16

Role of reproductive sciences in carnivore conservation

DAVID E. WILDT, JOGAYLE HOWARD AND JANINE BROWN

INTRODUCTION TO PROBLEMS AND ISSUES

What is the role of reproductive sciences in conservation?

Reproductive biology as a discipline is misunderstood in the conservation world. It generally is perceived as a set of high-tech solutions for producing offspring, usually in *ex situ* (zoo) conditions. The field is largely regarded as a highly applied science. The reality is that it is the goal of the reproductive biologist to understand the diverse and wondrous ways that living organisms (including carnivores) reproduce. Reproductive biology is pri-marily a basic science geared to generating new knowledge about reproductive mechanisms. If done systematically and appropriately, then resulting data can have applied benefits.

The origins of this misunderstanding probably hail to the late 1970s and two significant events. The first was the birth of Louise Brown, the first human infant to be conceived in a Petri dish (by *in vitro* fertilization). The second was the emergence and routine use of embryo transfer in the cattle industry, making it possible to produce 'litters' of calves from genetically superior cows that normally produce only a single calf per year. The idea that the technology could easily be extrapolated and extended to endangered species was proposed by a few intrepid zoo directors who contemplated assembly-line propagation of endangered species. A small cadre of reproductive physiologists, hired by a few zoos, began applying cattle technologies to zoo-held species.

The first significant birth, a gaur calf born to a Holstein cow after interspecies embryo transfer, occurred at the Bronx Zoo in the early 1980s (Stover & Evans, 1984). This was followed by a few other significant, usually one-time births at other zoos, primarily in Artiodactyla. There were just enough of these 'gee-whiz' events to capture the public's imagination and to stigmatize the discipline as one with the sole purpose of enhancing offspring production.

It was normal that animal managers and the public might become excited about producing wildlife using hi-tech reproduction. The potential benefits were significant (Holt *et al.*, 1996; Wildt *et al.*, 1997). Among other advantages, gamete and embryo technologies (artificial insemination, embryo transfer, *in vitro* fertilization) could offer a means of moving genetic material while eliminating stresses associated with transporting animals long distances. Assisted breeding would overcome problems associated with mate incompatibility, mate preference and pair bonding. In combination with the ability to cryopreserve sperm and embryos, these techniques would help avoid genetic drift, facilitate germplasm transport and provide frozen 'insurance' in the event of catastrophes capable of wiping out small populations.

Why assisted breeding is not routine in wildlife

If there are so many advantages to assisted breeding in wildlife management, why does the potential of these techniques continue to be discussed rather than seeing products from their use? That is, why are these techniques not being used routinely to help manage and conserve endangered species, including carnivores? The simple answer is that species are different physiologically as well as phenotypically. Assisted breeding techniques that are effective in humans and cows are not necessarily applicable to bears and hyaenas. On the contrary, reproductive mechanisms among animal groups are highly varied (Wildt *et al.*, 1992). Without understanding the fundamentals of reproductive processes, assisted breeding can never become consistently successful.

The following example may be useful for illustrating the dilemma. In the late 1970s, our laboratory became interested in the high proportion of zoo-maintained cheetahs that failed to naturally mate. We speculated that this management problem could simply be solved by artificial insemination. Sperm were collected, processed and cryopreserved from cheetahs in Africa using cattle technology. In the United States, sperm were deposited into hormonally treated cheetahs, again using cow technology. This highly applied study failed because, although most cheetahs ovulated, none became pregnant (Wildt *et al.*, 1986). Nonetheless, failure forced us to realize that a cheetah was not a cow! In retrospect, it was logical that cow technology would likely be inefficient in the highly specialized cheetah that had evolved for reasons considerably different than its bovine counterpart. It was clear that we needed more basic research on the fundamental biology of the cheetah. This realization resulted in more than 15 years of research on many physiological aspects of cheetah biology, including multidisciplinary collaborations with field biologists, behaviourists, geneticists, nutritionists and veterinarians. More than a dozen papers resulted on the reproductive biology of the cheetah (see reviews Wildt *et al.*, 1993; Brown *et al.*, 1996). A great deal was learned not only about cheetah reproduction, but also about the integrative biology of the species, findings that are contributing to enhanced management of cheetahs in zoos. One by-product was more effective production of cheetah cubs by assisted breeding (Wildt *et al.*, 1997). Furthermore, the technology has been transferred to the field in a partnership with the Cheetah Conservation Fund, a non-governmental organization in Namibia. Guidance and assistance are being provided to create a genome resource bank (a frozen repository of sperm, blood products, tissue and DNA) to help protect the largest disease-free population of cheetahs in Africa. Sperm are being collected and cryopreserved from wild-caught cheetahs, and its biological competence has been proven by the birth of cubs in the United States using sperm transported intercontinentally (Wildt *et al.*, 1997). This is one example of what the reproductive sciences are all about – curiosity driven research and increasing intellectual capital, with the results beginning to pay dividends in management and conservation.

