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Reproductive biotechnologies for endangered mammalian species

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Abstract — Assisted reproductive techniques (gamete cryopreservation, artificial insemination, embryo transfer, and in vitro fertilization) allow to propagate small fragmented populations of wild endangered species or domestic breeds. There are the best way for producing several offspring from selected genitors in order to avoid inbreeding depression. However, few mammalian species have been well studied for their reproductive biology whereas huge differences have been observed between these species. Furthermore, materials, methods and experimental designs have to be adapted for each case and each limiting factor (wildness, poor quantity of biological material, disparate locations). Genome resource banking is currently arising and the most applied reproductive biotechnology remains artificial insemination. Assisted reproductive techniques currently developed in domestic species (intracytoplasmic sperm injection, nuclear transfer) may offer new opportunities for the propagation of endangered species.

endangered species / artificial insemination / cryopreservation / in vitro fertilization / embryo

Résumé — Biotechnologies de la reproduction appliquées aux mammifères en voie de disparition. Les techniques de reproduction assistée (cryoconservation des gamètes, insémination artificielle, transfert embryonnaire, fécondation in vitro) permettent d'accroître des populations, parfois dispersées géographiquement, d'espèces sauvages ou domestiques en voie de disparition. Ces méthodes sont le meilleur moyen pour produire plusieurs descendants à partir de géniteurs sélectionnés de façon à éviter la consanguinité. La biologie de la reproduction est connue pour peu d'espèces de mammifères alors que de grandes différences ont pourtant été mises en évidence entre ces espèces. En outre, les matériels, les méthodes, ainsi que les schémas expérimentaux doivent être adaptés à chaque cas et pour chaque facteur limitant (animaux sauvages, peu de matériel biologique, populations dispersées). Des banques génétiques sont actuellement en voie de constitution mais la technique la plus utilisée reste l'insémination artificielle. Cependant, des biotechnologies de la reproduction en cours de mise au point chez les animaux domestiques (injection intra-cytoplasmique de spermatozoïde, transfert nucléaire) pourraient offrir de nouvelles possibilités pour l'accroissement de populations menacées.

espèces menacées / insémination artificielle / congélation / fécondation in vitro / embryon

1. INTRODUCTION

The pressure of selection has resulted in thousands of different mammalian species, each with its own genetic make-up and each adapted to its own environment. Extinction of species is part of the natural process of evolution and is irreversible, but is now occurring at a much higher rate than speciation because of human activities, such as habitat destruction, over-hunting, or competition with introduced herbivores. For some domestic species, extinction has been rather due to intensive selection of a few breeds imposed by management techniques and market demands. The aim of animal conservation is to maintain biodiversity because the removal of a single species can affect the functioning of entire ecosystems [25].

A species is endangered when its survival in the wild is unlikely if causal factors of extinction continue to operate. Threatened populations may be extinct in the wild and composed of less than 50 mature individuals raised in captivity. For domestic breeds, populations are considered as endangered when less than 1 000 females or less than 20 fertile males remain [25]. Therefore, factors that may reduce the population size of a small breeding group of animals may be the variations in litter sizes, a skewed sex ratio in offspring, preferential mating between individuals, random fluctuations in birth and death rates, and an overlap of generations [3]. Isolated populations have little or no genetic exchange between them and the main problem is the mating of closely related animals that increases homozygosity and inbreeding depression. The lack of genetic diversity leads to a bad adaptive capacity and risks of transmission of inherited diseases, congenital defects and fertility problems [57, 74].

Habitat preservation is virtually, the best way to conserve biodiversity [72], but small population propagation is part of multidisciplinary research including genetic and ecological characterizations needing further strategies. In situ conservation enables in live populations of animals in their adaptive environments to be maintained the usual strategy for endangered domestic breeds. But these efforts are sometimes insufficient for the propagation of small populations and maintaining of adequate genetic diversity. Thus, ex situ conservation is aimed at establishing a viable population in captivity for an eventual reintroduction and at cryopreserving animal genetic resources (gametes, embryos, DNA, serum). However, the reproduction process may be impaired in captivity by small space, health and husbandry problems, a non adapted diet, modified sexual behavior or infertility [40]. Therefore, field conservation and captive breeding need the help of assisted reproductive techniques (ART) including gamete cryopreservation, artificial insemination (AI), embryo transfer and in vitro fertilization (IVF). ART allow to obtain more offspring from selected genitors to ensure genetic diversity and may reduce the interval between generations. As we will analyze in the first part of this review the utilization of reproductive biotechnologies for endangered mammalian species is not easy because of the broad biological variability between species and the sparse knowledge about it. In a second part, the current status of ART in endangered domestic breeds and non-domestic species will be reviewed.

2. LIMITING FACTORS AND STRATEGIES WHEN USING REPRODUCTIVE BIOTECHNOLOGIES FOR ENDANGERED SPECIES

2.1. Great variability in reproductive physiology, anatomy and behavior

Success in producing new individuals with the help of ART requires, as a first step, a greater knowledge in the basic aspects of reproductive biology. Fewer than 100 mammalian species have been studied among more than 4 000 for the details of their reproductive biology. Many of them are livestock and laboratory animals [40, 72]. Unfortunately, there are few studies about wild species which differ enormously in physiology, anatomy and behavior.

