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Developing biological resource banks as a supporting tool for wildlife reproduction and conservation The Iberian lynx bank as a model for other endangered species

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Abstract

This work presents a Biological Resource Bank generated as a complementary supporting tool for the reproduction and the in situ and ex situ conservation of the Iberian lynx. In its design we prioritized the preservation of a maximum of the current genetic and biological diversity of the population, and the harmless collection of the samples. To provide future reproductive opportunities through any possible technique, we processed and cryopreserved germinal cells and tissues from dead animals, 7 males and 6 females, as well as somatic cells and tissues from 69 different individuals. This somatic cell reserve reflects a very

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important fraction of the population biodiversity which, furthermore, will allow the development of a wide variety of studies that can be easily extrapolated to the majority of the population. We have developed a new non-destructive method to isolate cells with stem-cell-like properties. If considered convenient in the future, and after proper research, such cells could permit therapeutic applications and perhaps be a good source to be used in somatic cell nuclear transfer. Samples of whole blood and its derivatives, hairs, urine and feces from many different individuals were also preserved. Proper storage of such samples is required to allow epidemiological studies to be performed for the testing of different etiological hypotheses or, in general, to develop any bio-sanitary study to improve conservation strategies within the natural habitat. This work describes the main aspects involved in the practical implementation of the Iberian lynx Biological Resource Bank, as a model that could be useful for the development of similar banks for other endangered species.

Keywords: Reproduction; Conservation strategies; Threatened species; Iberian lynx; Biological Resource Bank

1. Introduction

The aim of animal conservation is to maintain and, if possible, increase biodiversity. The ideal approach to achieve this objective is by preserving the natural habitat (Wildt et al., 1997; Loi et al., 2001). Nevertheless, in situ conservation strategies are sometimes insufficient for the propagation of small populations as well as for the maintenance of adequate genetic diversity (Comizzoli et al., 2000). It is then important to reinforce the above strategy with ex situ programs aimed at establishing a viable population through captive breeding and cryopreservation of animal genetic resources (Andrabi and Maxwell, 2007). Biological Resource Banks (BRB), also termed Genome or Genetic Resource Banks, are repositories of collected, processed and stored biological material. Their use in the management and conservation of endangered species is being promoted extensively (Wildt, 1992; Holt et al., 1996; Comizzoli et al., 2000). Some recent examples are the Frozen Ark Consortium (http://www.frozenark.org) and the BRB of Southern Africa's wildlife (Bartels and Kotze, 2006). If used properly, these reserves have the potential of preserving the current genetic diversity of populations, as well as providing future reproductive opportunities through different techniques.

Most of the cryobanks previously reported in the literature are mainly or exclusively specialized in gametes and embryos. Such reserves are therefore germplasm banks, primarily containing semen samples (Harnal et al., 2002; Crosier et al., 2006). Their basic finality is to obtain offspring using assisted reproductive techniques (ART), which include artificial insemination, in vitro fertilization and embryo transfer, among others. Such techniques have been successfully applied to domestic species and, since the pioneer work of Kraemer et al. (1976), they also appear as a promising tool for the conservation of threatened species. Although intensive study of the reproductive biology of wild animals is still required (Wildt et al., 1997), successful cryopreservation has been carried out for spermatozoa of different non-domestic species (Crosier et al., 2006; Pukazhenthi et al., 2006a,b; see also Andrabi and Maxwell, 2007 for a review). These sample types must then be included as a basic component of any Biological Resource Bank. Nevertheless, semen banks of wild endangered animals are poorly developed at the present, probably due to the difficulty of obtaining semen samples and, consequently, the lack of basic knowledge concerning species-specific cryopreservation procedures. The situation

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is even more difficult for oocyte cryopreservation, the collection of which is only feasible from recently dead or captive females. Consequently, the banking of a genetic pool representative of the population biodiversity is very difficult when only gametes from endangered species are preserved.

We considered that, in addition to gametes and embryos, Biological Resource Banks must also contain other biological sources such as somatic tissues and cells. These samples can be easily and harmlessly collected during biopsies and necropsies. Therefore, through their collection and processing, we can obtain the largest genetic biodiversity possible of both the male and female population. Tissues and somatic cells have wide-ranging applications, which include genetic, toxicological or epidemiological studies, among others. Another potential utility of somatic cells is their application in somatic cell nuclear transfer (SCNT), popularly known as cloning, as donors of the individual genetic inheritance. There are reports of 16 mammalian species that have been cloned successfully, giving birth to healthy offspring (Cibelli, 2007). These include sheep (Cambell et al., 1996; Wilmut et al., 1997), calves (Cibelli et al., 1998), mice (Wakayama et al., 1998), goats (Baguisi et al., 1999), pigs (Polejaeva et al., 2000), cats (Shin et al., 2002), rabbits (Chesne et al., 2002), mules (Woods et al., 2003), horses (Galli et al., 2003) and threatened mammal species such as bovids (Lanza et al., 2000), mouflons (Loi et al., 2001), felids (Gómez et al., 2003) or wolves (Kim et al., 2007). These offspring were born using intra and inter-species variants of this technique. Somatic cell nuclear transfer has thus been suggested as a potentially integral part of wildlife conservation programs (Wildt and Wemmer, 1999; Ryder, 2002; Andrabi and Maxwell, 2007). However, this technique has presently a very low efficiency rate, thus more studies and approaches are required.

The preservation of other biomaterials is also useful for wildlife conservation purposes. Collection and storage of blood and tissues was already considered by Wildt et al. (Wildt, 1992; Wildt et al., 1997) since these materials can be processed into serum, plasma, blood cells, DNA and tissue/cell cultures. These biomaterials can be used for the study of genetic variation, phylogeny, paternity, gene flow and/or selection. Fecal or hair samples are frequently used as non-invasive methods of genetic identification (Palomares et al., 2002; Pilot et al., 2007). Fecal samples are also used to identify gastrointestinal parasites (Rodríguez and Carbonell, 1998; Vicente et al., 2004; Dryden et al., 2005) as well as for the monitoring of reproductive activities through the measurement of different metabolites (Comizzoli et al., 2000; Jewgenow and Paris, 2006). Blood and urine are used in epidemiological studies (Hayes et al., 2002) and are also taken routinely for general sanitary check-ups. However, such biomaterials are usually collected separately to develop specific studies and are rarely preserved. We consider useful to systematically collect, process and preserve this kind of biomaterials in endangered animals to allow, when necessary, any study favoring the in situ conservation.

