine and ovine embryos.

Cryopreservation of ovarian tissue and manipulation of oocytes enclosed in preantral follicles

Cryopreservation of ovarian tissue and the manipulation of oocytes enclosed in preantral follicles are performed in only a few centers of animal genetic resources. These techniques could be of great interest in the conservation of endangered species in the future (Ledda *et al*., 2001). The main objective is the storage of primordial follicles that are located in the ovarian cortex, which represents the immature oocyte reserve. The potential recovery of thousands of viable oocytes is extremely important for IVP on a large scale (Figueiredo *et al*., 2007). In this context, freezing protocols have been improved over the past 20 years and high cell survival rates have been obtained (Dermici *et al*., 2003). Muruvi *et al*. (2009) demonstrated that sheep primary follicles isolated from cryopreserved neonatal ovarian tissue could be successfully grown in vitro using a serum-free, attachment-based culture system. Different standardized cryopreservation protocols need to be developed and tested to apply them to diverse species. Thus, priority must be given to the basic research as many key points need to be clarified, so the use of this technique could become realistic in endangered species.

Xenografting of ovarian tissue

Ovary xenografting is a valuable tool that may enable the generation of good quality oocytes from ovarian tissues recovered from endangered wildlife species. Oocytes recovered from grafts can be used in IVF to produce offspring. A major advantage of this biotechnology is the possibility of ovarian tissue to be transplanted irrespective of age, reproductive cycle or even *post-mortem* (Snow *et al*., 2001). Regardless of the differences between species, the recipient´s hormonal milieu allows the xenotransplantated ovarian tissue to reassume its normal function as well as its follicular development in a similar way of the donor species of ovarian tissue (Wolvekamp *et al*., 2001).

Conclusions

The erosion of genetic resources is increasing and it is clear that the problem of the growing extinction of biological species is far from an adequate solution. The use of ART in endangered species is limited, especially because favorable results depend on the knowledge of reproductive physiology of the species in question and few studies are available.

Usually, the technologies applied to endangered species are adapted from domestic breeds and, as a consequence, some biotechnologies have adjusted well, producing great results, while others were unsuccessful and frustrating. However, the positive results which were obtained are important to ensure that the use of these biotechnologies is a major tool for the conservation of endangered and threatened species. Finally, it is noteworthy that the interaction between the breeding program and GRB should be dynamic and interactive, maximizing each strategy's potential.

Acknowledgments

This study was sponsored by research funds and scholarship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brasília, Brazil), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (PNPD/CAPES, Brasília, Brazil) and Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP, Fortaleza, Brazil). Special thanks go to Christiane Garcia Vilela Nunes for critical reading of the manuscript and helpful comments.

Bibliography

Akiyama K, Kobayashi J, Sato Y, Sata R, Ohashi M, Sasaki E, Oda Y, Ogawa Y, Ueda S, Nabenishi H, Matoba S (2010). Calf production from vitrified bovine sexed embryos following in-straw dilution. Animal Science Journal, 81(4), 461-464.

Amstislavsky SY, Trukshin IS (2010). Cryobanking mammalian embryos: priorities and the optimal choice of reproductive technologies. Russian Journal of Developmental Biology, 41(1), 13-23.

Andrabi SMH, Maxwell WMC (2007). A review on reproductive biotechnologies for conservation of endangered mammalian species. Animal Reproduction Science, 99, 223-243.

Bartels P, Friedmann Y, Lubbe K, Mortimer D, Rasmussen

LA, Godke RA (2001). The live birth of an eland (*Taurotragus oryx*) calf following estrous synchronization and artificial insemination using frozen-thawed epididymal sperm. Theriogenology, 55, 381 [Abstract].

Bedford JM (1978). Influence of abdominal temperature on epididymal function in the rat and rabbit. American Journal of Anatomy, 152, 509-622.

Bettencourt EM, Bettencourt CM, Chagas e Silva J, Ferreira P, Manito CI, Matos CM, Romão RJ, Rocha A (2008). Effect of season and gonadotrophin preparation on superovulatory response and embryo quality in Portuguese Black Merinos. Small Ruminant Research, 74, 134-139.

Bettencourt EM, Bettencourt CM, Chagas e Silva J, Ferreira P, Matos CP, Romão RJ, Rocha A (2009). Fertility rates following the transfer of ovine embryos cryopreserved using three protocols. Small Ruminant Research, 82(2), 112-116.

