Conventional and Biotechnological Tools for Conservation of Local and Endangered Animal species

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The northeastern hill region has been in focus for its high biodiversity and this region has been a priority for conservation agricultural. The northeastern hill region rich in biodiversity not only in the plant but also in animal species and birds. The reasons for conservation of animal genetic resource are economic potential, scientific use and cultural interest of particular animal population/species. The aim of animal conservation is to maintain biodiversity because removal of single species can affect the functioning of global ecosystems. Habitat preservation is one of the best ways to conserve biodiversity. In situ conservation strategies enable live populations of animals to be maintained in their adaptive environments. However, these efforts are sometimes insufficient for the propagation of small populations and maintaining adequate genetic diversity.

Economic potential

Endangered populations should be conserved for their potential economic use in the future. Their economic potential may be the production of meat, milk, fiber, skin or draught power and it may be in diverse climatic and environmental conditions. Endangered populations with economic potential may have regional adaptation developed for the country of origin, or adaptations which may be beneficial in other areas of the world where similar or complementary conditions exist. Animals with distinct characteristics may be beneficially incorporated into the breeding programmes of other countries, for example, the prolific characteristics of the once rare Finnsheep (Maijala *et. al,* 1990), Twinning and triplet kidding in Assam hill goat and specific milk composition of yak and mithun in the NEH region. Economic potential cannot be measured by looking simply at performance. Rare or endangered breeds are often highly adapted and their performance should be measured comparatively, within their own environmental conditions. They should not be compared with other breeds in improved or modified conditions or under intensive management. Furthermore, they should be examined with respect to the products for which they were selected and valued in the conditions under which they evolved. There are many examples where growth rate, prolificacy, or milk production have been measured and used to illustrate the inferiority of purebred indigenous stock over that of exotic imported breeds or their crosses (Hodges, 1986).

Scientific use

Endangered populations should be conserved for their possible scientific use. This may include the use of conservation stocks as control populations, in order to monitor and identify advances and changes in the genetic makeup and production characteristics of selected stocks. They may include basic biological research into physiology, diet, reproduction or climatic tolerance at the physiological and genetic level. Genetically distinct breeds are needed for research into disease resistance and susceptibility, which could help in the development of better medication or management of disease. It could also help with the identification of specific genes involved in natural disease or parasite control. Some populations may also be used as research models in other species, including man. Example, isolation and identification of gene responsible for diseases resistant in Indian cattle.

Cultural interest

Many populations have played an important role in specific periods of national or regional history. There are also breeds which have been associated with social and cultural development; the Navajo-Churro sheep whose wool is essential in the production of the native rugs of the Navajo. Yak and mithun is essential for social and cultural identity of tribes in the NEH region. There are also many breeds which may be conserved for their aesthetic value. Cattle breeds in India and many of the ornamental and fighting/games for poultry breeds in India

Principles of conservation

The idea of conserving animal genetic resources focuses on two separate; the first is the conservation of 'genes' and the second, the conservation of 'breeds' or populations. But both are interlinked concepts. The conservation of 'genes' refers to action to ensure the survival of individual genetically controlled characteristics inherent within a population or group of populations. However, it does not require that the genetic function at the chromosome or DNA level be understood. Such a characteristic may in fact be a complicated biochemical function controlled by several sections of DNA on more than one chromosome, but provided the characteristic can be identified in the appearance or function of the animals that exhibit.

The conservation of populations or breeds refers to action to ensure the survival of a population of animals as defined by the range of genetically controlled characteristics that it exhibits. This form of conservation is applied to endangered species as well as to breeds and it ensure the conservation of all the characteristics inherent with a given population, including many which may not have been recognized, defined, identified or monitored. The differences between breeds may often be due to differences in the frequency of quantitative genes rather than the presence or absence of unique genes.

Candidates for conservation

Opinions may vary over the years to which animal genetic resources are candidates for conservation. Estimates have been influenced by the relative cost/benefit of conserving all genetic variation as compared to those that can be demonstrated to have predictable economic, scientific or cultural value.

Unique population: Uniqueness is difficult to define with respect to livestock populations. There are populations with obviously unique and clear characteristics or traits. But for the vast majority of populations their uniqueness is subjective. It refers to the fact that no other population has the same ancestry, environmental adaptation, human selection, appearance or production characteristics. In effect, the difference between two populations may only be a function of the relative frequencies of the same genes. From the point of view of conservation any population which is historically or geographically isolated or which has had little genetic influence from other breeds over a long period of time, or which exhibits unusual characteristics or traits should be considered to be a unique population.

Endangered species: In wildlife conservation, a population said to be endangered when the chance of the survival in the wild is unlikely unless action is taken to conserve that population. There is no simple numerical level at which a population is defined as being endangered or eligible for consideration as a candidate for conservation. Rather it is dependent upon a number of factors: the actual numbers of animals; the rate of decline in the population size; the closeness of relationship between individuals within the population; the geographical range and the rate of reduction of that range; special threats from introduced species; rapid changes in the environmental conditions including climate, predators and parasites.