With the aim of providing a supporting tool for reproduction and conservation programs, we created in 2002 a Biological Resource Bank for Spain's endangered wildlife, including mammals, fishes and many avian species (León-Quinto et al., 2005). This bank began as a reserve of cryopreserved tissues and somatic cells, and was later extended in the case of the most endangered species to also include other biological samples.

In this work we will focus on the Iberian lynx Biological Resource Bank, generated by our group in collaboration with the Environmental Council of the Regional Government of Andalusia and with members of the Captive Breeding Program. The Iberian lynx is probably the most endangered felid in the world, with less than 170 individuals probably located in only two metapopulations isolated from each other in the south of Spain. It is presently considered as critically endangered by

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the World Conservation Union (IUCN, 2004). In the design of such a biological reserve, we have considered as a main priority the preservation of a maximum of the current genetic and biological diversity of the population. To this end, all banked samples were harmlessly collected from as many individuals as possible. With these conservation approaches, a wide range of sample types were chosen, processed and preserved to: (a) allow for any possible bio-sanitary study addressed to improve the conservation of the existing individuals within their natural habitat; (b) provide future reproductive opportunities through all possible techniques, considering both gametes and somatic cells. Within this latter objective we have introduced a new approach to try to improve the efficiency of the somatic cell nuclear transfer. During the cell cultures, we searched for cells presenting two major properties of stem cells, high proliferation rate and the capability to form spherical colonies called embryoid bodies (Soria et al., 2000; Berná et al., 2001; Tuan et al., 2002; León-Quinto et al., 2004; Wagers and Weissman, 2004; Yang et al., 2007). Many reports (e.g., Tuan et al., 2002; Wagers and Weissman, 2004; Alberio et al., 2006; Yang et al., 2007) have recently shown the presence of stem cells in adult tissues. Stem cells undergo more replication cycles and have a greater plasticity than fully differentiated somatic cells (Tuan et al., 2002; Wagers and Weissman, 2004; Yang et al., 2007). Our hypothesis is that such features could favor the nuclear re-programming that must take place during the somatic cell nuclear transfer technique and thus we search for cells with stem-cell-like properties.

This complementary conservation strategy is being developed at the present as a supporting tool for reproduction and global conservation, both in situ and ex situ. In this article we describe the methodology used for the treatment of each component of the Iberian lynx BRB generated by our group during the 2003–2006 periods, attending to the objectives and conservation approaches exposed above. Our aim is to describe the main aspects involved in its practical implementation, as a model that could be useful for the development of similar banks for other endangered species.

2. Material and methods

2.1. Materials

All tissue culture media, supplements and reagents were purchased from Gibco/BRL (Grand Island, NY, USA), unless otherwise noted.

2.2. Resource sampling

The collection of samples for the Biological Resource Bank of the Iberian lynx has been performed under agreement with the Environmental Council of the Regional Government of Andalusia. We have implemented protocols to collect and send samples from dead and living animals and provided sampling kits with appropriate media. The samples were either personally transported or sent by urgent courier in refrigerated styro-foam containers. The time elapsed from the moment the samples were taken to its processing in the laboratory was under 24 h.

The samples taken from recently deceased animals were skin, muscle, oral mucosa, bone marrow, spine marrow, intestines and gonads. From live animals, millimeter-sized skin biopsies were isolated, always profiting from animal manipulation for other reasons (radio-tracking, sanitary check-ups, etc.). Whole blood (with EDTA or heparin and without such anticoagulants), urine, pulled hairs and feces were taken from cadavers as well as live animals when possible.

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2.3. Germ Cell Bank

2.3.1. Gamete cryopreservation

Since each germ sample of endangered wild animals is of great importance, we considered a non-threatened phylogenetically related species as an animal model to study the best suited procedure of gamete cryopreservation. We thus selected the domestic cat as felid model. By using gonads from domestic cats castrated at local veterinary clinics, we have carried out different studies and extrapolated the best results to the Iberian lynx.

For the cryopreservation of sperm cells obtained from cat epididymus we tested two of the most utilized glycerol concentrations for feline sperm, 7% and 4% (e.g., Tsutsui et al., 2000; Zambelli et al., 2002; Pukazhenthi et al., 2006a,b). Based on our results for sperm cells from cat epididymus (see Section 3.1 below), we used for the Iberian lynx a freezing medium that contained 4% glycerol as cryoprotectant, Tes (*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid)-Tris (2-amino-2-(hydroxymethyl)-1,3-propanediol) buffer as diluent and 20% egg yolk. Sperm cells were also isolated from part of the testicular tissue (Devroey and Van Steirteghem, 2004; Comizzoli et al., 2006) and preserved in the same freezing medium. The cryopreservation procedure of sperm cells included gradual cooling of the samples to +5 °C, equilibration at 5 °C for 20–30 min, packaging the sperm cells and exposing the samples to liquid nitrogen vapor for 10–30 min before immersion and storage in liquid nitrogen. The motility and viability of sperm cells was analyzed by using eosin-nigrosin staining assay.

We also used the cat model to test the cryopreservation procedure for oocytes and we isolated and froze immature oocytes from castrated cats. After thawing, we analyzed the effect of adding sucrose to the freezing medium described by Comizzoli et al. (2004) on the oocyte maturation rate. In the case of ovaries from Iberian lynx, part of the gonads was processed to release the follicle population by slicing with a scalpel blade. From our results obtained with the cat model (see Section 3.1), we have frozen half of the immature oocytes isolated from part of each gonad of the Iberian lynx without sucrose and the other half in presence of 0.1 M sucrose.

2.3.2. Gonadal tissue cryopreservation

For testicular tissue from the Iberian lynx, each remaining gonad after sperm cells isolation was divided into two parts. One half was cryopreserved in dimethylsulfoxide as previously described by Shinohara et al. (2002) for murine and rabbit. The other half was cryopreserved in glycerol as described for human testicular biopsy by Hovatta et al. (1996).

After oocytes isolation, the rest of the Iberian lynx ovary was divided into three parts and cryopreserved by using 1.4 M dimethylsulfoxide, 1.5 M dimethylsulfoxide with 0.1 M sucrose and 1,2-propanediol with sucrose as previously described in other species by Gunasena et al. (1997), Paris et al. (2003) and Fabbri et al. (2006).