Different reproductive strategies are used by eutherian mammals for the control of ovulation and pregnancy [2, 3, 17]: spontaneous ovulation (ruminants), ovulation induced by coitus (felids), luteal life span not prolonged by mating (canids), embryonic diapause (mustelids, roe deer, bears, seals), extra corpora lutea during pregnancy (equids, deer). Marsupials however, differ from eutherian species in several aspects of their reproduction [44]. Variations also exist in the reproductive regulatory processes within the same genus. In deer species, breeding seasons are not similar and not controlled in the same way; breeding is aseasonal in the axis deer (*Axis axis*), rusa deer (*Cervus timorensis*), and sambar (*Cervus unicolor*) whereas the red deer (*Cervus elaphus*) and sika deer (*Cervus nippon*) are seasonal breeders [31]. Melatonin treatment is an efficient strategy to control the circannual cycle of reproductive activity in the red deer [1] but not for all seasonal species. The length of the estrus cycle may vary from 18 days in the red deer to 27 days in the white-tailed deer (*Odocoileus virginianus*), and the gestation period lasts from 180 days in hydropotes (*Hydropotes inermis*) to 285 days in Pere David's deer (*Elaphurus davidianus*) [31]. Hormone profiles (ovarian activity) of different close species do not however differ enormously, as shown in felids [7]. In addition to the divergent physiology, oocytes, spermatozoa, embryos and cells from different species usually require different nutritive media for in vitro culture; these media have yet to be defined for most endangered species.

In wild species vaginal cytology is not well adapted to the assessment of the moment of the estrus cycle. Therefore, modern approaches to non invasive endocrine monitoring play an important role in optimizing the success of breeding programs [61]. Fecal steroid metabolite analysis have been used to estimate the pregnancy rate of free ranging herds [63], or to assess the reproductive status of males and females for various species such as the sika deer, wild black rhinoceros (*Diceros bicornis minor*) and clouded leopard (*Neofelis nebulosa*) [8, 22, 75]. The ovarian cycle may also be characterized by steroid and peptide analysis in the urine [55]. In Asian and African elephants (*Elephas maximus*, *Loxodonta africana*), ultrasonography is also a good tool for characterizing the female reproductive status, for monitoring ovarian function or assessing the male reproductive tract [27].

For AI, it is important to precisely know the appropriate site for sperm deposition (vagina, cervix or uterus) and the appropriate time during estrus. Because the actual time of ovulation may be difficult to assess, the best strategy is to control the ovarian functions in order to more easily detect and manipulate the sexually receptive period of females. Unfortunately, commercially available gonadotropin preparations are not efficient in all species; ovarian responsiveness to synchronization treatments may be variable [59, 68].

For every species, there are also technical limitations linked to various anatomies. In small size animals, as in the common marmoset monkey (*Callithrix jacchus*), sperm recovery by vaginal washing after copulation is the best collection technique [49]. When animals are too small for ultrasonography or blood collection, they may also benefit from ovarian monitoring or gestation diagnosis by fecal steroid metabolites as shown in the pygmy loris (*Nyctecebus pygmaeus*) [34]. In contrast, manual collection of elephant semen seems to be more efficient with the help of ultrasonography [27]. Genital tracts also have anatomical species-specific characteristics [59], especially in marsupials that have two separate uteri, each connected to lateral vaginae by twin cervices [44]. Transcervical embryo recovery or AI may be achieved in most large mammals, but some species have impenetrable cervices like the giraffe (*Giraffa giraffa*) and okapi (*Okapia johnstoni*) [42], thus laparoscopic methods offer a good alternative for these kinds of animals.

In wild species, sexual and social behaviors also play a key role in the application of reproductive biotechnologies. In the deer species, only the dominant stags may be collected because only these individuals produce good quality sperm. In a group of animals, only one female may be sexually active. To reduce the vulnerability to predators, some species (e.g. *Oryx dammah*) exhibit a small window of receptivity to mating [42]; thus monitoring of the ovarian activity by fecal steroid metabolites is once again a good strategy in this case. Captivity may also induce physiological or behavioral troubles, propagation may be impaired because of sexual incompatibility between paired individuals (aggressiveness), and sexual activity may also be modified in solitary animals [40]. In order to reduce handling stress that leads to pathologies or traumatism, manipulation of deer species is performed in the darkness. Semen collections from aggressive males is feasible with the help of internal artificial vaginas or vaginal condoms. Thus reduced handling and non-invasive methods (administration of drugs and hormones with projectile darts, non-surgical methods for AI or embryo recovery and transfer) are suitable for endangered wild species.

2.2. Few individuals are available and sometimes in widely disparate locations

In captivity, 200 to 250 individuals in disparate locations often compose populations of endangered wild species. For breeding programs, the number of founder animals should be as large as possible to maximize genetic diversity. Animals with difficulties in collecting, cryopreserving or transferring their gametes, or individuals dispersed geographically could not be discarded from the ART program [3]. Technical adaptations such as a portable incubator or mobile laboratory may solve the problem of time elapsed between the gamete recovery in the field and its treatment (cryopreservation, culture) [10].

The poor availability of biological material is a major limiting factor for the study of reproductive physiology and the set up of adapted ART in endangered species. Alternative methods are necessary to characterize some parameters and to select the best donors. We therefore used heterologous in vitro fertilization (IVF) with zona-free in vitro matured bovine oocytes in order to assess the fertilizing ability and the developmental potential of cryopreserved semen from different stags. In vitro capacitation of the Oryx sperm (*Oryx dammah*) [58] and fertilizing ability of different spermatozoa of the genus Bos [46] have been assessed by heterologous IVF with bovine oocytes. The oocyte penetration assay is also used for testing sperm in canids [26].