In common domestic species for which varieties, strains or breeds are in danger of extinction, the population levels at which action needs to be taken can be much lower. In these cases the common strains or breeds can be used for cross breeding, grading up or as surrogate mothers in an embryo transfer programme.

The International Union for the Conservation of Nature (IUCN) provides clear definitions for the variety of species in its international Red Data Books. These definitions relate to the survival chances of the populations and take all the variables of population structure and environmental factors into account. Wildlife conservation, based upon these categories is most commonly centered on the in situ conservation of populations in their natural environments. The population size must be sufficient to enable the necessary genetic diversity to survive within the population, so that it has a good chance of continuing to adapt and evolve over time. This reserve size can be calculated for target species by examining the population density in naturally occurring situations. The reserves must then be protected from intrusion or destruction by man, and against other catastrophes.

Rapid change in population: Rapid genetic changes in intensively selected breeds, often involving the use of high levels of advanced technology including artificial insemination (AI) and embryo transfer. These breeds are producing at a very high level under intensive management, veterinary care and feeding regimes. They include breeds likely to be affected by the introduction of transgenic technology. They are the intensively selected dairy cattle breeds of the temperate regions and the industrialized pig and poultry stocks. Conservation may be needed of samples of these populations as they change to ensure that alternative selection options exist. Collection of cryogenic samples would be a useful precaution enabling future changes in direction within these breeds and the establishment and maintenance of live control populations as in situ conservation.

Characterization of germplasm

Characterization of the breed which includes the measurement and description of external appearance, production characteristics, climatic adaptation, disease resistance, parasite tolerance, management and any other special feature. It may also involve the collection of biochemical information from blood types, milk proteins and the comparative analysis of DNA fragments.

Use of molecular markers

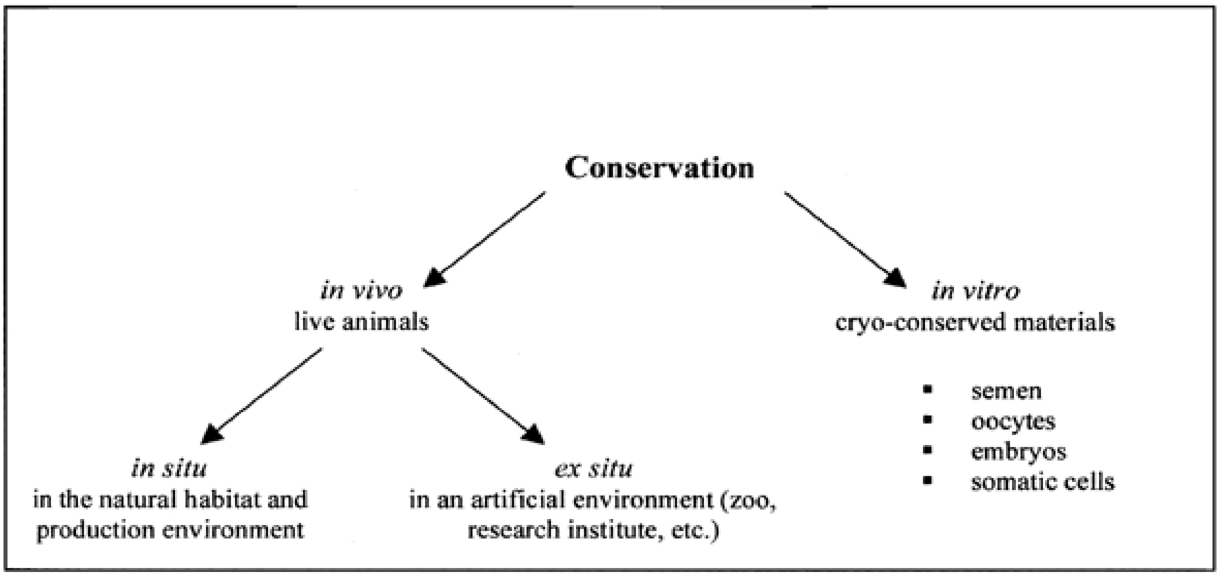
Molecular markers are a tool to study diversity on the genetic level. The most widespread use of molecular markers in this context is the assessment of diversity within and between breeds.

In addition, one might also consider markers associated with so-called quantitative trait loci (QTL), i.e. markers that reflect the genetic potential of an animal for a given quantitative or qualitative trait. Farm animal research focuses very strongly on mapping QTLs and single genes so that such markers will be increasingly available in the future. Of special interest will be markers linked to disease-resistance QTL, such as trypanotolerance in cattle (Hanotte *et. al.,* 2003), nematode resistance in sheep (Coltman *et. al*., 2001), and E.coli-resistance in pigs (Meijerink *et. al.,* 2000).Molecular markers are an indispensable tool to understand the genetic structures of populations.