Boettcher PJ, Stella A, Pizzi F, Gandini G (2005). The combined use of embryos and semen for cryogenic conservation of mammalian livestock genetic resources. Genetics Selection Evolution, 37, 657-675.

Chagas e Silva J, Lopes da Costa L, Cidadão R, Robalo Silva J (2003). Plasma progesterone profiles, ovulation rate, donor embryo yield and recipient embryo survival in native Saloia sheep in the fall and spring breeding seasons. Theriogenology, 60(3), 521-532.

Cheng FP, Wu JT, Chan JPW, Wang JS, Fung HP, Colenbrander B, Tung KC (2004). The effect of different extenders on post-thaw sperm survival, acrosomal integrity and longevity in cryopreserved semen of Formosan Sika deer and Formosan Sambar deer. Theriogenology, 61, 1605-1616.

Coloma MA, Toledano-Díaz A, López-Sebastián A, Santiago-Moreno J (2010). The influence of washing Spanish ibex (*Capra pyrenaica*) sperm on the effects of cryopreservation in dependency of the photoperiod. Theriogenology, 73, 900-908.

Comission on Genetic Resources – FAO. The State of the World's Animal Genetic Resources for Food and Agriculture. Rome: 2007. 524p.

Comizzoli P, Mermillod P, Cognié Y, Chai N, Legendre X, Mauget R (2001). Successful *in vitro* production of embryos in the red deer (*Cervus elaphus*) and the sika deer (*Cervus nippon*). Theriogenology, 55, 649–659.

Comizzoli P, Mermillod P, Mauget R (2000). Reproductive biotechnologies for endangered mammalian species. Reproduction Nutrition Development, 40, 493–504.

Coonrod SA, Flores-Foxworth G, Moreno JF, Westhusin M, Byrd SR, Kraemer DC (1994). Birth of Armenian red sheep (*Ovis orientalis*) lambs to domestic sheep (*Ovis aries*) following interspecific transfer of IVM-IVF derived embryos. Theriogenology, 41, 182. [Abstract].

Demirci B, Lornage J, Salle B, Poirel MT, Guerin JF, Franck M (2003). The cryopreservation of ovarian tissue: uses and indications in veterinary medicine. Theriogenology, 60, 999-1010.

Demmers KJ, Jabbour HN, Deakin DW, Flint APF (2000). Production of interferon by red deer (*Cervus elaphus*) conceptuses and the effects of roIFN-tau on the timing of luteolysis and the success of asynchronous embryo transfer. Journal of Reproduction and Fertility, 118, 387-395.

Durrant BS (2009). The importance and potential of artificial insemination in CANDES (companion animals, non-domestic, endangered species). Theriogenology, 71, 113-122.

Fernandez-Arias A, Folch J, Alabart JL, Ramon J (1996). Successful interspecific embryo transfer between Spanish ibex (*Capra pyrenaica hispânica*) and domestic goat (*Capra hircus*) using microosmotic pumps for FSH administration. Theriogenology, 45, 247. [Abstract].

Figueiredo JR, Celestino JJH, Rodrigues APR, Silva JRV (2007). Importância da biotécnica de MOIFOPA para o estudo da foliculogênese e produção *in vitro* de embriões em larga escala. Revista Brasileira de Reprodução Animal, 31(2), 143-152.

Folch J, Cocero MJ, Chesné P, Alabart JL, Domínguez V, Cognié Y, Roche A, Fernández-Arias A, Martí JI, Sánchez P, Echegoyen E, Beckers JF, Sánchez Bonastre A, Vignon X (2009). First birth of an animal from an extinct subspecies (*Capra pyrenaica pyrenaica*) by cloning. Theriogenology, 71, 1026-1034.

Freitas VJF, Melo LM (2010). *in vitro* embryo production in small ruminants. Revista Brasileira de Zootecnia, 39, 409-413.

Galal S (2005). Biodiversity in goats. Small Ruminant Research, 60, 75-81.

Glowka L, Burhenne-Guilmin F, Synge H (1994). A guide to the convention on biological diversity, IUCN. IUCN – the World Conservation Union (Gland, Switzerland).

Holt WV, Pickard AR (1999). Role of reproductive technologies and genetic resource banks in animal conservation. Reviews of Reproduction, 4, 143-150.

Holt WV, Abaigar T, Jabbour HN (1996a). Oestrous synchronization, semen preservation and artificial insemination in the Mohor gazelle (*Gazella dama mhorr*) for the establishment of a genome resource bank programme. Reproduction, Fertility and Development, 8, 1215-1222.