2.4. Somatic Cell Bank

After proper washing in aseptic conditions, somatic tissues were divided into two halves. One half was cryopreserved as tissue using the methods previously described by Erdag et al. (2002), Castagnoli et al. (2003) and Kuznetsov et al. (2003), and the other half was processed and cultured to obtain cells. In the latter case, somatic tissue samples underwent enzymatic digestion or were cut into small 1–5 mm^2 pieces. In both cases, pieces of tissues and dissociated cells were transferred onto stem cell growth medium as previously described by León-Quinto et al. (2004). Briefly, tissues and cells were cultured on gelatin-coated Petri dishes with high-glucose DMEM

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containing 15% fetal bovine serum (Biochrom AG), 1% non-essential amino acids, 0.1 mmol/l 2-mercaptoethanol, 1 mmol/l sodium pyruvate, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 1000 U/ml of leukaemia inhibitory factor (LIF). The presence of LIF in the medium retains the pluripotent feature of the stem cells (Duval et al., 2000; Zandstra et al., 2000; Sekkai et al., 2005). Therefore, we submitted cells and tissues to specific stem cell medium, which potentially enhances the growth of such cells, if they are present in the small fragments of tissues received. We searched for cells presenting a higher proliferation rate than fully differentiated somatic cells and discerned such a feature by the presence of cell clusters. When observed, clusters were isolated, dissociated into single cells by trypsinization and sent onto non-treated dishes to form embryoid bodies, structures formed by embryonic stem cells. When spherical colonies (SC) similar to embryoid bodies were formed, they were collected after 4 days, dissociated into single cells and plated onto 35 mm coated Petri dish for another 7–14 days. After new clusters appeared, they were isolated and the process of forming SC and plating was repeated once again. In parallel, cells that did not form clusters in the first cell culture were separated in equivalent number than those forming the clusters, and used as controls by placing them in the same conditions as the clusters and SC during the whole process. The proliferation rate of the cells in the clusters was evaluated by using trypan blue staining and compared with the controls.

Cells derived from the clusters and SC were cryopreserved as cells with stem-cell-like properties using the freezing media previously reported by De Bari et al. (2001) and Bakken et al. (2003). Cultures that did not contain clusters were grown in culture medium without LIF. Cells were expanded up to 3 passages and cryopreserved after 1–3 passages according to the procedure described by Yokomuro et al. (2003) and Falanga et al. (2004).

2.5. Other biological reserves

Other preserved biological samples were whole blood (with EDTA or heparin and without such anticoagulants), pulled hairs, urine and feces. Concerning the whole blood, its derivatives such as plasma and serum were obtained in all cases. Serum was separated from whole blood without anticoagulant after centrifugation at 2800 rpm for 10 min. Plasma was obtained from whole blood with EDTA or heparin after centrifugation at 2500 rpm for 10 min. Serum and plasma were preserved at −80 °C once isolated. Whole blood with anticoagulant was also used to cryopreserve the living cells present in the blood, especially the lymphocytes, using the protocol described by Hayes et al. (2002). Urine was centrifuged at 1500 rpm for 5 min and the supernatant was preserved at −80 °C. Pulled hairs were cryopreserved in dimethylsulfoxide in order to preserve the living cells. Fecal samples were obtained from the natural habitat or collected during anesthesia and were separated in three parts, in order to carry out a correct conservation making possible all kind of analyses. In this sense, one third was preserved in 10% formalin, another was preserved directly at −80 °C and the last part in absolute ethanol (Da Silva et al., 1999).

3. Results

3.1. Germ Cell Bank

3.1.1. The domestic cat as felid model

Sperm cells from cat epididymus previously frozen in 7% and 4% glycerol were thawed. As shown in Table 1, a 4% glycerol concentration resulted in a greater viability and motility after thawing than 7% (62 ± 2% vs. 51 ± 3% and 53 ± 3% vs. 42 ± 3% respectively, $p < 0.05$ in both cases).

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Table 1
Effect of the glycerol concentration on the viability and mobility of epididymal cat sperm cells

N	Viability (%)		Mobility (%)		Glycerol (%)
	Fresh	Thawed	Fresh	Thawed	
12	88 ± 2	51 ± 3[a]	81 ± 1	42 ± 3[b]	7
12	86 ± 3	62 ± 2[a]	79 ± 2	53 ± 3[b]	4

Data are mean ± S.E.M. Values marked with similar letter differ significantly from each other at $p < 0.05$

Immature oocytes from cat ovaries that have been previously frozen were thawed and their maturation rate was analyzed. As shown in Table 2, the absence or presence of 0.1 M sucrose in the freezing medium did not significantly ($p > 0.05$) affect the oocyte maturation after thawing (55 ± 4 vs. 53 ± 5, respectively).

3.1.2. Iberian lynx Germ Cell Bank

We have collected gonads from Iberian lynx's cadavers since August 2005. Between August 2005 and the end of 2006 we received testes from 7 males, although most of them were subadults or did not die during the reproductive season. The post-mortem interval (from the moment of death to the sample processing in the laboratory) was from 24 to 60 h, and the age of the animals ranged from 8 weeks to 5 years. Living sperm cells were successfully isolated only in two cases, from the epididymus ($n = 2$) and from part of the testicular parenchyma ($n = 1$). Furthermore, the amount of sperm cells obtained was very small (196 000 ± 87 327, $n = 3$) and with little or no movement. Taking into account the previous results obtained by us in cats, sperm cells from Iberian lynxes were cryopreserved in freezing medium containing 4% of glycerol. Half of each remaining gonad was cryopreserved in glycerol ($n = 7$) and the other half in dimethylsulfoxide ($n = 7$).

We received ovaries from 6 Iberian lynxes for the same period as commented above. The post-mortem interval was from 24 to 72 h and the age ranged from 1 day to 7 years. Oocytes were not isolated in the ovary from the 1-day-old lynx. In the rest of cases, the average number of immature oocytes isolated from a part of the ovarian cortex received was 29 ± 8 ($n = 5$). According to our previous results obtained in the cat, we have frozen half of the immature oocytes isolated from each gonad of the Iberian lynx without sucrose ($n = 5$) and the other half in presence of 0.1 M sucrose ($n = 5$). The rest of the ovary was divided into three parts and cryopreserved as tissue by using the freezing media described in Section 2.3.2.