Conservation methods

The terms conservation, preservation, ex situ and in situ are used here according to the definition given by FAO (1992). There are several ways, differing in efficiency, technical feasibility and costs, to conserve animal genetic resources. Developing and utilising a genetic resource is considered the most rational conservation strategy. However, there are cases where ex-situ approaches are the only alternatives. Ex-situ approaches include: maintenance of small populations in domestic animal zoos; cryopreservation of semen (and ova); cryopreservation of embryos; and some combinations of these. Cryopreservation of gametes, embryos or DNA segments can be quite an effective and safe approach for breeds or strains whose populations are too small to be conserved by any other means.

Systematic overview of basic conservation schemes for farm animals



Regeneration of offspring following transfer of frozen-thawed embryos has been successful for all major domestic species, Respective pregnancy rates of 58 and 50% for fresh and frozen-thawed in vitro produced embryos have been reported (Lu *et. al* 1990). Also, calves have been produced from transfer of both split and frozen-thawed in vitro produced embryos.

Development in genetic engineering, cryobiology, cell biology and embryology will provide techniques that may enhance our ability to preserve germplasm in vitro. Techniques such as transfer of DNA within and between species and the production of viable transgenic animals are far from practical application. However, biotechnology will certainly contribute newer and cheaper methods for preservation such as storage of catalogued DNA. At present, other than live animal and embryo preservation, the other techniques do not allow preservation of genomes in a form which can be reactivated in toto at a later stage, but they permit the preservation of individual genes or gene combinations for possible future regeneration.

Conservation of indigenous animal genetic resources should be one of the priority livestock development activities for developing countries. The critical importance of these resources to their owners in developing countries need not be emphasised. Their importance to developed countries is also becoming evident as indicated by the increasing importation of tropical germplasm by these countries. It is highly likely that these resources will become of increasing importance to the industrialised countries either as sources of unique genes or when environmental concerns necessitate change in production systems. Developed countries should, thus, assist in the conservation and development of these resources. Technology for cryopreservation of semen and embryo is sufficiently developed and to be applied in developing countries.

Ex situ versus *in situ* conservation methods

Ex situ preservation involves the conservation of plants or animals in a situation removed from their normal habitat. It is used to refer to the collection and freezing in liquid nitrogen of animal genetic resources in the form of living semen, ova or embryos. It may also be the preservation of DNA segments in frozen blood or other tissues. In situ conservation is the maintenance of live populations of animals in their adaptive environment or as close to it as is practically possible. For domestic species the conservation of live animals is normally taken to be synonymous with in situ conservation.

Ex situ and in situ conservation are not mutually exclusive. Frozen animal genetic resources or captive live zoo populations can play an important role in the support of in situ programmes.

1. *Ex situ* conservation /Cryogenic Preservation

a. Advantages

- Relatively low cost for collection, freezing and storing frozen material, as compared to maintaining large scale live populations.
- The cost of maintaining a cryogenic store is minimal. Such banks require little space and few trained technicians.
- A very large number of frozen animals from a large number of populations can be stored in a single facility.
- Cryogenically preserved populations suffer no genetic loss due to selection or drift.
- A sample in suspended animation and that sample remain genetically identical from the time of collection to the time of use.
- The effects of long term radiation are considered to be negligible.

b. Disadvantages

- The principal disadvantages of *ex situ*, or cryogenic preservation is the availability of the necessary technology and access to the frozen populations.
- It requires a guaranteed supply of liquid nitrogen which may be costly.
- Cryogenic stores have no intrinsic value with respect to financial income unless material can be sold for research and development.
- It does not produce food or other agricultural commodities and might therefore be deemed to be expensive luxuries in periods of financial austerity.
- If sampling method and collection of genetic material from a limited number, cryogenic storage can result in an initial genetic drift.
- Shift in gene frequencies between the original population and the cryogenically conserved sample population.
- There is a potential danger in cryogenic storage, from large scale loss of material due to serious accidents due to human error, power failure, loss of liquid nitrogen, fire, flood, storm, earthquake or war.
- Cryogenically preserved populations are not able to adapt through gradual selection, to changes in the climate or disease background of the local or global environment.

2. *In situ* Conservation

a. Advantages

- *In situ* conservation relate to the availability of technologies and the utilization of the breeds.