Holt WV, Bennett PM, Volobouev V (1996b). Genetic resource banks in wildlife conservation. Journal of Zoology, 238, 531-544.

Holt WV, Moore HDM, North RD, Hartman TD, Hodges JK (1988). Hormonal and behavioural detection of oestrus in blackbuck, *Antilopre cervicapra*, and successful artificial insemination with fresh and frozen semen. Journal of Reproduction and Fertility, 82, 717-725.

Johnston LA, Parrish JJ, Monson R, Leibfried- Rutledge L, Susko-Parrish JL, Northey DL, Rutledge JJ, Simmons LG (1994). Oocyte maturation, fertilization and embryo development *in vitro* and *in vivo* in the gaur (*Bos gaurus*). Journal of Reproduction and Fertility, 100, 131-136.

Ledda S, Leoni G, Bogliolo L, Naitana S (2001). Oocyte cryopreservation and ovarian tissue banking. Theriogenology, 55, 1359-1371.

Li XC, Yue H, Li CY, He XH, Zhao QJ, Ma YH, Guan WJ, Ma JZ (2009). Establishment and characterization of a fibroblast cell line derived from Jining Black Grey goat for genetic conservation. Small Ruminant Research, 87, 17-26.

Locatelli Y, Cognié Y, Vallet JC, Baril G, Verdier M, Poulin N, Legendre X, Mermillod P (2005). Successful use of oviduct epithelial cell coculture for *in vitro* production of viable red deer (*Cervus elaphus*) embryos. Theriogenology, 64, 1729-1739.

Locatelli Y, Vallet JC, Huyghe FP, Cognié Y, Legendre X,

Mermillod P (2006). Laparoscopic ovum pick-up and *in vitro* production of sika deer embryos: Effect of season and culture conditions. Theriogenology, 66, 1334-1342.

Lopes Jr ES, Maia ELMM, Paula NRO, Teixeira DIA, Villaroel ABS, Rondina D, Freitas VJF (2006). Effect of age of donor on embryo production in Morada Nova (white variety) ewes participating in a conservation programme in Brazil. Tropical Animal Health and Production, 38(7-8), 555-561.

Loskutoff NM, Bartels P, Meintjes M, Godke RA, Schiewe MC (1995). Assisted reproductive technology in nondomestic ungulates: a model approach to preserving and managing genetic diversity. Theriogenology, 43, 3-12.

Mara L, Pilichi S, Sanna A, Accardo C, Chessa B, Chessa F, Dattena M, Bomboi G, Cappal P (2004). Sexing of *in vitro* produced ovine embryos by duplex PCR. Molecular Reproduction and Development, 69, 35-42.

Mariante AS, Cavalcante N (2006). Animals of the Discovery. Domestic breeds in the history of Brazil. Clécia M. 2nd Ed. Brasília: Embrapa Recursos Genéticos e Biotecnologia.

Mariante AS, Albuquerque MSM, Egito AA, McManus C, Lopes MA, Paiva SR (2009). Present status of the conservation of livestock genetic resources in Brazil. Livestock Science, 120, 204-212.

Martínez-Pastor F, Martínez F, Álvarez M, Maroto-Morales A, García-Alvarez O, Soler AJ, Garde JJ, Paz, P, Anel L (2009). Cryopreservation of Iberian red deer (*Cervus elaphus hispanicus*) spermatozoa obtained by electroejaculation. Theriogenology, 71, 628-638.

Morrow CJ, Wolfe BA, Roth TL, Wildt DE, Bush M, Blumer ES, Atkinson MW, Monfort SL (2000). Comparing ovulation synchronization protocols for artificial insemination in the scimitar-horned oryx (*Oryx dammah*). Animal Reproduction Science, 59, 71-86.

Muruvi W, Picton HM, Rodway RG, Joyce IM (2009). *In vitro* growth and differentiation of primary follicles isolated from cryopreserved sheep ovarian tissue. Animal Reproduction Science, 112(1), 36-50.

Niasari-Naslaji A, Mosaferi S, Bahmani N, Gerami A, Gharahdaghi AA, Abarghani A, Ghanbari A (2007). Semen cryopreservation in Bactrian camel (*Camelus bactrianus*) using SHOTOR diluent: Effects of cooling rates and glycerol concentrations. Theriogenology, 68, 618-625.