Background data are often sparse and opportunities for research may be limited. Another way to solve the problem could be the use of a closely related non endangered species as a model for the study of physiological parameters or set up of techniques. For example, a third of the world deer species is rare or endangered but the reproductive physiology is supposed to be close to the common species studied for farming [3]. There are other models such as domestic cattle for wild oxen [62], domestic cats for endangered felids [54], domestic dogs for foxes [17], common marmoset monkeys for endangered callithrix species [43], and South American camelids for endangered camelids [6]. The domestic ferret (*Mustela putorius furo*) and domestic rabbit (*Oryctolagus cuniculus*) have also been studied for developing non-surgical methods of embryo collection and transfer in small species [35].

Since the number of individuals is often poor, the number of recipient mothers for embryo transfer is also a limiting factor for a breeding program. Interspecies embryo transfer is therefore a key technique in the conservation of endangered species by choosing appropriate related surrogate species with similarity between body size, estrus cycle and gestation pattern. Embryo transfer of gaur (*Bos gaurus*) embryos in Holstein cows was the first successful interspecific embryo transfer [64]. Other examples concern wild horse embryos transferred into domestic horses [65], Indian desert cat embryos (*Felis silvestris*) transferred into domestic cat (*Felis catus*) [54], mouflon embryos (*Ovis orientalis*) into domestic sheep (*Ovis aries*) [19]. Successful interspecific-bispecific transfers (Spanish ibex embryos (*Capra pyrenaica*) + goat embryos) into the domestic goat have also been reported [18]. Even if treatment with interferon could reduce embryonic loss due to asynchrony between the embryo and the recipient mother [15], the immunological barrier remains a major restriction for interspecific pregnancies. In deer species, common related subspecies are easier to find. In our endangered deer breeding program [45], Japanese sika deer hinds (*Cervus nippon nippon*) will serve as surrogate mothers for Vietnamese sika deer (*Cervus nippon pseudaxis*) or Formosan sika deer embryos (*Cervus nippon taiouanus*). When there is no related surrogate species (e.g. panda, *Ailuropoda melanoleuca*), interspecific embryo transfer could be feasible by creating chimeras (trophectoderm of the recipient mother with inner cell mass of the endangered species) as previously shown in ovine chimeras [9]. Further studies about the sexual preference of offspring after interspecific birth following embryo transfer will, however, be required.

Physiological, anatomical, and behavioral knowledge are not limiting factors when using reproductive biotechnologies for endangered domestic breeds. Indeed, more common related breeds are often studied. Individuals are not in disparate locations, and appropriate surrogate mothers are easy to find for intraspecific embryo transfer. The main problem is to propagate a small population avoiding genetic drift.

2.3. Regulations and institutional support

In addition to practical and technical limiting factors, institutional and economical constraints should also be considered. Even if the "Convention on International Trade in Endangered Species" (CITES) play a key role in animal conservation, rules sometimes bring new problems to conservationists by limiting the acquisition of animals that are needed to maintain traditional captive breeding programs. Furthermore, embryo importation and cryobanking strategies for wildlife species are currently not well defined [60]. Institutional supports are important (e.g. Conservation Breeding Specialist Groups, Species Survival Plan, Taxon Advisory Group, World Watch List of FAO for domestic animal diversity, European global databank for farm animal genetic resources). However, the species to save may be chosen according to different interests (political, cultural, economic) sometimes outside the control of biologists. The maintenance of large captive populations of wild animals in parallel to a reintroduced stock represents considerable problems in terms of costs. This, however, provides a back up for successive releases if there are problems in the reintroduced population. The most attractive strategy with respect to costs and low inbreeding is that involving cryopreservation of semen plus a breeding herd [41]. Long term financial support is also necessary as shown in the European Union (EEC 2078/92) which supports breeders of endangered domestic breeds, but the questions are: which intervention strategies will have the desired effects for a precise situation, what is the cost/benefit ratio? Even if embryo transfer or AI are not the most efficient methods to quickly propagate small populations, they may sometimes be more suitable than sophisticated techniques (a good AI program versus a poorly efficient IVF program).

3. CURRENT STATUS OF REPRODUCTIVE BIOTECHNOLOGIES FOR ENDANGERED MAMMALIAN SPECIES

Kraemer [38] used ART in a wild species (embryo transfer in baboon *Papio* sp.) for the first time. In Europe, ART have been used for endangered domestic breeds for more than 10 years.

3.1. Genome resource banking

Genome resource banking (GRB) refers to the collection, processing, storage and use of gametes, embryos and other biological material. GRB is in combination with ART, an interface for in situ and ex situ conservation [29]. It is currently more developed for rare domestic breeds (bovine, ovine, caprine, porcine), but the concept of using GRB to facilitate the management and conservation of endangered species is being promoted extensively [72]. If used properly, GRB has the potential to decelerate the loss of gene diversity in captive populations by reintroducing original genetic material, without removing genetically valuable individuals from the wild. As for the set up of ART, the factors that need to be considered in developing GRB are: the conservation justification, knowledge of life history and natural reproduction, knowledge of assisted reproduction, demographic distribution of donors and recipients, accessibility of donors for banking, type and amount of biomaterials to be stored. A small space for storage is needed but the liquid nitrogen supply must be efficient. Furthermore, cryobanks have to be held in two different sites in order to avoid the risk of total destruction.