REVIEW OF OTHER CASE STUDIES

The value of fundamental reproductive studies in carnivores

A primary advantage to studying carnivores is the opportunity to discover new mechanisms in previously unstudied species. Almost all reproductive biologists today study reproductive systems of humans, livestock and laboratory animals. Therefore, conventional wisdom on how reproduction occurs mechanistically is precariously derived from only a few species. This provincial approach ignores thousands of wildlife (and hundreds of carnivore) species that hold a wealth of information about novel reproductive phenomena. This is a resource not to be taken casually because of its fundamental importance, as well as its potential economic, social and even human health value.

One illustrative example in carnivores is the phenomenon of teratospermia, a condition in which high proportions of pleiomorphic (or malformed) sperm are consistently ejaculated. Certain species, subspecies or populations within the Felidae family have a predilection for teratospermia. Cheetahs (in zoos and in the wild) routinely ejaculate *c.* 75% structurallyabnormal sperm (Wildt *et al.*, 1983; Wildt, 1994). Studies also have revealed that lions isolated in the Serengeti's Ngorongoro Crater as well as the remnant population of Asian lions in the Gir Forest Sanctuary of western India also are teratospermic (Wildt *et al.*, 1987). The most provocative example has involved the Florida panther, an isolated subspecies in southern Florida that consistently ejaculates more than 90% pleiomorphic spermatozoa (Barone *et al.*, 1994).

But what is the significance of the high number of malformed sperm observed in certain felids? Reproductive techniques, especially *in vitro* fertilization, have been used to examine teratospermia in the context of sperm fitness and function. Early studies determined that there was a direct relationship between the proportion of sperm in the ejaculate that were normally-shaped and *in vitro* fertilization success (see review Wildt, 1994). Thus, fertilization and embryo cleavage occurred readily in species such as the domestic cat and tiger (normospermic species), compared to teratospermic counterparts such as the cheetah, Florida panther and clouded leopard. Similarly, following *in vitro* culture was the discovery that the deformed sperm never advanced into the egg proper, either failing to bind or penetrate the glycoprotein coat of the egg (the zona pellucida) (Howard *et al.*, 1993). Of more basic interest was the function of the normal-shaped sperm from teratospermic ejaculates. Are these cells really normal? Again detailed technical studies revealed that structurally-normal sperm from teratospermic males were also compromised at the subcellular level, including in capacitation (Long *et al.*, 1996) and tyrosine kinase signalling (Pukazhenthi *et al.*, 1996) (biochemical processes that assist in achieving fertilization). More recent data reveal that normal-appearing sperm from teratospermic donors also have unstable chromatin (Anzalone *et al.*, 1998).

In addition to addressing a fascinating phenomenon (sperm morphology and function), these studies can have applied relevance. We suspect that the cause of teratospermia is related to a lack of genetic diversity within populations (in the case of the lion and Florida panther; Roelke *et al.*, 1993a) or entire species (in the case of the cheetah; O'Brien *et al.*, 1985), although a clear cause and effect relationship has yet to be established. Nonetheless, it is firmly documented that these sperm do not participate efficiently in the fertilization process. Furthermore, it is apparent that these studies of wild carnivores have provided a new perspective on teratospermia, a condition that commonly occurs in humans (Kholkute *et al.*, 1992). Therefore, these findings have human health implications and is one reason why this work in our laboratory has been supported by the National Institutes of Health.