- The in situ conservation of live populations requires no advanced technology.
- The farmers of every region and nation know how to manage and maintain their local strains.
- It can ensure that financial commitment to the conservation of animal genetic resources involves helping to improve the livelihood of farming communities associated with the breeds targeted for conservation.
- Live conservation projects involve animal utilization and are net producers of food, fibre and draught power.
- It does not require the importation of expensive materials, skills or equipment.
- Live conservation programmes may survive major political or environmental upheaval, wars, or climatic disasters.
- Sufficient numbers of breeding units must be established and maintained, however, for each conserved population.
- It enable breeds to be properly characterized and evaluated in their own and related localities.
- It allow for comparative trials, research and crossing experiments.
- This method of conservation also allows populations to adapt to changing environmental conditions and endemic diseases.

b. Disadvantages

- Lack of complete control over the many factors which influence the survival of individuals and therefore the genetic makeup of the conserved population.
- In situ conservation requires land and people which are limited resources in some regions.
- It dependent upon unpredictable financial and political change particularly and capacity to produce agricultural commodities and sell livestock to supplement their budgets.
- Genetic drift is an inevitable feature of all live animal conservation projects, even when steps are taken to minimize the problem.
- Selection and the resultant shift in the gene frequencies within a population are a real possibility.
- Selection is a particular concern when it is applied to populations being maintained under modified environmental conditions
- *In situ* conservation incurs the possible threat of disease eliminating whole or substantial parts, of a conserved population, particularly if the conserved herd is in a single or only a few linked locations.
- Diseases may also act as a major selection pressure within a population, and may substantially change its characteristics. Finally, live animal conservation programmes do not assist in the easy international transfer of animal genetic resources as compared to the movement of frozen material and it is relatively more expensive and there are international restrictions on the movement of animals to control disease.

3. Co-ordination between *in situ* conservation and *ex situ* conservation

Cryogenic methods allow for animal genetic resource material to be suspended, unchanged, for long periods of time. Live conservation efforts enable breeds to be properly evaluated, monitored and used in the present changing agro-economic climate as well as being available for future farmers and livestock breeders. The two strategies are not mutually exclusive and should be considered as complimentary strategies which may be easily and beneficially linked. Collecting and freezing of semen is far simpler in most species than collecting and freezing of embryos. Recent development in the technology to mature ova from the ovaries of slaughtered females has produced a relatively cheap and easy method for the collection of haploid cells from females to parallel the collection of sperm. It is likely that this technique will become increasingly useful as the methods become more widely available.

Conventional techniques /steps to be taken

- To undertake systematic cataloguing of animal germplasm and to establish a data bank and information service on animal genetic resources.
- Identification, evaluation, cataloguing and conservation of herds or flocks identified as valuable for purposes of conservation consisting of important indigenous breeds of livestock and poultry in the country.
- To undertake survey for the evaluation of merits or attributes of breeds discovered recently or threatened with extinction.
- Formulation of criteria and parameters to enable identification of animals and flocks of superior genetic merit or worthiness for conservation. To take steps for the preservation of germplasm both as live animals or by setting up frozen semen and embryo banks.
- Documentation of pertinent information in regard to identity of herds and flocks on a computer readable format.
- Processing of information collected under surveys carried out for the identification of valuable animal resources material.
- Dissemination of information in a cogent manner to enable individuals and agencies to use information in regard to the available animal genetic resources,
- Maintenance of national/international liaison with institutions concerned with similar work.
- Rendering financial assistance to universities, IGAR institutes and government and private bodies, where maintenance of such valuable germplasm is considered desirable.
- Monitoring of the entire improvement programme and maintenance of rare breeds/herds/ flocks in the country.
- Monitoring of new introduction of animal germplasm and new synthetics.
- To stimulate programmes for improvement in the various breeds and to give adequate financial and technical support to these.

Application of biotechnological tools for conservation of endangered species

Biotechnological methods offer many advantages to conventional captive breeding procedures currently in use today. For one, the animals do not have to be moved around, procedures that often cause severe stress and affect fecundity of the individuals. Samples can be taken from animals in the wild without removing them from their environment. Space is also a limiting factor in most zoos around the world and taking only samples from the animals would help that situation. The procedures that are in current use in animal farming have been shown to be relatively harmless to the animals. Storage of the genetic information will help to preserve biodiversity and counter the effect of genetic drift on small populations. The genetic material is available to many generations not just one. Even if the animal dies its genes are still available. Even animals that have been dead for 24 hours are still useful because their gametes can be extracted. Successful protocols could possibly be used to re-inject diversity back into wild populations that have become very small.

The main disadvantage is that research on these techniques for endangered animals is still at a very early stage and is very costly. The amount of resources available for conservation of species is very limited and the main argument is whether or not to invest in such research. The resources are needed for habitat preservation. Some argue that the best way to help endangered animals is to protect their environment not freeze them. Entire ecosystems cannot be frozen because of our limited knowledge of the role of the different organisms. If the habitat is destroyed it can never be recreated in exactly the same way so it is useless to bring some of the species back. It is also very arguable whether just preserving the genes will be enough to conserve the entire animal. Many animals need to learn behaviour in order to survive. This technique does not allow that. It is also believed that public perception will be to accept these techniques as complete solutions thereby giving people further excuses to destroy habitats.