A bovine breed could be saved with 1 000 sperm doses collected on 25 different males or 300 embryos (non-sexed) from 90 donors. Cryopreservation of embryos is currently not routinely possible in pigs but there is a semen bank for endangered breeds in Europe [39]. Another example is given by the embryo bank of the White Caceres cattle breed [4]. In wild species, a GRB program (semen) has been initiated for the Siberian tiger (*Panthera tigris altaica*) [29], and the Wildlife Breeding Resource Center has established the first GRB in Africa.

3.2. Sperm collection and cryopreservation, artificial insemination

Semen collection may be achieved by artificial vaginas, electroejaculation or flushing of the epididymis. These methods have been successfully used in deer species [3]. Additionally, post-coital sperm recovery has also been described in marmoset monkeys or in rhinoceros (*Dicerorhinus sumatrensis*, *Diceros bicornis*) [49, 50]. Epididymal sperm has been successfully cryopreserved in chinchilla (*Chinchilla laniger*) [52] and in red deer [21]. This method is suitable for the cryopreservation of spermatozoa after death of the male or after the rut period.

Cryopreservation techniques are well controlled in domestic ruminants, but less in equids and pigs. For wild species, standard domestic animal extenders (TRIS-buffer + egg yolk) have been tried. We are currently cryopreserving ejaculated and epididymal sperm from different deer species using a protocol developed for ram semen [11]. However, physico-chemical requirements differ between species as shown by glycerol concentration tolerances: 5% in cattle, no more than 4% in deer, 3% in pigs, 1.75% in mice, 6% in Chinchilla, and large differences are also observed in marsupials [30]. For white rhinoceros (*Ceratotherium simum*) sperm cryopreservation, however, glycerol does seem not to be acceptable [73].

AI allows the controlled propagation of genetic material from selected males and it is the most extensively applied ART. Examples include wild bovids, cervids, canids and wild felids [28, 32, 48, 54]. Intrauterine laparoscopic insemination is necessary when catheter insertion through the cervix is not possible or is ineffective, as demonstrated in felids [67]. In the USA, implementation of an AI program for the black footed ferret (*Mustela nigripes*) has allowed significant propagation and the reintroduction of this threatened animal [72].

3.3. Induction of ovulation, superovulation, embryo collection and transfer

In the sable antelope (*Hippotragus niger*) and in other wild ruminants, ovulation may be induced artificially by PGF2α injection or by removal of progesterone-releasing implants. Fecal steroid monitoring has been performed for assessing the effectiveness of the treatment and adapting the doses [68]. For felids, the induction of ovulation is possible at any stage of the reproductive cycle by using eCG and hCG injections but immunological reactions may impair the stimulation [67].

The advantage of superovulation is to propagate female genetic material. This has already been performed in various species such as African antelopes, giraffe, deer, wild cattle, wood bison (*Bison bison*) and camelids [42, 51]. Unfortunately, as in domestic animals, exogenous gonadotropins may lead to abnormal oocyte or follicle development, immunization, and variable ovarian response.

For large mammals (bovids, cervids, equids), transcervical embryo collection or transfer are used [42, 59, 69]. However, Laparoscopic embryo transfer is however, performed in various species (silver fox *Vulpes vulpes*, bear *Ursus americanus*, swine breeds) when non-surgical methods are not possible [5, 32, 56]. In wild species, scarce knowledge on the kinetics of embryo development and foeto-maternal recognition may lead to asynchrony between the transferred embryo and the recipient mother. In a recent study on the red deer, treatment of the recipient mother with interferon reduced significantly embryonic loss after asynchronous transfer [15]. For endangered wild species, cryopreservation of embryos remains to be developed. We are currently trying to develop a vitrification procedure adapted to producing in vitro deer embryos.

3.4. In vitro production of embryos

This is the most efficient technique for the propagation of small populations but it is also the most expensive method. There are different steps: gamete recovery, in vitro maturation (IVM) of the oocytes, sperm in vitro capacitation, in vitro fertilization (IVF), and in vitro development (IVD) of the resulting embryos. Immature oocyte recovery (by transvaginal or laparoscopic Ovum Pick-Up (OPU) on living females) avoids the problem of the timing of ovulation and allows to collect dead or sick females (e.g. with obstruction of the genital tract), prepubertal or pregnant animals. In contrast to AI, handling is reduced and more embryos may be produced with the same semen dose.

IVM or IVF have been tried in various species such as the mink whale (*Balaenoptera acutorostrata*) [20], African elephant (*Loxodonta africana*) [36], gorilla (*Gorilla gorilla*) [53], and zebra (*Equus burchelli*, *Equus zebra*) [47]. In our deer preservation program, we developed a method for repeated immature oocyte recovery on live sika deer hinds by laparoscopic OPU. We defined standard conditions adapted from domestic ruminants for IVM/IVF and IVD in the red deer and the sika deer before application to related endangered subspecies [14, 45]. Maturation rates were about 75–80%, and fertilization rates were 60% (with ejaculated or epididymal sperm). Finally, 20% of the fertilized oocytes reached the blastocyst stage after 6 days of culture in SOF medium. We noted that supplementation of media with biological fluids from the same species (follicular fluid, serum) was not necessary.