3.2. Somatic Cell Bank

3.2.1. Cells with stem-cell-like properties

After 7–14 days of culture in stem cell medium, some clusters ($n = 79$) of cells were observed in samples from 25 different individuals. These clusters were isolated and sent onto non-treated Petri dishes to form embryoid bodies. Most of the isolated clusters (86.1%) were capable of forming

Table 2
Effect of adding 0.1 M sucrose to the freezing medium on the rate of cat oocyte maturation after thawing

N	−Sucrose	+Sucrose
14	55 ± 4	53 ± 5

Data are mean ± S.E.M. Values do not differ significantly from each other, $p > 0.05$

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SC similar to embryoid bodies, and after 4 days were dissociated into single cells and plated onto treated Petri dishes to study if they continued to form clusters, in order to isolate the cells presenting the highest proliferation rate. From the SC population plated, new clusters appeared in 69.1% of the cultures ($n = 47$) which were isolated and cultured to form SC once again, to analyze if these cells maintained the capability of forming such structure. All these second generation clusters formed SC ($n = 47$), which were dissociated after 4 days and plated to expand the cells with stem-cell-like properties. The replication rate for the clusters was a factor 1.8 ± 0.4 ($n = 47$) higher than that for cells not forming clusters but isolated from the first cell culture presenting them.

We finally isolated cells with stem-cell-like properties from 18 different lynxes. Such cells appeared in samples from pups as well as in those from subadult and adult individuals, but they were found only in the shortest post-mortem intervals. The tissues of origin were mostly skin and muscle, however these cells were also found in the rest of the tissues received.

3.2.2. Somatic cells

Most of the cell cultures did not present clusters. In these cases, after the first trypsinization, the cells were expanded in culture medium without LIF. The majority of the cells obtained in the first 1,2 passages, together with the cells with stem-cell-like properties, were cryopreserved and reserved to a possible future use in somatic cell nuclear transfer. On the other hand, about 1/10th of the cells obtained in the two first passages were expanded up to a maximum of 3 passages, for their use in any bio-sanitary study. Most of the cells obtained from the tissues were fibroblasts, however depending on the tissue it was possible to obtain a heterogeneous population of cells.

Somatic cells and cells with stem-cell-like properties were obtained from 56 biopsies and 24 cadavers of a total of 69 different individuals, for the period 2003–2006.

3.3. Other biological reserves

We obtained whole blood, pulled hairs, urine and feces since October 2005. In all cases whole blood was sent with EDTA or heparin and without anticoagulant in order to obtain and preserve serum, plasma and the cell fraction of the whole blood. All the feces were preserved by using the three ways described in Section 2.5. As detailed in Table 3, we have processed and preserved whole blood and its derivatives, pulled hairs, urine and feces from 58, 45, 34 and 39 different individuals, respectively.

3.4. The Iberian lynx Biological Resource Bank: global composition

At the end of 2006, the global composition of the Iberian lynx Biological Resource Bank reflected the following qualitative and quantitative biodiversity. It contained gonads from 13

Table 3
Number of individuals from which biomaterials were processed and preserved during 2005 and 2006

Preserved samples	Individuals (2005)	Individuals (2006)	Different individuals (2005 + 2006)
Whole blood and its derivatives	12	56	58
Pulled hairs	9	48	45
Urine	10	31	34
Feces	13	40	39

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Table 4
The Iberian lynx Biological Resource Bank at the end of 2006

Preserved samples	Males (*N*)	Females (*N*)	Total individuals (*N*)
Gonads	7	6	13
Gametes	2	5	7
Somatic tissues	35	34	69
Somatic cells	31	32	63
Cells with SCLP	11	7	18
Blood and derivatives	28	30	58
Hairs	20	25	45
Urine	19	15	34
Feces	25	14	39

The abbreviation SCLP means stem-cell-like properties.

Table 5
Somatic Cell Bank

	Individuals (*N*)	Cryotubes/individual	Total of cryotubes
Necropsies	24	34 ± 14	~800
Biopsies	56	≥ 3	≥ 168

Average of cryotubes preserved at the end of 2006.

individuals, gametes from 7, somatic tissues from 69, somatic cells from 63, cells with stem-cell-like properties from 18, whole blood and derivatives (plasma and serum) from 58, hairs from 45, urine from 34 and feces from 39 (see Table 4). Furthermore, the amount of preserved samples for each individual was in some cases very relevant for the Somatic Cell Bank. As shown in Table 5, at least three cryotubes were preserved from each biopsy, corresponding to tissues that have been directly preserved without suffering culture, other tissues that have been cultured for several days, and the resulting cells from the tissue cultured. The number of cryopreserved samples from necropsies was much more variable, depending of both quantity and tissue types taken.

4. Discussion

In this work we have presented a BRB for the Iberian lynx, generated by our group for the period 2003–2006. This complementary conservation strategy is being developed as a supporting tool for the reproduction and the global conservation, both in situ and ex situ. In the design of such a biological reserve, we have considered as a main priority the preservation of a maximum of the current genetic and biological diversity of the population. To that end, all banked samples (germinal and somatic cells, as well as the rest of biomaterials) were harmlessly collected from as many individuals as possible. With this conservation approach, we chose a wide range of sample types, which were taken from necropsies or from live animals, in the latter case always profiting from the animal manipulation for other reasons. The main goals have been to avoid the irreversible loss of biodiversity from each dead individual and to dispose of a maximum of representation from the population biodiversity.

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4.1. Germinal Cell Bank

Although the use of reproductive technologies to enhance reproduction in humans and domestic species is significant, these techniques have so far had a negligible impact on the genetic management of wildlife species (Pope, 2000; Pukazhenthi and Wildt, 2004). Despite a few offspring that have been produced in several wildlife species, we agree with Pukazhenthi et al. (2006a,b) in considering such successes as mere demonstrations of the potential of each technology, because no species is being routinely propagated. In any case, we considered that gametes and gonads must be taken into account as a basic component of any Biological Resource Bank.