In situ and *ex situ* conservation programs for some endangered mammalian species can benefit from modern reproductive biotechnologies or assisted reproductive techniques (ART) including artificial insemination (AI), embryo transfer (ET), in vitro fertilization (IVF), gamete/embryo micromanipulation, semen/embryo sexing and genome resource banking (GRB). With more knowledge emerging on the basic biology of reproduction, cloning or somatic cell nuclear transfer (SCNT) have been suggested as a potentially integral part of wildlife conservation programs. To date, however, natural breeding coupled with traditional ART has been the preferred method for increasing endangered animal populations, due to the poor efficiency of SCNT. With future progress in the field of cloning, this technology will also become helpful for saving species at risk of extinction. It is believed that modern biotechnologies or ART for mammalian species threatened with extinction will allow more offspring to be obtained from selected parents to ensure genetic diversity and may reduce the interval between generations.

Strategies for application of reproductive biotechnologies in endangered species

Within the past few decades, a powerful new approach has emerged for conservation of threatened wildlife species, through in situ and ex situ conservation programs. Hanks (2001) in his review on conservation strategies suggested that zoo-based captive breeding programs should also be regarded as a supplement rather than an alternative to in situ conservation activities. Also captive breeding programs should essentially be guided by rational priorities for ex situ conservation, ideally focusing on threatened species or groups with which zoos already have husbandry experience. One of the major problems with the implementation of in situ and ex situ conservation programs is the lack of availability of the biological material which is required for a better understanding of reproductive patterns as well to maximize reproductive efficiency. This constraint arises from the strict procedures adopted for restraining or anaesthetizing free-living animals for collection of biological/reproductive samples. However, this has been partially resolved by the development of viable methods for assessment of hormonal profiles from voided urine and faeces, also termed as non-invasive hormonal monitoring.

Many studies have highlighted the possibilities for non-invasive, remote monitoring of reproductive status in a number of endangered mammalian species (Broen, 2000). Further development of techniques that allows the instantaneous assessment of the endocrine status of animals living in nature would offer exciting opportunities to interrelate their physiology, especially that of reproduction, with their natural environment. This information would also help to apply available ART like AI and ET more efficiently for in situ or zoo-based conservation of endangered species. Other developments have taken place on the collection of biological material, like semen from aggressive males, by the use of internal artificial vaginas or vaginal condoms, collection from the epididymes following the death of an animal post-coital sperm recovery. For embryo recovery, non-surgical or less invasive methods like transcervical embryo collection have been applied in the recent past. Ultrasonography is another non-invasive technology that can be helpful for monitoring female ovarian function, reproductive tract morphology, pregnancy, foetal growth and assessing the male reproductive tract in many non-domestic species.

Reproductive biotechnological tools

A number of reproductive biotechnologies are available that are being widely applied for in situ and ex situ conservation of endangered species. The main technologies that have been used or considered are artificial insemination, embryo transfer (and its combination with in vitro fertilization) and sexing, gamete and embryo micromanipulation, sperm sexing, genome resource banking, and cloning.

1. Artificial insemination

One of the major applications of AI in conservation is to avoid genetic depression caused by fragmentation of groups in free-living species. Therefore, for some species living in small populations, it may be feasible to capture females for short periods for AI with sperm collected from zoo-maintained healthy males. Alternatively, for some species it would be more appropriate to capture free-ranging males for short time for semen collection, and AI captive females. Another possible application of AI, for in situ or zoo-based conservation, is to circumvent the poor natural mating behaviour in some species like giant pandas (Wildt *et. al*., 2003). Additionally, AI can be applied in captive non-domestic species to avoid the challenges of translocation of the animals for breeding purposes (Pukazhenthi and Wildt, 2004).

Besides the vast potential of AI in conservation programs there are also significant boundaries for AI technology in wildlife. Although it is generally possible to collect semen from most nondomestic mammals by artificial vagina, vaginal condoms, digital masturbation of penile bulb or electroejaculation (under anaesthesia), these processes remain difficult for some species, including the rhinoceros, non-domestic equids, certain great apes, canids and marsupials (Pukazhenthi and Wildt, 2004). Another challenge is the poor knowledge on female reproductive physiology and anatomy in many non-domestic species, particularly a comprehensive understanding of female reproductive cycles and determination of the most suitable site for semen deposit in female tract, which are very necessary for successful AI.