3.5. Other biotechnologies and future applications

Assisted hatching, embryo bisection, sperm or embryo sexing are not routinely used in domestic species. There are currently no references in endangered species. However, intra-cytoplasmic sperm injection (ICSI) is an alternative to IVF and may be a useful technique for endangered species when no motile sperms are retrieved from cadavers [37]. This ART is now performed in felids [54, 72], equids [23] and in the Rhesus monkey (*Macaca mulata*) [66]. This last example does not only have a laboratory interest but could also be useful for endangered primates.

Restoration of species by transfer of somatic nuclei into enucleated recipient oocytes has already been considered. Calves of an endangered breed of cattle (Enderby Island) adapted to extreme climatic conditions were born after nuclear transfer of granulosa cells into enucleated oocytes from domestic cows and transfer of the resulting embryos into domestic cow recipients [70]. Additionally, a pregnancy was observed after the transfer of embryos reconstructed from cells of argali *Ovis ammon* and enucleated oocytes of domestic sheep [71]. In the giant panda, blastocysts have also been obtained after nuclear transfer of panda cells into rabbit enucleated oocytes [12]. Furthermore, it has clearly been demonstrated that bovine oocyte cytoplasm could also serve as recipient for somatic cells from different mammalian species [16]. Cloning might even serve a useful purpose with species that have never bred in captivity such as the giant armadillo (*Priodontes giganteus*), or the saola (*Pseudoryx nghetinhensis*).

Germline preservation (male and female) followed by transplant in the SCID mouse could also be an interesting alternative when unexpected death of valuable individuals (complementary to oocyte recovery, epididymal flushing and somatic cell collection). Moreover, gonad rescuing techniques (preantral follicle culture) have already been tested in non-domestic felids [33]. Finally, antral follicle development in xenografted cryopreserved elephant ovarian tissue [24] or spermatogonial sperm cell transplantation after thawing in mice followed by restoration of spermatogenesis [13] could be future reproductive biotechnologies for endangered species.

4. CONCLUSION

The application of reproductive biotechnologies for the preservation of endangered mammalian species is limited by several factors. Production of embryos and offspring depends on the existing knowledge of the reproductive physiology of each particular species and little is known about the physiology of most wild animals. Captivity and poorly available biological material (often in disparate locations) increase obstacles for research progress. Thus, ART progress for endangered species depends on multidisciplinary research. ART for endangered species are adapted from technologies developed in domestic species even though all problems have not been solved in these species (e.g. variable ovarian response to hormonal stimulation). Additionally, wild species are more sensitive to stress as compared with domestic ones and require reduced handling of individuals. Furthermore, the methods and materials used have to be adapted to allow the work in field conditions. Implementation of an ART program for endangered wild species are more rare than for endangered domestic breeds. However, all over the world there are endangered species or domestic breeds which may be candidates for conservation programs. An optimal genetic management system would consist of a captive population and a cryopreserved genetic resource bank in constant dynamic interaction. But ART are not the only solution for animal conservation. Education of people and habitat preservation are essential, and it is important to consider that a species requires a conservation action even if it is not threatened.

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REFERENCES

[1] Asher G.W., Veldhuizen F.A., Morrow C.J., Duganzich D.M., Effects of exogenous melatonin on prolactin secretion, lactogenesis and reproductive seasonality of adult female red deer (*Cervus elaphus*), J. Reprod. Fertil. 100 (1994) 11–19.

[2] Atkinson S., Ragen T.J., Gilmartin W.G., Becker B.L., Johanos T.C., Use of a GnRH agonist to suppress testosterone in wild male Hawaiian monk seals (*Monachus schauinslandi*), Gen. Comp. Endocrinol. 112 (1998) 178–182.

[3] Bainbridge D.R., Jabbour H.N., Potential of assisted breeding techniques for the conservation of endangered mammalian species in captivity: a review, Vet. Rec. 143 (1998) 159–168.

[4] Bartolome Garcia B., Garcia Barreto L.J., A conservation programme for the White Caceres cattle breed, Archivos de Zootecnia 47 (1998) 371–380.

[5] Boone W.R., Catlin J.C., Casey K.J., Dye P.S., Boone E.T., Schuett R.J., Live birth of a bear cub following nonsurgical embryo collection, Theriogenology 51 (1999) 519–529.

[6] Brown B.W., A review on reproduction in South Amerisan camelids, Anim. Reprod. Sci. 58 (2000) 169–195.

[7] Brown J.L., Wasser S.K., Wildt D.E., Graham L.H., Comparative aspects of steroid hormone metabolism and ovarian activity in felids, measured noninvasively in feces, Biol. Reprod. 51 (1994) 776–786.

[8] Brown J.L., Wildt D.E., Graham L.H., Byers A.P., Collins L., Barrett S., Howard J.G., Natural versus chorionic gonadotropin-induced ovarian responses in the clouded leopard (*Neofelis nebulosa*) assessed by fecal steroid analysis, Biol. Reprod. 53 (1995) 93–102.

[9] Butler J.E., Anderson G.B., BonDurant R.H., Pashen R.L., Penedo M.C. Production of ovine chimeras by inner cell mass transplantation, J. Anim. Sci. 65 (1987) 317–324.

[10] Byrd S.R., Flores-Foxworth G., Applewhite A.A., Westhusin M.E., In vitro maturation of ovine oocytes in a portable incubator, Theriogenology 47 (1997) 857–864.