Non-invasive hormone monitoring for tracking reproductive status

One of the most powerful, contemporary technologies for studying reproduction in carnivores is the measurement of gonadal hormonal metabolites in excreted faeces or urine. This technique has no direct role in propagating offspring, yet provides information on important reproductive characteristics such as onset of puberty, seasonality, oestrous cycle duration, time and type (induced versus spontaneous) of ovulation, pregnancy and even parturition prediction. Hormones drive reproductive success and give an indication of the reproductive status of an individual, a population or even a species. Until recently, the sole means of assessing endocrinological status in wildlife was through the measurement of hormones in blood samples collected invasively, usually at anaesthesia. In addition to disrupting an animal's normal routine, there was always the potential for the anaesthetic drugs to perturb normal endocrine patterns that, in turn, could influence subsequent processes, such as ovulation. However, assessing hormonal metabolite content in faeces or urine avoids these pitfalls and permits long-term sampling with no animal disturbance. Validation studies have proven that metabolite profiles generated from serial collections of carnivore faeces reflect physiological hormone fluctuations in the peripheral circulation (see review, Brown & Wildt, 1997).

Whether the predominant hormones are excreted in faeces or urine is species-specific and is determined during the validation process. Once this information is known, it is possible to design studies that assess hormonal-reproductive status acutely or long-term. Particularly valuable studies have been possible under controlled situations in zoos where frequent excreta collection is a simple by-product of routine enclosure cleaning. Projects have demonstrated that certain species (e.g., ocelot) produce consistent reproductive cycles throughout the year (Figure 16.1), whereas others (e.g., Pallas' cat) exhibit marked seasonality (Figure 16.2). The gonads of both male and female Pallas' cats generally are quiescent from late spring to early fall. Beginning in October, the male begins to excrete androgens in the faeces, no doubt reflecting the need for testosterone to stimulate spermatogenesis for the impending, but brief, breeding season. In late December, the female begins to excrete gradually increasing, baseline concentrations of oestrogen metabolites followed by cyclic and peak oestrogen patterns that coincide with maximal androgen excretion by the male (Figure 16.2). Particularly interesting is the brevity of peak gonadal activity in both sexes, with an abrupt and sharp decline to nadir hormone production by late February or early March. To-date, no other such felid species shows this remarkably brusque seasonality.