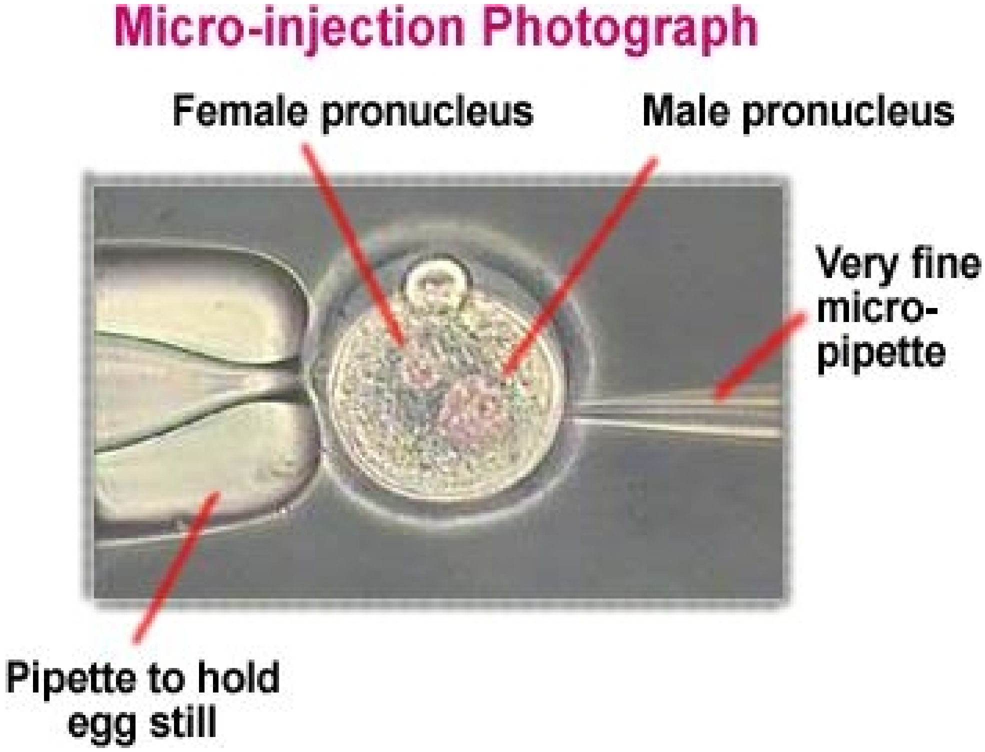
2. Embryo transfer and in vitro fertilization

Despite some early successes ET like AI has not been fully applied to the genetic management of wildlife populations. The key conservation strategy in this regard has been to pave the way for interspecies ET. In this case, embryos from an endangered species are cryopreserved and/or transferred to a more common surrogate of a different species. In wildlife ET, sexing pre-implantation embryos could be a useful conservation tool. Currently there are no references on endangered species, but techniques for sexing bovine and ovine embryos in breeding programs to manipulate the sex ratios of offspring could be modified for application in wildlife species of the same families. A recent study on sexing of in vitro produced sheep embryos describes the advancements and advantages with embryo sexing by duplex PCR (Mara *et. al*., 2004). This, demonstrated the possibility of transferring fresh sexed embryos on the same day of biopsy, a feature extremely important when the animals are in synchronized oestrus and recipient dams are available for immediate ET. This could eliminate the tedious step of embryo cryopreservation. In cases where recipients are not available, sexed embryos could be frozen after selective retention of the most likely the female embryos and discarding the unwanted males (Mara *et. al*., 2004). In vitro fertilization has been also tried in various wildlife species but with sporadic success.

Another potential application of IVF in endangered wildlife species is the use of in vitro oocyte maturation (IVM) to save the germplasm from females that die unexpectedly or accidentally (Pukazhenthi and Wildt, 2004). In a recent review by Galli *et. al*. (2003) this strategy is termed "genetic recovery". The oocytes recovered from the ovaries of a dying individual would be matured *in vitro* and subsequently utilized for *in vitro* production of embryos.

Gamete and embryo micro-manipulation

The importance of intra-cytoplasmic sperm injection (ICSI) to wildlife or rare animal preservation appears limitless, as even non-viable sperm in cattle has resulted in the birth of live calves (Goto *et. al*., 1991). The development of ICSI in humans has lead to its application in non-human primate species, a number of which are endangered. Similarly, improvements in ICSI techniques in some domesticated animals could be useful for conservation programs of endangered species belonging to the same families. It should be noted that for the fertilization of feline and murine oocytes, ICSI has been less effective than sub-zonal insemination (SUZI; Pope *et. al*., 1995; Yanagimachi, 1998), raising the possibility that ICSI, like most of the other reproductive biotechnologies, is also species-specific. Despite this, there is a preference for applying ICSI to wildlife conservation.



The different procedures used for sperm insertion (ICSI and SUZI) will have an important role to play in future conservation efforts, particularly for endangered species in which males might develop a higher proportion of abnormal sperm and no other method except ICSI or SUZI would be available for successful IVF. This is particularly important for some feline species, such as the clouded leopard and cheetah, where a high level of abnormalities have been detected in the spermatozoa of animals in captive populations (Wildt *et. al*., 1986; Roth *et. al*., 1994, 1995). Drilling holes on the zona pellucida aims to facilitate earlier hatching of embryos from the zona pellucida when it has been hardened by ovarian stimulation and/or embryo culture. Results obtained by Loskutoff *et. al*. (1999) indicates that partial zona dissection improves the hatching frequencies of bovine blastocysts produced in vitro and co-culture conditions can affect survival after thawing. Hence, it is probable that the embryos of endangered wildlife species will also benefit from micro-manipulated hatching techniques.