[11] Chemineau P., Cognié Y., Guérin Y., Orgeur P., Vallet J.C., Collection and preservation of spermatozoa, in: Training manual on artificial insemination in sheep and goats, Rome, FAO Animal Production and Health Paper 83 (1991) 115–130.

[12] Chen D.Y., The giant panda (*Ailuropoda melanoleuca*) somatic nucleus can dedifferentiate in rabbit ooplasm and support early development of the reconstructed egg, Science in China, Serie C, Life Sci. 42 (1999) 346–353.

[13] Clouthier D.E., Avarbock M.R., Maika S.D., Hammer R.E., Brinster R.L., Rat spermatogenesis in mouse testis, Nature 381 (1996) 418–421.

[14] Comizzoli P., Mermillod P., Legendre X., Mauget R., Successful in vitro production of embryos in the red deer (*Cervus elaphus*) and the sika deer (*Cervus nippon*), Theriogenology (in press).

[15] Demmers K.J., Jabbour H.N., Deakin D.W., Flint A.P.F., 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, J. Reprod. Fertil. 118 (2000) 387–395.

[16] Dominko T., Mitalipova M., Haley B., Beyhan Z., Memili E., McKusick B., First N.L., Bovine oocyte cytoplasm supports development of embryos produced by nuclear transfer of somatic cell nuclei from various mammalian species, Biol. Reprod. 60 (1999) 1496–1502.

[17] Farstad W., Assisted reproductive technology in canid species, Theriogenology 53 (2000) 175–186.

[18] Fernandez-Arias A., Alabart J.L., Folch J., Beckers J.F., Interspecies pregnancy of Spanish ibex (*Capra pyrenaica*) fetus in domestic goat (*Capra hircus*) recipients induces abnormally high plasmatic levels of pregnancy-associated glycoprotein, Theriogenology 51 (1999) 1419–1430.

[19] Flores-Foxworth G., Coonrod S.A., Moreno J.F., Byrd S.R., Kraemer D.C., Westhusin M., Interspecific transfer of IVM IVF-derived red sheep (*Ovis orientalis gmelini*) embryos to domestic sheep (*Ovis aries*), Theriogenology 44 (1995) 681–690.

[20] Fukui Y., Mogoe T., Ishikawa H., Ohsumi S., In vitro fertilization of minke whale (*Balaenoptera acutorostrata*) follicular oocytes matured in vitro, Theriogenology 47 (1997) 399.

[21] Garde J.J., Ortiz N., Garcia A.J., Gallego L., Landete-Castillejos T., Lopez A., Postmortem assessment of sperm characteristics of the red deer during the breeding season, Arch. Androl. 41 (1998) 195–202.

[22] Garniera J.N., Green D.I., Pickard A.R., Shaw H.J., Holt W.V., Non-invasive diagnosis of pregnancy in wild black rhinoceros (*Diceros bicornis minor*) by faecal steroid analysis, Reprod. Fertil. Dev. 10 (1998) 451–458.

[23] Guignot F., Ottogalli M., Yvon J.M., Magistrini M., Preliminary observations in in vitro development of equine embryo after ICSI, Reprod. Nutr. Dev. 38 (1998) 653–663.

[24] Gunasena K.T., Lakey J.R., Villines P.M., Bush M., Raath C., Critser E.S., McGann L.E., Critser J.K., Antral follicles develop in xenografted cryopreserved African elephant (*Loxodonta africana*) ovarian tissue, Anim. Reprod. Sci. 53 (1998) 265–275.

[25] Henson E.L., In situ conservation of livestock and poultry, FAO Animal Production & Health Paper 99 (1992) 112 p.

[26] Hewitt D.A., England G.C., The canine oocyte penetration assay; its use as an indicator of dog spermatozoal performance in vitro, Anim. Reprod. Sci. 50 (1998) 123–139.

[27] Hildebrandt T.B., Hermes R., Jewgenow K., Göritz F., Ultrasonography as an important tool for the development and application of reproductive technologies in non-domestic species, Theriogenology 53 (2000) 73–84.

[28] Holt W.V., Abaigar T., Jabbour H.N., Oestrous synchronization, semen preservation and artificial insemination in the Mohor gazelle (*Gazella dama mhorr*) for the establishment of a genome resource bank programme, Reprod. Fertil. Dev. 8 (1996) 1215–1222.

[29] Holt W.V., Pickard A.R., Role of reproductive technologies and genetic resource banks in animal conservation, Rev. Reprod. 4 (1999) 143–150.

[30] Holt W.V., Fundamental aspects of sperm cryobiology: the importance of species and individual differences, Theriogenology 53 (2000) 47–58.

[31] Jabbour H.N., Hayssen V., Bruford W., Conservation of deer: contributions from molecular biology, evolutionary ecology, and reproductive physiology, J. Zool. Lond. 243 (1997) 461–484.

[32] Jalkanen L., Lindeberg H., Successful embryo transfer in the silver fox (*Vulpes vulpes*), Anim. Reprod. Sci. 54 (1998) 139–147.

[33] Jewgenow K., Blottner S., Lengwinat T., Meyer H.H., New methods for gamete rescue from gonads of nondomestic felids, J. Reprod. Fertil. Suppl. 51 (1997) 33–39.

[34] Jurke M.H., Czekala N.M., Jurke S., Hagey L.R., Lance V.A., Conley A.J., Fitch-Snyder H., Monitoring pregnancy in twinning pygmy loris (*Nycticebus pygmaeus*) using fecal estrogen metabolites, Am. J. Primatol. 46 (1998) 173–183.