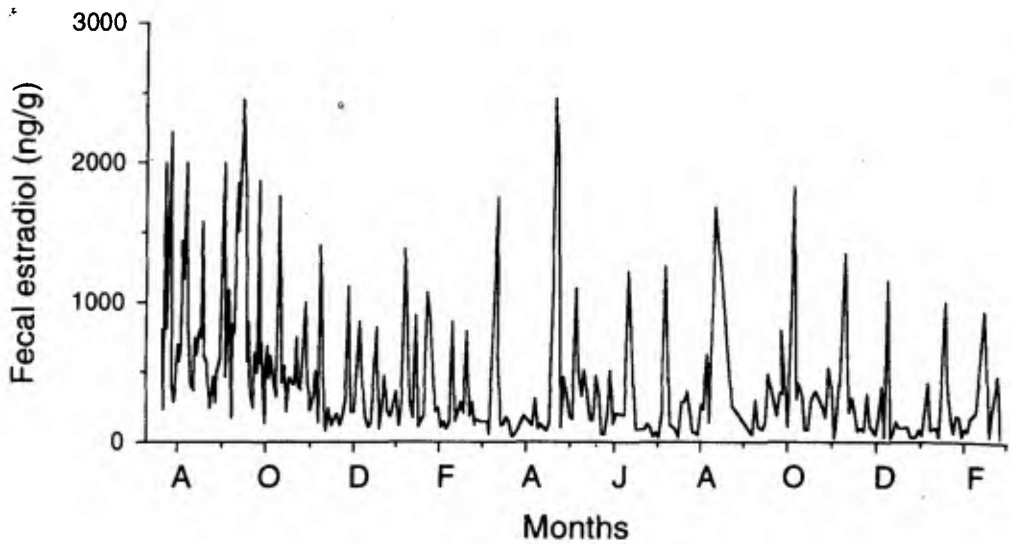

Figure 16.1. Faecal oestradiol profile in an adult female ocelot over time illustrating continuous patterns of ovarian activity.

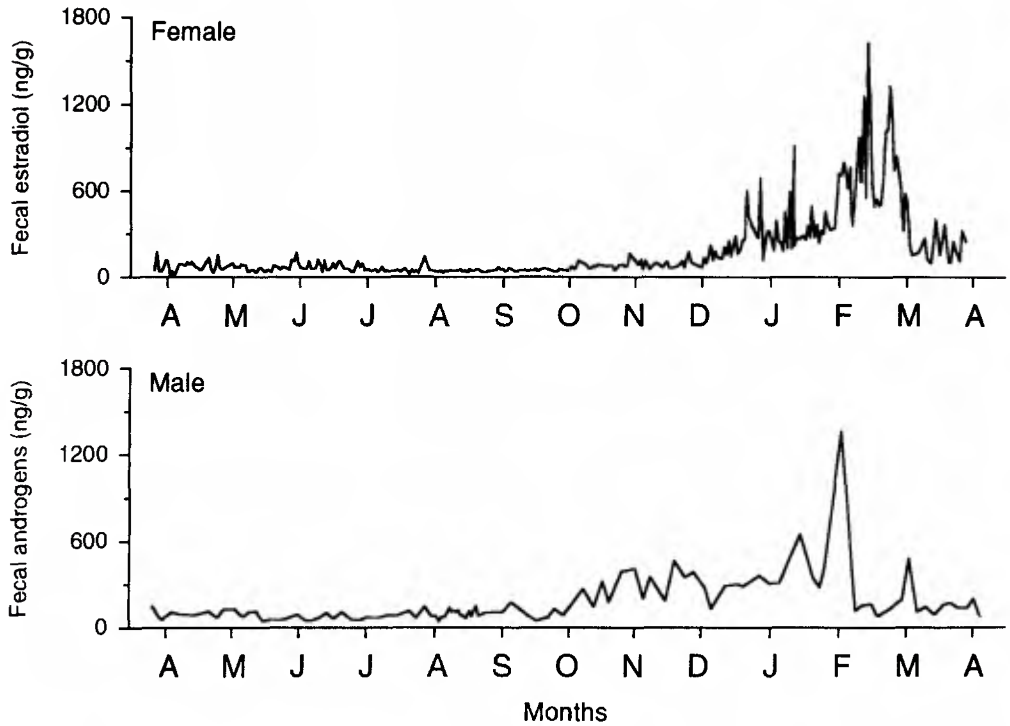

Figure 16.2. Profiles of faecal oestradiol excretion in an adult female (top panel) and faecal androgen excretion in an adult male (lower panel) Pallas' cat, illustrating marked gonadal seasonality.

Such fundamental data can have applied value, including discovering that excessive artificial light in one zoo (as a result of an early winter evening activity, 'Festival of Lights') disrupted the onset of normal seasonality in the Pallas' cat, thereby preventing breeding and kitten production (Swanson & Brown, unpub. data). Isolating this pair from the extended artificial lighting in the subsequent year resulted in normal hormone patterns in both the female and male, breeding and the production of kittens. Another example involves the impact of pairing behaviourally incompatible female cheetahs, a practice that can disrupt normal ovarian cyclicity (Wielebnowski, Wildt & Brown, unpub. data). Separating zoo-held cheetahs and maintaining them singly (as cheetahs normally live in nature) has been shown to provoke the reinitiating of normal reproductive cycles. Other management applications include: identifying the causes of reproductive inactivity (senescence, cystic ovarian follicles, retained corpora lutea); improving hormone treatments useful to increasing the efficiency of ovulation induction for artificial insemination; and assessing the level of physiological stress for the purpose of evaluating and improving habitats to enhance reproductive success and animal well-being. The latter possibility holds great promise because the technology has advanced to allow accurate measures of adrenal corticoid hormones, often used as indices of 'stress' (Graham & Brown, 1996).