3. Sperm sexing

Sex pre-selection of offspring through the use of sexed spermatozoa has great potential as a captive population management strategy for endangered wildlife, particularly those species with single-sex dominated social structures. Moreover, unbalanced sex ratios, especially excessive male births, can play havoc with small population management of wildlife (Pukazhenthi and Wildt, 2004). Producing predominantly female offspring is advantageous in accelerating the re-population rate, especially in species that are notorious for slow reproduction (Maxwell *et. al*., 2004). Thus, recent advances in sexing mammalian sperm on the basis of the differences in DNA content in X-compared with Y-chromosome bearing sperm deserves consideration for wildlife conservation (Pukazhenthi and Wildt, 2004). Recently, an AI study of sexed and thawed elk sperm has been conducted that produced 11 offspring, nine of which were of the predicted sex based on the use of predominantly X- or Y- bearing sperm in the inseminates (Schenk and De Grofft, 2003).

A significant challenge in using the sperm sexing technique for controlling gender in wildlife breeding programs will be the often low sperm densities encountered and/or the tendency for males to produce pleiomorphic spermatozoa (Pukazhenthi and Wildt, 2004). Also for use of sex-sorted semen in AI, insemination close to the site of fertilization and time of ovulation is critical for successful fertilization and ongoing pregnancy (Maxwell *et. al*., 2004).

4. Genome resource banking

There have been a number of approaches proposed to slow or halt the rate of species decline. One suggestion is to undertake a program aimed at preserving genetic material or ex situ cryoconservation of germplasm, specifically spermatozoa, oocytes or embryos, and other cells/tissues or DNA from endangered species. Often termed as genetic resource banking (GRB), the aim is to create depositories of germplasm as an interface between in situ and ex situ conservation programs (Holt and Pickard, 1999). Therefore, GRB can be tool for managing the exchange of genetic diversity among endangered species by facilitating the creation of a global gene pool (Hanks, 2001).

a. Semen banks: Systematic cryopreservation and storage of semen from endangered species can facilitate maintenance of genetic heterozygosity, while minimizing movement of living animals between captive areas/zoos/research centers or countries (Johnston and Lacy, 1995). Using frozen-thawed spermatozoa would facilitate the infusion of new genetic material across populations by AI. The use of frozen sperm from semen banks increases the generation interval indefinitely and allows fewer males to be held in captivity because some of the genetic diversity is maintained strictly as frozen spermatozoa. Having sperm samples preserved from representative free-living males protects the existing diversity from unforeseen danger and eliminates the need to remove males from their natural habitats to support in situ or zoo-based breeding programs.

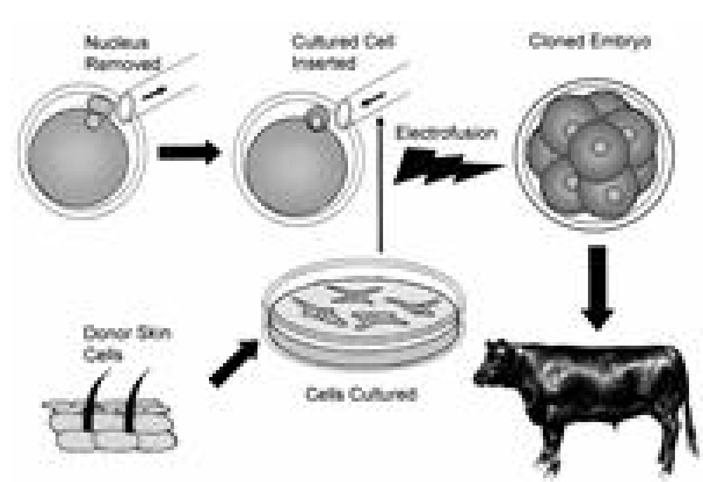
b. Embryo or oocyte banks: Embryo cryopreservation and storage allows conservation of the full genetic complement of the sire and dam and thus has enormous potential for protecting and managing species and population integrity and heterozygosity. However, the success of applying this technology to wildlife will be dictated by the uniqueness of the embryo of each species (Pukazhenthi and Wildt, 2004). Furthermore, the differences among embryos in cryosensitivity are substantial, as demonstrated by the variance between the freezable bovine compared with the difficult to freeze swine embryos (Nieman and Rath, 2001). In the case of carnivores, there has been some progress in that a baseline protocol for embryo freezing and thawing has been established for the domestic cat (Pope, 2000). Conventional freezing and thawing procedures for embryos are time-consuming and require the use of biological freezers and a microscope. Complicated embryos freezing procedures may soon be replaced by a relatively simple procedure called vitrification. However, its greatest advantage is its simplicity, because to date vitrification is only used experimentally in embryos from domestic animal breeds like cattle et cetera (reviewed by Vajta, 2000). Therefore, the biggest challenge is to establish a standardized vitrification method, which can be successfully applied for cryopreservation of embryos at different developmental stages of endangered mammalian species.