We have successfully isolated living sperm cells in testes from two animals of a total of seven males, although in small amounts and with little or no movement. Such a modest rate of success could be explained by factors such as the long post-mortem interval (from 24 to 60 h) and the fact that most of the individuals were subadults or died outside the reproductive season. We nevertheless preserved them because special techniques can be used with immobile but mature sperm cells, such as the intra-cytoplasmic sperm injection (Comizzoli et al., 2006), whereas immature sperm cells can be latter matured in vitro or in vivo (Snedaker et al., 2004; Axner, 2006). In this sense, research is under way in our laboratory on in vitro and in vivo maturation of the frozen testicular tissue and testicular cell suspensions.

In order to try to rescue some living oocytes from the received ovaries, we isolated immature oocytes and quickly preserved them in five cases by using protocols previously tested by us in the domestic cat. As in male's cadavers, a negative non-negligible factor affecting to the survival of oocytes is the large post-mortem interval in same cases, ranged from 24 to 72 h. Studies are under way in ovaries from cats to examine the fraction of living oocytes that remain after post-mortem intervals similar to those of lynx ovaries, as well as their developmental potential to achieve maturation in vitro.

Recently, a great interest in the storage of intragonadal gametes by ovarian or testicular tissue cryopreservation (Luvoni, 2006; Pukazhenthi et al., 2006a,b; Jewgenow and Paris, 2006) has been stimulated, even though it is still considered strictly experimental. This represents an important strategy for the preservation of germ cells of young individuals that die before reaching sexual maturity or without offspring. However, there is a gap of species-specific knowledge in this respect and much more studies are needed. In order to try to rescue the most amount of living cells after thawing, we thus chose to simultaneously use more than one cryopreservation protocols for each gonad, testes and ovaries, which were successful in other species.

Summarizing, we have exclusively used non-invasive methods to collect the gametes. Therefore, germinal cells were obtained only from dead animals. Such a procedure has some limitations, such as the usually long delay between the moment when the animal dies, the timing of necropsy and the transfer of the samples to the laboratory, as well as the fact that some animals were subadult or died outside the reproductive season. In spite of this, we managed to rescue some living gametes and gonadal tissues from all dead animals, and with them immature gametes within their natural architecture were cryopreserved. Future work to mature preserved immature sperm cells and oocytes by means of both in vitro and/or in vivo methods will be needed.

4.2. Somatic Cell Bank

The collection and processing of somatic tissues allows the preservation of a maximum of genetic biodiversity, from prematurely dead adults, juvenile individuals, pups and even fetus. For the period 2003–2006, we have obtained somatic tissues and cells from a total of 69 different

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individuals. Since the current Iberian Lynx population is about 170 individuals, such a Somatic Cell Bank reflects a very important fraction of the population biodiversity currently existing for this species.

A reserve of cryopreserved cells opens the possibility for a wide range of studies in genetics, toxicology, epidemiology, phylogeny or, in general, to develop any other study addressed to improve Iberian lynx conservation. Indeed, due to the lack up to now of such a reserve, different types of studies such as genetic identification were performed by using feces or hair (Pilot et al., 2007), where the correct extraction and amplification of the DNA is not always possible. This kind of problems can be overcame by using as source of DNA living somatic cells that can be expanded in cultures. In addition, a cryopreserved somatic cell reserve representing a relevant fraction of the biodiversity population makes possible to easily extrapolate the results from any study to the majority of the population.

Another potential utility of somatic cells is their application in SCNT. The first cloned animal was obtained only 11 years ago and, therefore, such a technique is still in a developing stage and presently has a low efficiency rate. In spite of this, 16 different mammalian species have been cloned (see Cibelli, 2007 for a review). Although abnormalities have been observed in some cloned animals (Cibelli et al., 2002), most offspring resulting from cloning appear healthy and produce healthy offspring. Fundamental research in the field is needed (Campbell et al., 2005; Cibelli, 2007) as well as new approaches to try to ameliorate the effectiveness of this technique.

Stem cells undergo more replication cycles and have a greater plasticity than fully differentiated somatic cells (Tuan et al., 2002; Wagers and Weissman, 2004; Yang et al., 2007). In principle, such features could favor the nuclear re-programming that must take place during the somatic cell nuclear transfer technique. Although further research is needed to prove that stem cells (all or specific types of them) could help to improve the effectiveness of SCNT, we have considered that this type of cells is especially valuable for this and other potential applications. Therefore, all the different tissues were processed with an optimal medium favoring the growth of stem cells if they are present in the small fragments of tissues received. To clearly show the presence of such cells, immunostaining techniques are usually applied (see, e.g., Yang et al., 2007). Since such techniques imply the death of the cells, we chose to isolate living cells that possessed at least the stem cell properties such as high replication rate and the ability to form SC, structures similar to embryoid bodies formed by embryonic stem cells. We have then developed a new non-destructive method to isolate cells with stem-cell-like properties. Future research will be performed in order to discern if such stem cells characteristics present in these cells make of them a better source for the SCNT than other cell types. Karyotyping such cells is considered a previous step of particular relevance before starting SCNT, as well as some other molecular characterisation.

4.3. Other biological reserves

We have also considered necessary to contemplate the specific preservation of other biomaterials such as whole blood, from which to obtain derivatives such as serum and plasma, urine, pulled hairs and feces. Blood and urine are used in epidemiological studies (Hayes et al., 2002) and routinely taken to general sanitary check-ups. Samples of feces or hair are frequently used as non-invasive methods of genetic identification (Palomares et al., 2002; Pilot et al., 2007). Fecal samples are also used to identify gastrointestinal parasites (Rodríguez and Carbonell, 1998; Vicente et al., 2004; Dryden et al., 2005) or monitoring reproductive activities through the measurement of different metabolites (Comizzoli et al., 2000).

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Systematic collection, processing and proper storage of such samples are needed in epidemiological studies to provide material for the testing of different etiological hypotheses or, in general, to develop any bio-sanitary study to improve the Iberian lynx conservation within its habitat. When necessary, from the samples preserved, we will be able to analyze the presence of antibodies against specifics antigens or the sexual hormones from the serum; the global physiology of organs such as the pancreas, liver, kidney, etc. from biochemical analyses developed in plasma; the lymphocytes population which inform about the state of the immune system of the animal from whole blood; or to know the general state of the animal as well as possible problems associated with the genito-urinary system from urine analyses. Such samples will also serve to repeat analyses if necessary or to start new investigations. In this sense, we have recently started a new study concerning the intestinal parasites present in the Iberian lynx population from the fecal samples preserved in the bank.