There also is a growing database suggesting that non-invasive hormone metabolite monitoring has applications in field studies of carnivores. Creel *et al.* (1992) first described the potential of conditioning free-living dwarf mongooses in the Serengeti National Park to scent mark rubber pads, thus providing a source of urine for hormonal assessments. Especially important was the finding that alpha females in mongoose packs excreted more oestrogen that was a likely means of suppressing reproduction in subordinate counterparts. In contrast, the alpha male had indistinguishable androgen profiles from subordinates. A similar approach was extrapolated to African wild dogs of the Selous Game Reserve, but in this case using faeces (Creel *et al.*, 1997a). It was possible to positively correlate wild dog reproductive behaviours and dominance hierarchies to oestrogen excretion (in the case of females) but not androgen excretion (in the case of males). Also of significance was that adrenal status could be assessed in wild dogs by measuring faecal corticosterone concentrations (Creel *et al.*, 1997a). It was discovered that there was an 'hyperadrenal cost' to dominance status within the pack (alpha males and females excreted more corticosterone). This was intriguing because it was widely believed that reproductive suppression in subordinates was caused by social stress, a hypothesis that now could be rejected on the basis of non-invasive assessment of adrenal status. In sum, hormonal metabolite monitoring combined with conventional behavioural ecology is providing new, more holistic information of relevance to conservation.

Using reproductive technology to assist in the recovery of an endangered carnivore, the black-footed ferret

When sufficient fundamental knowledge was secured (after years of basic research), reproductive technologies became routine in propagating livestock and humans. In theory, the same scenario should be possible for carnivores given a similar strategic approach. Although there is a growing number of examples using artificial insemination and even *in vitro* fertilization and embryo transfer to produce carnivore offspring (see reviews, Wildt *et al.*, 1998; Howard, 1999), there is one species in which reproductive technologies are routinely contributing to recovery and reintroduction – the black-footed ferret, a small, charismatic mustelid whose original natural range was the Great Plains (USA).

The black-footed ferret's survival was linked to the common prairie dog, its primary prey. As the Great Plains were settled and converted to farmlands, the government actively supported massive poisonings of prairie dogs. The decline of black-footed ferrets followed and, by the 1960s and 1970s, the species was largely eliminated from its native range. No captive breeding programmes existed in zoos, and by the late 1970s the species was believed to be extinct. A small population was discovered in 1981, but eventually suffered a disease epidemic that caused the death of all but 18 individuals. These last remaining ferrets were brought into captivity and were the subject of an enormous collaborative effort by the State of Wyoming, the U.S. Fish & Wildlife Service, zoos throughout North America, the Conservation Breeding Specialist Group and the American Zoo and Aquarium Association. With the production of ferret kits in Wyoming, animals were eventually distributed to six other facilities to assist in the breeding programme.

There have been two areas of focus at the Conservation and Research Center in northern Virginia. Originally, kits were produced solely by natural breeding. Since 1986, our laboratory also has been involved in learning the basics about ferret reproductive biology while exploring the potential use of assisted breeding to accelerate offspring production. Original studies centered on the common European ferret and Siberian polecat as 'research models', developing an understanding about sperm physiology and optimal methods for processing, culturing and cryopreserving semen. These species also were valuable for developing a laparoscopic intrauterine artificial insemination technique, because an early study demonstrated that vaginal insemination was ineffective (Wildt *et al.*, 1989). Studies over time demonstrated that European ferrets and Siberian polecats could be produced routinely by artificial insemination (Table 16.1; Howard, 1998). The