Othen LS, Bellem AC, Gartley CJ, Auckland K, King WA, Liptrap RM, Goodrowe KL (1999). Hormonal control of estrous cyclicity and attempted superovulation in wood bison (*Bison bison athabascae*). Theriogenology, 52, 313-323.

Penfold LM, Monfort SL, Wolfe BA, Citino SB, Widlt DE (2005). Reproductive physiology and artificial insemination studies in wild and captive gerenuk (*Litocranius walleri walleri*). Reproduction, Fertility and Development, 17, 707-714.

Ptak G, Clinton M, Barboni B, Muzzeddu M, Cappai P, Tischner M, Loi P (2002). Preservation of the wild european mouflon: the first example of genetic management using a complete program of reproductive biotechnologies. Biology of Reproduction, 66, 796-801.

Pukazhenthi BS, Wildt DE. (2004). Which reproductive technologies are most relevant to studying, managing and conserving wildlife? Reproduction, Fertility and Development, 16, 33-46.

Santiago-Moreno J, Toledano-Díaz A, Pulido-Pastor A, Gómez-Brunet A, López-Sebastián A (2006). Birth of live Spanish ibex (*Capra pyrenaica hispanica*) derived from artificial insemination with epididymal spermatozoa retrieved after death. Theriogenology, 66, 283-291.

Saragusty J, Gacitua H, King R, Arav A (2006). *Post-mortem* semen cryopreservation and characterization in two different endangered gazelle species (*Gazella gazella* and *Gazella dorcas*) and one subspecies (*Gazella gazelle acaiae*). Theriogenology, 66, 775-784.

Snow M, Cleary M, Cox SL, Shaw JM, Paris MCJ, Jenkin G (2001). Comparison of the effects of *in vitro* and *in situ* storage on the viability of mouse ovarian tissue collected after death. Reproduction, Fertility and Development, 13, 389-394.

Soler AJ, Astore V, Sestelo A, Rivolta M, Jácome LN, Garde JJ (2003). Effect of thawing procedure on cryosurvival of deer spermatozoa: work in progress. Theriogenology, 60, 511-520.

Solti L, Crichton EG, Loskutoff NM, Cseh S (2000). Economical and ecological importance of indigenous livestock and the application of assisted reproduction to their preservation. Theriogenology, 53, 149-162.

Stover J, Evans J, Dolensek EP (1981). Inter species embryo transfer from the gaur to domestic Holestein. In: Annual Meeting of American Association of Zoo Veterinarians, Woodland Park Zoo, USA. Proceedings… 122-124.

Succu S, Leoni GG, Berlinguer F, Madeddu M, Bebbere D, Mossa F, Bogliolo L, Ledda S, Naitana S (2007). Effect of vitrification solutions and cooling upon *in vitro* matured prepubertal ovine oocytes. Theriogenology, 68, 107-114.

Thundathil J, Whiteside D, Shea B, Ludbrook D, Elkin B, Nishi J (2007). Preliminary assessment of reproductive technologies in wood bison (*Bison bison athabascae*): Implications for preserving genetic diversity. Theriogenology, 68, 93-99.

Tsai TC, Wu SH, Chen HL, Tung YT, Cheng WT, Huang JC, Chen CM (2011). Identification of Sex-Specific Polymorphic Sequences in Goat Amelogenin Gene for Embryo Sexing. Journal of Animal Science, (89), 2407-14.

Vajta G. (2000). Vitrification of the oocytes and embryos of domestic animals. Animal Reproduction Science, 60-61, 357-364.

Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS (1997). Viable offspring derived from fetal and adult mammalian cells. Nature, 385, 810-813.

Wirtu G, Pope CE, Paccamonti DL, Godke RA, Dresser BL (2009). Ultrasonographic-guided retrieval and *in vitro* maturation of eland (*Taurotragus oryx*) and bongo (*Tragelaphus eurycerus isaaci*) antelope oocytes. Animal Reproduction Science, 111(2-4), 160-172.

Wolvekamp MC, Cleary ML, Cox SL, Shaw JM, Jenkin G, Trounson AO (2001). Follicular development in cryopreserved Common Wombat ovarian tissue xenografted to Nude rats. Animal Reproduction Science, 65(1-2), 135-147.

Zoheir KM, Allam AA (2010). A rapid method for sexing the bovine embryo. Animal Reproduction Science, 119(1-2), 92-96.