MAINTAINING POPULATIONS WITH THE AID OF ASSISTED BREEDING TECHNIQUES

D.J. Blyde

Western Plains Zoo, P.O. Box 831, Dubbo, NSW 2830

SUMMARY
Assisted breeding techniques can be used in conjunction with natural breeding to maintain and enhance populations of rare and endangered populations of exotic and native animals. These techniques have generally been adapted from those used in domestic animals although some have been adapted from the field of human reproduction. Many examples exist of the successful use of assisted breeding techniques in exotic species. Assisted breeding techniques are now being evaluated for their potential use in native species.
Keywords: Semen collection, oestrous synchronisation, artificial insemination, embryo transfer, invitro fertilisation

INTRODUCTION
Assisted breeding techniques have been used in domestic species to enhance reproduction for many years. Techniques such as artificial insemination (AI), embryo transfer (ET) and in-vitro fertilisation (IVF) are now commonplace in a number of domestic animal species. These techniques have been adapted and modified to some degree for use in certain native and exotic species where they are proving to be important tools in the management of small and geographically distant populations.

Populations of non-domestic species kept in zoological institutions tend to be relatively small and geographically isolated. For example, the entire population of *Przewalski's horse (Equus przewalski)* is thought to number only one thousand world-wide. This population is spread over more than one hundred institutions in four continents (Boyd and Houpt 1994). The planning and execution of translocating megafauna between continents is extremely difficult and involved as illustrated by Kelly *et al* 1995 in the case of transporting *Black rhinoceros (Diceros bicornis)* from Zimbabwe to Australia .

The goal of most captive breeding programs is to maintain 90% of the genetic variation available in the wild population for 200 years. It has been estimated that 100-300 individuals must be maintained if greater than 80% of a breeding population's genetic diversity is to be sustained for 100-200 years (Frankel and Soule 1981). The costs of such programs, especially for megafauna are substantial and the genetic management of these programs could be facilitated by genome storage. Although assisted breeding is in its infancy in non-domestic species, there has been enough success in this field to indicate that these techniques will play a significant role in the conservation of endangered and threatened species. Habitat preservation and regeneration, natural captive breeding programs and public education will also be necessary if these endangered species are to survive.

The use of assisted breeding techniques has the following advantages in a captive breeding program:

i) decreasing the risk of transportation of live, sometimes nervous animals, likely to injure themselves.

ii) overcoming incompatibility in selected breeding pairs

iii) the use of post-mortem material from recently deceased animals, thereby maintaining the genetic diversity available

iv) the use of physically impaired animals unable to mate naturally or of terminally ill animals.

v) the ability to produce offspring quickly from selected matings using the technique of ET

vi) reducing the risk of disease transmission

SEMEN COLLECTION AND PRESERVATION

The ability to collect and store semen is vital to most assisted breeding techniques. Semen can be collected in domestic animals by electro-ejaculation, manual manipulation and the use of an artificial vagina. Similarly, semen can be collected in non-domestic animals by these techniques. By far the most common way of collecting semen from non-domestic species is by the use of electro-ejaculation. This is generally accomplished under anaesthesia, sedation, immobilisation or physical restraint. Examples of species where semen collection and preservation has been successful include *Black rhinoceros, Koalas (Phascolarctus cinereus)* and *Cheetah (Acinonyx jubatus)*. There still remain a number of species where long-term preservation of semen has not been accomplished. Although semen can be collected readily from a number of macropod species e.g. *Eastern grey kangaroos (Macropus giganteus)* and *Red kangaroos (Macropus rufus)*, long-term preservation has not been successful to date.

The use of an artificial vagina for the collection of semen has had some success in certain species. Individual animals can be conditioned to serve an artificial vagina e.g. *Cheetah, Black rhinoceros*. Certain species will serve an artificial vagina without prior conditioning. Johnston *et al* 1996a found that naive *Koalas* served an artificial vagina regularly in the breeding season.

Epididymal semen can be collected from recently dead animals, animals to be euthanased or animals that are to be castrated. Epididymal semen is easily obtained and has much potential in the preservation of exotic and native species for this reason.

Semen may be collected from animals in the wild, stored and used to inseminate captive females. This has the advantage of increasing the genetic diversity in the captive population without depleting numbers in the wild. It also overcomes the problems of transportation, quarantine and pair incompatibility. A recent example of this was the collection of semen from wild *Cheetah* in Namibia. Semen was collected under immobilisation by electro-ejaculation, frozen and transported to the United States where it was used to inseminate females in various captive institutions. Viable offspring were produced from this program.

OESTROUS SYNCHRONISATION

In much the same way as it is vital to be able to successfully collect and store semen in order to use assisted breeding techniques, it is also vitally important that the oestrous period of the female can be predicted or synchronised to allow the maximum beneficial use of collected semen. Many non-domestic animals have domestic animal counterparts and the oestrous cycle can be identified in the same way as it is in the domestic analogue. Oestrous synchronisation can be performed using similar techniques and drug combinations as those used for domestic animals. A protocol for the induction of oestrous in the *Cheetah* was developed by Wildt *et al* 1981 and is similar to that used for the domestic cat. Other species, however, have no domestic animal counterparts and much research needs to be undertaken in order to characterise the reproductive biology of the species in question.