Table 16.1. Ferrets produced by laparoscopic intrauterine artificial insemination using fresh or frozen-thawed spermatozoa

Species	Sperm treatment	No. of pregnancies (%)	No. of kits	Mean (± SEM) no. kits/litter
European ferret	fresh	17/24 (70.8)	85	5.2 ± 0.5
	thawed	7/10 (70.0)	31	4.4 ± 1.0
Siberian polecat	fresh	1/1 (100.0)	6	6.0
	thawed	5/6 (83.3)	26	5.2 ± 1.2
Black-footed ferret	fresh	11/14 (78.6)	32	2.9 ± 0.4
	thawed	2/3 (66.7)	1	1.5 ± 0.5

Adapted from Howard, 1998.

significance of having assisted breeding technology available for black-footed ferrets eventually became highly apparent.

A 1995 survey of breeding records of the black-footed ferret species survival plan revealed that more than 50% of males failed to breed naturally, a serious problem in capturing all genetic diversity from the small number of original founders. Since then our research has focused on potential causes for this lack of reproductive fitness, which has revealed at least two problems. The first is a relatively high rate of sexual incompatibility that was expressed as excessive male aggression, apathy or poor copulatory positioning (Wolf *et al.*, 1998b). Males with this problem that are from important, underrepresented genetic lineages have been relegated to being sperm donors for artificial insemination, and kits are now being produced routinely using fresh or frozen-thawed sperm (Table 16.1; Figure 16.3; Howard *et al.*, 1997).

The second complication originated with the assumption that yearling males approaching their first breeding season were 'prime breeders'. Collaborators at the National Black-Footed Ferret Conservation Center (Wyoming) had already confirmed that yearling black-footed ferrets had comparable testes sizes and libido to two- and three-year-old counterparts. However, systematic semen collection and evaluation revealed that the yearlings produced sperm for a shorter time period during the breeding season than their older counterparts (Wolf *et al.*, 1998b; Howard *et al.*, 1999). Thus, yearlings were undergoing abbreviated spermatogenesis in the face of normal sexual drive, so mating was occurring often in the absence of sperm release, which resulted in a high rate of pseudopregnancy (sterile matings). When males were selected for breeding in 1998 on the basis of sperm production (rather than testes volume), whelping success increased 20%, and an additional 59 kits were produced by natural matings

Figure 16.3. Black-footed ferret kits produced by artificial insemination from genetically valuable parents.

(Howard *et al.*, 1999). Thus, a modest use of reproductive technologies boosted the efficiency of the black-footed ferret breeding programme. The result has been more animals available for the reintroduction programme in progress in five western USA states. This is the first example of consistent accelerated propagation of an endangered species, which for reintroduction programmes includes the use of assisted breeding technology.

CONCLUSIONS AND RECOMMENDATIONS FOR CONSERVATION

Reproductive biology is an important component of wildlife research. If 'to conserve' means 'to preserve and protect', then reproduction (the essence of continued species existence) is a high priority study area. However, reproductive biology now almost always has a high-tech nuance. There is a perception that the discipline largely involves assisted breeding techniques, such as artificial insemination, *in vitro* fertilization and/or embryo transfer. But reproductive biology does not have to be defined by, or closely linked to, technology. Furthermore, reproductive techniques often are most valuable for producing new knowledge, and not necessarily for generating offspring. This has been the case for several carnivores, especially for a diverse array of felids, a few canids and a mustelid, the black-footed ferret. Fundamental studies have revealed a wealth of information on topics ranging from gonadal–hormonal–behavioural relationships, to the early events of fertilization/embryogenesis, to factors influencing reproductive fitness. Especially interesting have been studies of novel phenomena (e.g., teratospermia) and the recognition of species-specific differences in reproductive mechanisms, even among closely related taxa. Powerful tools also have been developed: (1) to measure hormonal patterns from steroidal metabolites excreted in faeces or urine; and (2) to systematically collect and cryostore sperm, both of which are applicable to certain wild carnivore populations. Once this fundamental reproductive information is known, then wildlife can be propagated using improved natural or assisted breeding. The black-footed ferret, discussed earlier, is a prime example of how reproductive technologies are being used to enhance management and increase offspring numbers for reintroduction into nature.