5. Conclusions

The Iberian lynx Biological Resource Bank presented in this work is an important tool to avoid the irreversible loss of biodiversity from each dead individual and to dispose of a maximum of representation from the population biodiversity. The goal of this article was to describe the main aspects involved in its practical implementation, as a model that could be useful for the development of similar banks for other endangered species.

To provide future reproductive opportunities through all possible techniques, we processed and cryopreserved germinal and somatic cells and tissues, obtained exclusively using non-invasive methods from dead animals and biopsies. The somatic cell reserve reflects a relevant fraction of the population biodiversity which, furthermore, will allow the extrapolation of the results from any study to the majority of the population. We have developed a new non-destructive method to isolate cells with stem-cell-like properties. Such cells were cryopreserved as a potentially better source of cells than fully differentiated somatic cells, in order to be used in the Somatic Cell Nuclear Transfer if it is considered convenient in the future. Samples of whole blood and derivatives, pulled hairs, urine and feces were systematically processed as well, to develop any study at any time in order to improve the Iberian lynx conservation within its habitat. We continue working at present on the Iberian lynx Biological Resource Bank, with the same conservation objectives and approaches exposed in this report.

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References

Alberio, R., Campbell, K.H.S., Johnson, A.D., 2006. Reprogramming somatic cells into stem cells. Reproduction 132, 709–720.

Please cite this article in press as: Leon-Quinto, T., et al., Developing biological resource banks as a supporting tool for wildlife reproduction and conservation, Anim. Reprod. Sci. (2008), doi:10.1016/j.anireprosci.2008.05.070

Andrabi, S.M.H., Maxwell, W.M.C., 2007. A review of reproductive biotechnologies for conservation of endangered mammalian species. Anim. Reprod. Sci. 99, 223–243.

Axner, E., 2006. Sperm maturation in the domestic cat. Theriogenology 66, 14–24.

Baguisi, A., Behboodi, E., Melican, D.T., Pollock, J.S., Destrempes, M.M., Cammuso, C., Williams, J.L., Nims, S.C., Porter, C.A., Midura, P., Palacios, M.J., Ayres, S.l., Denniston, R.S., Hayes, M.L., Ziomek, C.A., Meade, H.M., Godke, R.A., Gavin, W.G., Overstrom, E.W., Echelard, Y., 1999. Production of goats by somatic cell nuclear transfer. Nat. Biotechnol. 17, 456–461.

Bakken, A.M., Bruserud, O., Abrahamsen, J.F., 2003. No differences in colony formation of peripheral blood stem cells frozen with 5% or 10% dimethyl sulfoxide. J. Hematother. Stem Cell Res. 12, 351–358.

Bartels, P., Kotze, A., 2006. Wildlife biomaterials banking in Africa for now and the future. J. Environ. Monitor. 8, 779–781.

Berná, G., León-Quinto, T., Enseñat-Waser, R., Montanya, E., Martín, F., Soria, B., 2001. Stem cells and diabetes. Biomed. Pharmacother. 55, 206–212.

Cambell, K.H.S., McWhir, J., Ritchie, W.A., Wilmut, I., 1996. Sheep cloned by nuclear transfer from a cultured cell line. Nature 380, 64–66.

Campbell, K.H.S., Alberio, R., Choi, I., Fisher, P., Kelly, R.D.W., Lee, J.-H., Maalouf, W., 2005. Cloning: eight years after Dolly. Reprod. Domest. Anim. 40, 256–268.

Castagnoli, C., Alotto, D., Cambieri, I., Casimiri, R., Aluffi, M., Stella, M., Alasia, S.T., Magliacani, G., 2003. Evaluation of donor skin viability: fresh and cryopreserved skin using tetrazolium salt assay. Burns 29, 759–767.

Chesne, P., Adenot, P.G., Viglietta:, C., Baratte, M., Boulanger, L., Renard, J.P., 2002. Cloned rabbits produced by nuclear transfer from adult somatic cells. Nat. Biotechnol. 20, 366–369.

Cibelli, J., 2007. A decade of cloning mystique. Science 316, 990–992.

Cibelli, J., Campbell, K.H.S., Seidel, G.E., West, M.D., Lanza, R.P., 2002. The health profile of cloned animals. Nat. Biotechnol. 20, 13–14.

Cibelli, J.B., Stice, S.L., Golueke, P.J., Kane, J.J., Jerry, J., Blackwell, C., de León, F.A.P., Robl, J.M., 1998. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. Science 280, 1256–1258.

Comizzoli, P., Mermillod, P., Mauget, R., 2000. Reproductive biotechnologies for endangered mammalian species. Reprod. Nutr. Dev. 40, 493–504.

Comizzoli, P., Wildt, D.E., Pukazhenthi, B.S., 2004. Effect of 1,2-propanediol versus 1,2-ethanediol on subsequent oocyte maturation, spindle integrity, fertilization, and embryo development in vitro in the domestic cat. Biol. Reprod. 71, 598–604.

Comizzoli, P., Wildt, D.E., Pukazhenthi, B.S., 2006. In vitro development of domestic cat embryos following intracytoplasmic sperm injection with testicular spermatozoa. Theriogenology 66, 1659–1663.

Crosier, A.E., Pukazhenthi, B., Henghali, J.N., Howard, J.G., Dickman, A.J., Marker, L., Wildt, D.E., 2006. Cryopreservation of spermatozoa from wild-born Namibian cheetahs (*Acinonyx jubatus*) and influence of glycerol on cryosurvival. Cryobiology 52, 169–181.

Da Silva, A.J., Bornay-Llinares, F.J., Mour, I.N.S., Slemenda, S.B., Tuttle, J.L., Pieniazek, N.J., 1999. Fast and reliable extraction of protozoan parasite DNA from fecal specimens. Mol. Diagn. 4, 57–64.

De Bari, C., Dell'Accio, F., Tylzanowski, P., Luyten, F.P., 2001. Multipotent mesenchymal stem cells from adult human synovial membrane. Arth. Rheum. 44, 1928–1942.

Devroey, P., Van Steirteghem, A., 2004. A review of ten years experience of ICSI. Hum. Reprod. 10, 19–28.