c. Tissue graft banks: Although little research has been directed towards wildlife species, the cryopreservation and subsequent use of gonadal tissue offers fascinating opportunities. This has particularly been the case since the news of a live birth following orthotopic transplantation of cryopreserved ovarian tissue in humans. Recent developments in the auto-grafting and xeno-grafting of ovaries and testes clearly demonstrate the potential value of cryopreserving gonadal tissue (Oktay and Yih, 2002; Tibary *et. al.,* 2005). The aim of ovarian and testicular tissue cryo-preservation is to store primordial follicles and spermatogonial cells, respectively (Pukazhenthi andWildt, 2004). A similar phenomenon has occurred in immunodeficient (nude) rats receiving transplants of thawed wombat ovarian tissue (Wolvekamp *et. al*., 2001). This could have a huge impact in wildlife conservation, as ovarian tissues could even be collected and preserved from young females who had died due to unknown etiology. The stage has probably not been reached where cloning technology is ready for application to maintain population viability or conserve species but in the future tissue samples (somatic cells) collected and stored from endangered species could be exploited by nuclear transfer. Research is required now to identify suitable sources of cells which could be exploited for banking and future cloning-based conservation programs. Therefore, the establishment of worldwide tissue graft banks, to store reproductive/somatic tissue and cells collected opportunistically from threatened wildlife species, could be a milestone in conservation planning. This is particularly the case in situations where population numbers are critically low, other options have failed and conservationists are faced with the need to rescue all extant genetic diversity, including from dying neonates.

5. Cloning

Somatic cell nuclear transfer (SCNT) is a process by which the nucleus (DNA) is moved from a donor cell to an enucleated recipient cell to create an exact genetic match of the donor. If this happens to be a viable embryo that proceeds to term, the resulting offspring has the same genetic complement as the original donor, except for the mitochondrial DNA, which is derived from the recipient (Wolf *et. al*., 2001; Yang *et. al*., 2004). Conservation has been highlighted recently as an area where SCNT may be useful.

Transfer of a somatic cell nucleus into the enucleated egg of a genetic stock, a closely related species or another subspecies can potentially allow the recovery of the entire nuclear genetic complement of the donor without the genetic dilution that would occur in producing biparental hybrids (Corley-Smith and Brandhorst, 1999). Moreover, SCNT may preserve and propagate endangered species that reproduce poorly in captivity until natural habitats can be restored and populations reintroduced to their ecological units (Tong *et. al*., 2002), and may even allow the resurrection of extinct species from appropriately preserved tissue.



A major practical objection to using cloning technology in wildlife conservation is the fundamental lack of information about the basic physiology of endangered species. While it is obvious that the species requiring most urgent protection and conservation are those that are considered endangered, it may be less obvious to some that these are the very same species for which the least background biological information exists (Holt *et. al*., 2004). For the present, it has been suggested that SCNT should be only considered as a useful tool for basic research for the investigation of cell biology and reprogramming (Van Heyman, 2005). In present circumstances, where rapid advances in cloning technology are being made, perhaps it is more appropriate to focus on developing realistic strategies for using these methods in wildlife conservation and ensuring that scarce resources are deployed where they will be most effective (Holt *et. al*., 2004).

Conclusion

- Precise and reliable estimates of different genetic components of variability of important economic traits of indigenous breeds of livestock and wild animals should be obtained.
- Properly designed selection experiments should be carried out for important indigenous breeds which are not involved in genetic improvement experiments through crossbreeding.
- The present evaluation of different breeds and breed crosses is being done only under intensive management system. Such evaluation should be done under intensive, medium and low input (as close to the existing practices in the farmers fields as possible) so that the most efficient genotypes for each of these management levels could be identified.
- A number of native breeds, strains or varieties are, or may be, in danger of genetic dilution through indiscriminate crossbreeding with exotic breeds. Such native breeds should be identified, so that they can be evaluated before this process leads to their essential loss.
- Some native breeds are in danger of losing genes for high production because high-performing animals are withdrawn from breeding populations for use in units of high production and/or subsequent slaughter (e.g. city milk production in India or slaughter animals closed because of large size). Such breeds should be identified and breeding units kept intact.
- Application of conventional and biotechnological tool for endangered free-living animals is rarer than for endangered domestic breeds. Progress in ART for non-domestic species will continue at a slow pace due to limited resources, but also because the management and conservation of endangered species is biologically quite complex. In practice, biotechnologies are species-specific or inefficient for many endangered animals.