[35] Kidder J.D., Roberts P.J., Simkin M.E., Foote R.H., Richmond M.E., Nonsurgical collection and nonsurgical transfer of preimplantation embryos in the domestic rabbit (*Oryctolagus cuniculus*) and domestic ferret (*Mustela putorius furo*), J. Reprod. Fertil. 116 (1999) 235–242.

[36] Kidson A., Loskutoff N.M., Raath C., Wood C.A., Williams K.R., van Schalkwyk J.O., Dyche W.K., Barry D.M., Bartels P., Age- and parity-dependent differences in ovarian activity and oocyte maturity in the African elephant (*Loxodonta africana*), Theriogenology 43 (1995) 246.

[37] Kishikawa H., Tateno H., Yanagimachi R., Fertility of mouse spermatozoa retrieved from cadavers and maintained at 4 degrees C, J. Reprod. Fertil. 116 (1999) 217–222.

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

[39] Labroue F., Luquet M., Guillouet P., Bussière J.F., Glodek P., Wemheuer W., Gandini G., Pizzi F., Delgado J.V., Poto A., Ollivier L., Gene banks for European endangered breeds of pigs. The situation in France, Germany, Italy and Spain, Proc. Jour. Rech. Porcine France 32 (2000) 419–427.

[40] Lasley B.L., Loskutoff N.M., Anderson G.B., The limitation of conventional breeding programs and the need and promise of assisted reproduction in nondomestic species, Theriogenology 41 (1994) 119–132.

[41] Lomker R., Simon D.L., Conservation strategies for endangered cattle breeds in view of costs and inbreeding, Stocarstvo. 49 (1995) 159–166.

[42] Loskutoff N.M., Bartels P., Meintjes M., Godke R.A., Schiewe M.C. Assisted reproductive technology in nondomestic ungulates: amodel approach to preserving and managing genetic diversity, Theriogenology 43 (1995) 3–12.

[43] Marshall V.S., Kalishman J., Thomson J.A., Nonsurgical embryo transfer in the common marmoset monkey, J. Med. Primatol. 26 (1997) 241–247.

[44] Mate K.E., Molinia F.C., Rodger J.C., Manipulation of the fertility of marsupials for conservation of endangered species and control of over-abundant populations, Anim. Reprod. Sci. 53 (1998) 65–76.

[45] Mauget R., Legendre X., Comizzoli P., Mermillod P., Assisted reproductive technology in sika deer: a programme to preserve endangered deer subspecies, in: Zomborsky Z. (Ed.), Advances in deer biology. Kasposvar: Proc. 4th Int. Deer Biology Congress, 1998, pp. 185–186.

[46] McHugh J.A., Rutledge J.J., Heterologous fertilization to characterize spermatozoa of the genus Bos, Theriogenology 50 (1998) 185–193.

[47] Meintjes M., Bezuidenhout C., Bartels P., Visser D.S., Meintjes J., Loskutoff N.M., Fourie F.L., Barry D.M., Godke R.A., In vitro maturation and fertilization of oocytes recovered from free-ranging Burchell's zebra (*Equus burchelli*) and Hartmann's zebra (*Equus zebra hartmannae*), J. Zoo. Wildl. Med. 28 (1997) 251–259.

[48] Monfort S.L., Asher G.W., Wildt D.E., Wood T.C., Schiewe M.C., Williamson L.R., Bush M., Rall W.F., Successful intrauterine insemination of Eld's deer (*Cervus eldi thamin*) with frozen-thawed spermatozoa, J. Reprod. Fertil. 99 (1993) 459–465.

[49] Morrell J.M., Nubbemeyer R., Heistermann M., Rosenbusch J., Kuderling I., Holt W., Hodges J.K., Artificial insemination in Callithrix jacchus using fresh or cryopreserved sperm, Anim. Reprod. Sci. 52 (1998) 165–174.

[50] O'Brien J.K., Roth T.L., Post-coital sperm recovery and cryopreservation in the Sumatran rhinoceros (*Dicerorhinus sumatrensis*) and application to gamete rescue in the African black rhinoceros (*Diceros bicornis*), J. Reprod. Fertil. 118 (2000) 263–271.

[51] Othen L.S., Bellem A.C., Gartley C.J., Auckland K., King W.A., Liptrap R.M., Goodrowe K.L., Hormonal control of estrous cyclicity and attempted superovulation in wood bison (*Bison bison athabascae*), Theriogenology 52 (1999) 313–323.

[52] Ponce A.A., Aires V.A., Carrascosa R., Fiol de Cuneo M., Ruiz R.D., Lacuara J.L., Functional activity of epididymal Chinchilla laniger spermatozoa cryopreserved in different extenders, Res. Vet. Sci. 64 (1998) 239–243.

[53] Pope C.E., Dresser B.L., Chin N.W., Liu J.H., Loskutoff N.M., Behnke E.J., Brown C., McRae M.A., Sinoway C.E., Campbell M.K., Cameron K.N., Owens O.M., Johnson C.A., Evans R.R., Cedars M.I., Birth of a western lowland gorilla (*Gorilla gorilla gorilla*) following in vitro fertilization and embryo transfer, Am. J. Primatol. 41 (1997) 247–260.