Dryden, M.W., Payne, P.A., Ridley, R., Smith, V., 2005. Comparison of common fecal flotation techniques for the recovery of parasites eggs and oocytes. Vet. Ther. 6, 15–28.

Duval, D., Reinhardt, S., Kedinger, C., Boeuf, H., 2000. Role of suppressors of cytokine signalling (socs) in leukemia inhibitory factor (LIF)-dependent embryonic stem cell survival. FASEB J. 14, 1577–1584.

Erdag, G., Eroglu, A., Morgan, J.R., Toner, M., 2002. Cryopreservation of fetal skin is improved by extracellular trehalose. Cryobiology 44, 218–228.

Fabbri, R., Pasquinelli, G., Bracone, G., Orrico, C., Paradisi, R., Seraccioli, R., Venturoli, s., 2006. Fetal calf serum versus human serum: ultraestructural evaluation of protein support influence on human ovarian tissue cryopreservation. Ultrastruct. Pathol. 30, 253–260.

Falanga, J., Fiore, D., Kouttab, N., Paiva, C., Carson, P., Yufit, T., 2004. Viabilitty and apostosis of wound fibroblast after cryopreservation. Wounds 16, 283–287.

Frozen Ark Consortium, 2005. http://www.frozenark.org.

Galli, C., Lagutina, I., Crotti, G., Colleoni, S., Turini, P., Ponderato, N., Duchi, R., Lazzari, G., 2003. Pregnancy: a cloned horse born to its dam twin. Nature 424, 635.

Please cite this article in press as: Leon-Quinto, T., et al., Developing biological resource banks as a supporting tool for wildlife reproduction and conservation, Anim. Reprod. Sci. (2008), doi:10.1016/j.anireprosci.2008.05.070

Gómez, M.C., Jenkins, J.A., Giraldo, A., Harris, R.F., King, A., Dresser, B.L., Pope, C.E., 2003. Nuclear transfer of synchronized African wild cat somatic cells into enucleated domestic cat oocytes. Biol. Reprod. 69, 1032–1041.

Gunasena, K.T., Villines, P.M., Critser, E.S., Critser, J.k., 1997. Live birth after autologous transplant of cryopreserved mouse ovaries. Hum. Reprod. 12, 101–106.

Harnal, V.K., Wildt, D.E., Bird, D.M., Monfort, S.L., Ballou, J.D., 2002. Computer simulations to determine the efficacy of different genome resource banking strategies for maintaining genetic diversity. Cryiobiology 44, 122–131.

Hayes, R.B., Smith, C.O., Huang, W.-Y., Read, Y., Kopp, W.C., 2002. Whole blood cryopreservation in epidemiological studies. Cancer Epidemiol. Biomarkers Prev. 1, 1496–1498.

Holt, W.V., Bennett, P.M., Volobouev, V., Watson, P.F., 1996. Genetic resource banks in wildlife conservation. J. Zool. 238, 531–544.

Hovatta, O., Foudila, T., Siegberg, R., Hohansson, K., Smitten, K.V., Reima, I., 1996. Pregnancy resulting from intracytoplasmic injection of spermatozoa from a frozen-thawed testicular biopsy specimen. Hum. Reprod. 11, 2472–2473.

IUCN, 2004. IUCN red list of threatened species. http://www.redlist.org.

Jewgenow, K., Paris, M.C.J., 2006. Preservation of female germ cells from avaries of cat species. Theriogenology 66, 93–100.

Kim, M.K., Jang, G., Oh, H.j., Yuda, F., Kim, H.J., Hwang, W.S., Hossein, M.S., Kim, J.J., Shin, N.S., Kang, S.K., Lee, B.C., 2007. Endangered wolves cloned from adult somatic cells. Cloning Stem Cells 9, 130–137.

Kraemer, D.C., Moore, G.T., Kramen, M.A., 1976. Baboon infant produced by embryo transfer. Science 192, 1246–1247.

Kuznetsov, A.V., Kunz, W.S., Saks, V., Usson, Y., Mazat, J.P., Letellier, T., Gellerich, F.N., Margreiter, R., 2003. Cryopreservation of mitochondria and mitochondrial function in cardiac and skeletal muscle fibres. Anal. Biochem. 319, 296–303.

Lanza, R.P., Cibelli, J., Díaz, F., Moraes, C.T., Farin, P.W., Farin, C.E., Hammer, C.J., West, M.D., Damiani, P., 2000. Cloning of an endangered species (*Bos gaurus*) using interspecies nuclear transfer. Cloning 2, 79–90.

León-Quinto, T., Jones, J., Skoudy, A., Burcin, M., Soria, B., 2004. In vitro directed differentiation of mouse embryonic stem cells into insulin-producing cells. Diabetologia 47, 1442–1451.

León-Quinto, T., Jones, J., Soria, B., 2005. Nuevas estrategias de biotecnología para la conservación de fauna amenazada: los bancos celulares somáticos y su aplicación al quebrantahuesos. In: Margalida, A., Heredia, R. (Eds.), Biología de la conservación del quebrantahuesos *Gypaetus barbatus* en España. Parques Nacionales. Ministerio de Medio Ambiente, pp. 305–314.

Loi, P., Ptak, G., Borboni, B., Fulka, J., Cappai, P., Clinton, M., 2001. Gentic rescue of an endangered mammal by cross-species nuclear transfer using post-mortem somatic cells. Nat. Biotechnol. 19, 962–964.

Luvoni, G.C., 2006. Gamete cryopreservation in the domestic cat. Theriogenology 66, 101–111.

Palomares, F., Godoy, J.A., Píriz, A., O'rien, S.J., Johnson, W.E., 2002. Faecal genetic analysis to determine the presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx. Mol. Ecol. 11, 2171–2182.

Paris, M.C.J., Snow, M., Cox, S.-L., Shaw, J.M., 2003. Xenotransplantation: a tool for reproductive biology and animal conservation? Theriogenology 61, 277–291.

Pilot, M., Gralak, B., Goszczynski, J., Posluszny, M., 2007. A method of genetic identification of pine marten (*Martes martes*) and stone marten (*Martes foina*) and its application to faecal samples. J. Zool. 271, 140–147.