Many exotic and native species exhibit overt signs of oestrous e.g. *Koalas*, most felid species and the majority of canid species. Other species have much less overt signs of oestrous and it may be difficult to detect the heat period. Techniques are now available for monitoring reproductive hormone levels in blood (Taya *et al* 1991) , urine (Ramsay *et al* 1994), faeces (Czekela *et al* 1994) and even saliva (Czekela and Callison 1996) in certain species. Smears of the vagina or urogenital sinus have been used to monitor the oestrous cycles in animals that can be handled easily, for example the *Greater bilby (Macrotis lagotis)* as described by McCracken 1990.

As with domestic species, ultrasonography is increasingly being utilised to define oestrous cycles and determine the exact time of ovulation. Adams *et al* 1991 performed transrectal ultrasound in *Black rhinoceros, Asian elephants (Elaphus maximus), African elephants (Loxodonta africana), Banteng (Bos javanicus), Gaur (Bos taurus), Giraffe and Bactrian camels (Camelus bactrianus).* This study concluded that transrectal ultrasound provides a method for reproductive management and assisted fertilisation programs of large nondomestic species. For this technique to be effective, animals must be conditioned to accept the procedure without causing injury to themselves or the operator. This means that restraint facilities must be constructed for this purpose and that the animals are properly conditioned. Although, the process of conditioning animals for restraint purposes is tedious, the advantages of having tractable, cooperative animals are extremely beneficial. Thorne and Whalen 1996 describe the conditioning of Black rhinoceros for reproductive manipulation.

ARTIFICIAL INSEMINATION

In order to perform artificial insemination it must first be possible to obtain semen from the male and to either detect oestrous in the female or to synchronise oestrous in that species. Most artificial insemination is performed by laparoscope. This technique has the advantages of introducing the semen directly into the uterine horns as well as being able to view the ovary and determine the exact stage of the oestrous cycle. Vaginal delivery of semen has been attempted in various species but the results obtained from this method are generally inferior to those obtained via laparoscopy.

AI can be extremely useful in those species that are generally solitary in the wild. The males of these species can be quite aggressive to the females outside of the oestrous period. In species such as the *Clouded leopard (Neofelis nebulosa)* and the *Fishing cat (Felis viverrinus)* it is not uncommon for males to kill females, even after they have been cohabitating for some considerable length of time. AI can avoid these problems by allowing the different sexes to be housed separately whilst still allowing females to conceive and increase the numbers of animals in the captive situation.

EMBRYO TRANSFER

As with domestic animals, ET allows the rapid production of genetically similar animals. This can be particularly important in endangered species, particularly if the embryos are a product of under represented animals within that population. Cross species ET has the advantage of using more common species as surrogate mothers for highly endangered species. This technique has been accomplished using Welsh-type pony mares as surrogate mothers for *Przewalski's horse* embryos (Summers *et al* 1987). Although this technique has been successful in the past, it is not highly repeatable. Other species with which success has been achieved include, *Bongo (Tragelaphus eurycerus)* embryos implanted in *Eland (Tragelaphus oryx)* and *Sand cat (Felis margarita)* embryos implanted in *domestic cats (Felis domestica)*.

INVITRO FERTILISATION

Although IVF has not been used to any great degree in exotic and native animals, it has enormous benefits. It may allow the use of post-mortem material to contribute to the survival of the species. Sperm may be able to be harvested up to forty-eight hours post-mortem and still be useful for IVF programs. A technique known as intracytoplasmic sperm microinjection (Trounson 1994) can be used to fertilise mature eggs by direct microinjection of sperm recovered from the testes and epididymis of recently deceased males of the same species. Oocytes may be harvested from post-mortem material and matured in-vitro prior to fertilisation (Trounson *et al* 1994).As stochastic events are not uncommon in exotic animals, the ability to use post-mortem material is especially important, particularly when numbers of individuals are low. IVF has been successfully performed in *Cheetah* (Donoghue *et al* 1992), *Gorillas (Gorilla gorilla)* and *Tigers (Panthera tigris)* (Donoghue *et al* 1990), amongst others and could prove to be a significant technique for management of captive species.

DISCUSSION

Assisted breeding techniques have been useful in the maintenance of many genetically diverse captive populations of exotic animals. However, their use has been somewhat limited in populations of Australian native animals. This is due to the fact that the collection of semen from and the manipulation of the oestrous cycle of many native species has been unsuccessful. Much research is needed in these areas for assisted breeding techniques to become a useful tool. Although assisted reproduction has been shown to be useful in the slowing of the extinction process it is not the entire answer to the loss of biodiversity. Other areas need to be promoted including habitat preservation, captive animal techniques and public education if conservation efforts are to be maximised.

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