[54] Pope C.E., Embryo technology in conservation efforts for endangered felids, Theriogenology 53 (2000) 163–174.

[55] Pryce C.R., Jurke M., Shaw H.J., Sandmeier I.G., Doebeli M., Determination of ovarian cycle in Goeldi's monkey (*Callimico goeldii*) via the measurement of steroids and peptides in plasma and urine, J. Reprod. Fertil. 99 (1993) 427–435.

[56] Rátky J., Treuer Á., Szabó P., Döbrentei B., Soós F., Seregi J., Solti L., Brüssow K.-P., Propagation of an endangered swine breed by laparoscopic embryo transfer, Theriogenology 47 (1997) 405.

[57] Roldan E.R., Cassinello J., Abaigar T., Gomendio M., Inbreeding, fluctuating asymmetry, and ejaculate quality in an endangered ungulate, Proc. R. Soc. Lond. B Biol. Sci. 265 (1998) 243–248.

[58] Roth T.L., Weiss R.B., Buff J.L., Bush L.M., Wildt D.E., Bush M., Heterologous in vitro fertilization and sperm capacitation in an endangered African antelope, the scimitar-horned oryx (*Oryx dammah*), Biol. Reprod. 58 (1998) 475–482.

[59] Schiewe M.C., Bush M., Phillips L.G., Citino S., Wildt D.E., Comparative aspects of estrus synchronization, ovulation induction, and embryo cryopreservation in the scimitar-horned oryx, bongo, eland, and greater kudu, J. Exp. Zool. 258 (1991) 75–88.

[60] Schiewe M.C., Hollifield V.M., Kasbohm L.A., Schmidt P.M., Embryo importation and cryobanking strategies for laboratory animals and wildlife species, Theriogenology 43 (1995) 97–104.

[61] Schwarzenberger F., Mostl E., Palme R., Bamberg E., Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals, Anim. Reprod. Sci. 42 (1996) 515–526.

[62] Solti L., Crichton E.G., Loskutoff N.M., Cseh S., Economical and Ecological Importance of Indigenous Livestock and the Application of Assisted Reroduction to their Preservation, Theriogenology 53 (2000) 149–162.

[63] Stoops M.A., Anderson G.B., Lasley B.L., Shideler S.E., Use of fecal steroid metabolites to estimate the pregnancy rate of a free-ranging herd of tule elk, J. Wildl. Manag. 63 (1999) 561–569.

[64] Stover J., Evans J., Dolensek E.P., Inter species embryo transfer from the gaur to domestic Holstein, Proc. Ann. Meet. Am. Assoc. Zoo Vet. Woodland Park Zoo USA (1981) 122–124.

[65] Summers P.M., Shephard A.M., Hodges J.K., Kydd J., Boyle M.S., Allen W.R., Successful transfer of the embryos of Przewalski's horses (*Equus przewalskii*) and Grant's zebra (*E. burchelli*) to domestic mares (*E. caballus*), J. Reprod. Fertil. 80 (1987) 13–20.

[66] Sutovsky P., Hewitson L., Simerly C.R., Tengowski M.W., Navara C.S., Haavisto A., Schatten G., Intracytoplasmic sperm injection for Rhesus monkey fertilization results in unusual chromatin, cytoskeletal, and membrane events, but eventually leads to pronuclear development and sperm aster assembly, Hum. Reprod. 11 (1996) 1703–1712.

[67] Swanson W.F., Horohov D.W., Godke R.A., Production of exogenous gonadotrophin-neutralizing immunoglobulins in cats after repeated eCG-hCG treatment and relevance for assisted reproduction in felids, J. Reprod. Fertil. 105 (1995) 35–41.

[68] Thompson K.V., Monfort S.L., Synchronisation of oestrous cycles in sable antelope, Anim. Reprod. Sci. 57 (1999) 185–197.

[69] Vendramini O.M., Bruyas J.F., Fieni F., Battut I., Tainturier D., Embryo transfer in Poitou donkeys, preliminary results, Theriogenology 47 (1997) 409.

[70] Wells D.N., Misica P.M., Tervit H.R., Vivanco W.H., Adult somatic cell nuclear transfer is used to preserve the last surviving cow of the Enderby Island cattle breed, Reprod. Fertil. Dev. 10 (1998) 369–378.

[71] White K.L., Bunch T.S., Mitalipov S., Reed W.A., Establishment of pregnancy after the transfer of nuclear transfer embryos produced from the fusion of argali (*Ovis ammon*) nuclei into domestic sheep (*Ovis aries*) enucleated oocytes, Cloning 1 (1999) 47–54.

[72] Wildt D.E., Rall W.F., Critser J.K., Monfort S.L., Seal U.S., Genome resource banks: living collections for biodiversity conservation, Bioscience 47 (1997) 689–698.

[73] Williams K.R., Dyche W.K., Brinders J., Molteno F., van der Lanken M., Armstrong D.L., Simmons L.G., Longevity in vitro and glycerol toxicity of epididymal sperm recovered from a white rhinoceros (*Ceratotherium simum*), Theriogenology 43 (1995) 353.

[74] Willis K., Use of animals with unknown ancestries in scientifically managed breeding programs, Zoo Biol. 12 (1993) 161–172.

[75] Yamauchi K., Hamasaki S., Takeuchi Y., Mori Y., Assessment of reproductive status of sika deer by fecal steroid analysis, J. Reprod. Dev. 43 (1997) 221–226.