Polejaeva, I.A., Chen, S.H., Vaught, T.D., Page, R.L., Mullins, J., Ball, S., Dai, Y.F., Boone, J., Walker, S., Ayares, D.l., Colman, A., Campbell, H.H.S., 2000. Cloned pigs produced by nuclear transfer from adult somatic cells. Nature 407, 86–90.

Pope, C.E., 2000. Embryo technology in conservation efforts for endangered felids. Theriogenology 53, 163–174.

Pukazhenthi, B.S., Wildt, D.E., 2004. Which reproductive technologies are most relevant to studing, managing and conserving wildlife? Reprod. Fertil. Dev. 16, 33–46.

Pukazhenthi, B.S., Comizzoli, P., Travis, A.J., Wildt, D.E., 2006a. Applications of emerging technologies to the study and conservation of threatened and endangered species. Reprod. Fertil. Dev. 18, 77–90.

Pukazhenthi, B.S., Laroe, D., Crosier, A., Bush, L.M., Spindler, R., Pelican, K.M., Bush, M., Howard, J.G., Wildt, D.E., 2006b. Challenges in cryopreservation of clouded leopard (*Neofelis nebulosa*) spermatozoa. Theriogenology 66, 1790–1796.

Rodríguez, A., Carbonell, E., 1998. Gastrointestinal parasites of the Iberian lynx and other wild carnivores from central Spain. Acta Parasitol. 43, 128–136.

Ryder, O.A., 2002. Cloning advances and challenges for conservation. Trends Biotechnol. 20, 231–232.

Sekkai, D., Gruel, G., Herry, M., Moucadel, V., Constantinescu, S.N., Albagli, O., Roux, D.T.L., Vainchenkera, W., Bennaceur-Griscelli, A., 2005. Microarray analysis of LIF/stat3 transcriptional targets in embryonic stem cells. Stem Cells 23, 1634–1642.

Please cite this article in press as: Leon-Quinto, T., et al., Developing biological resource banks as a supporting tool for wildlife reproduction and conservation, Anim. Reprod. Sci. (2008), doi:10.1016/j.anireprosci.2008.05.070

Shin, T., Kraemer, D., Pryor, J., Liu, L., Rugila, J., Howe, L., Buck, S., Murphy, K., Lyons, L., Westhusin, M., 2002. A cat cloned by nuclear transplantation. Nature 415, 859.

Shinohara, T., Inoue, K., Ogonuki, N., Kanatsu-Shinohara, M., Miki, H., Nakata, K., Kurome, M., Nagashima, H., Toyokuni, S., Kogishi, K., Honjo, T., Ogura, A., 2002. Birth of offspring following transplantation of cryopreserved immature testicular pieces and in-vitro microinsemination. Hum. Reprod. 17, 3039–30445.

Snedaker, A.K., Honaramooz, A., Dobrinski, I., 2004. A game of cat and mouse: xenografting of testis tissue from domenstic kittens results in complete cat spermatogenesis in a mouse host. J. Androl. 25, 926–930.

Soria, B., Roche, E., Berná, G., León-Quinto, T., Reig, J.A., Martín, F., 2000. Insulin-secreting cells derived from embryonic stem cells normalyce glycemia in streptozotocin-induced diabetic mice. Diabetes 49, 157–162.

Tsutsui, T., Tanaka, A., Takagi, Y., Nakagawa, K., Fujimoto, Y., Murai, M., Anzai, M., Hori, T., 2000. Unilateral intrauterine horn insemination of frozen semen in cats. J. Vet. Med. Sci. 62, 1247–1251.

Tuan, R.S., Boland, G., Tuli, R., 2002. Adult mesenchymal stem cells and cell-based tissue engineering. Arthritis Res. Ther. 5, 32–45.

Vicente, J., Palomares, F., de Ibañez, R.R., Ortiz, J., 2004. Epidemiology of Ancylostoma spp. in the endangered Iberian lynx (*Lynx pardinus*) in the Doñana national park, south-west Spain. J. Helminthol. 78, 179–183.

Wagers, A.J., Weissman, I.L., 2004. Plasticity of adult stem cells. Cell 116, 639–648.

Wakayama, T., Perry, A.C.F., Zuccotti, M., Johnson, K.R., Yanagimachi, R., 1998. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature 394, 369–374.

Wildt, D.E., 1992. Genetic Resource Banks for conserving wildlife species. Justification, examples and becoming organized on a global basis. Anim. Reprod. Sci. 28, 247–257.

Wildt, D.E., Rall, W.F., Critser, J.K., Monfort, S.L., Seal, U.S., 1997. Genome resource banks. Bioscience 47, 689–698.

Wildt, D.E., Wemmer, C., 1999. Sex and wildlife: the role of reproductive science in conservation. Biodivers. Conserv. 8, 965–976.

Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J., Cambell, K.H.S., 1997. Viable offspring derived from fetal and adult mammalian cells. Nature 385, 810–813.

Woods, G.L., White, K.L., Vanderwall, D.K., Li, G.p., Aston, K.I., Bunch, T.D., Meerdo, L.N., Pate, B.J., 2003. A mule cloned from fetal cells by nuclear transfer. Science 301, 1063.

Yang, X., Qu, L., Wang, X., Zhao, M., Li, W., Hua, J., Shi, M., Moldovan, N., Wang, H., Dou, Z., 2007. Plasticity of epidermal adult stem cells derived from adult goat ear skin. Mol. Reprod. Dev. 74, 386–396.

Yokomuro, H., Mickel, D.A.G., Weisel, R.D., Li, R.K., 2003. Optimal conditions for heart cell cryopreservation for transplantation. Mol. Cell. Biochem. 242, 109–114.

Zambelli, D., Caneppele, B., Castagnetti, C., Belluzzi, S., 2002. Cryopreservation of cat semen in straws: comparison of five different freezing rates. Reprod. Domest. Anim. 37, 310–313.

Zandstra, P.W., Le, H.V., Daley, G.Q., Griffith, L.G., Lauffenburger, D.A., 2000. Leukemia inhibitory factor (LIF) concentration modulates embryonic stem cell self-renewal and differentiation independently of proliferation. Biotechnol. Bioeng. 69, 607–617.

Please cite this article in press as: Leon-Quinto, T., et al., Developing biological resource banks as a supporting tool for wildlife reproduction and conservation, Anim. Reprod. Sci. (2008), doi:10.1016/j.anireprosci.2008.05.070