FUTURE RESEARCH AND NEEDS

It is apparent that the reproductive sciences are more than high-tech assisted breeding procedures to be used for a 'quick fix'. Rather, this is a broad-based discipline that can range from recording reproductive behaviours to the use of the most sophisticated laboratory techniques for understanding the subcellular mechanisms that allow the creation of embryonic life. There is no doubt that the reproductive sciences can contribute to carnivore conservation, but the first priority always must be the production of new fundamental knowledge that eventually may have management application.

Wildt & Wemmer (1999) have summarized other high priority areas that will allow reproductive research to contribute more to wildlife management:

Studbooks as sources of reproductive data

Many zoo populations are tracked by means of a studbook, a catalogue of living (and deceased) animals. Such documents (in computerized and hard copy form) hold a wealth of information on specific life history variables. These data not only have a fundamental value (e.g., on pubertal onset, gestation/generation interval, seasonality and reproductive senescence), but also have relevance in simulation modelling for *in situ* situations, especially in the context of assessing stochastic events on population dynamics and extinction risks (Lacy, 1993b).

Partnerships among disciplines

Tools in reproductive technology become even more powerful when merged with other conservation biology disciplines. This chapter already has offered some illustrative examples in the context of animal behaviour and the simultaneous assessment of gonadal or adrenal hormones (see discussion of free-living dwarf mongooses and behaviourally incompatible cheetahs). Non-invasive hormone monitoring has enormous potential for other interdisciplinary studies of carnivores (e.g., stress versus disease, mortality and environmental conditions). Additionally, gamete technologies have had an application in understanding reproductive fitness, including in wild populations associated with suspected losses in genetic variation (e.g., Florida panther; Roelke *et al.*, 1993a). Certainly, wildlife managers should be confident about collaborating with reproductive physiologists to assess animal fitness (in nature or in zoos) while looking for links to other variables (behaviour, genetics, disease and nutrition, and environmental factors, including stress). Some fascinating connections can be expected. Also, when aggressive genetic intervention is necessary, managers should consider exploiting techniques such as artificial insemination or genome resource banking to accelerate population growth, infuse genetic vigor or simply to bank germplasm as 'insurance' for the future.

Consistently controlling reproductive capacity

As emphasized throughout this chapter, reproductive technology has an application in carnivore conservation, but there is a continuing problem with *consistency*. Newspapers occasionally report an anecdotal story of a tiger produced by *in vitro* fertilization or a bear by embryo transfer. But there are too few studies in which the technology has become so reliable that wildlife managers actually want to incorporate more reproductive technologies into recovery programmes. That is why the consistent success of artificial insemination in the black-footed ferret is so important as a model to demonstrate what is possible. More such examples are needed among carnivores, and are likely to be achieved only after fundamental reproductive mechanisms are established. Likewise, the ability to effectively *reduce reproductive capacity* is as serious as the more common interest of enhancing fecundity. Contraception in carnivores is a high priority for research. An ability to routinely and safely prevent reproduction is key to the successful genetic management of zoo populations, so that precious space is not wasted on generic animals.

Promoting the reproductive sciences

Finally, there is a need for educating the public, scientific colleagues in the conservation community and our fellow reproductive biologists in academia about the true role of this discipline in conservation biology. Hopefully this chapter will be a first step in this direction. Ironically, the greatest challenge is not necessarily with scientists in other conservation disciplines, but with conventional reproductive biologists in academia. What is interesting is the evolution of the reproductive sciences in universities, where it is much more common to find researchers exploring the molecular biology of a cell rather than enjoying the satisfaction of working with whole, living animals. The graduate student who has either experienced the thrill of handling a wild carnivore, or ever seen one in nature is a rarity. This problem possibly is prevalent in the other life sciences, but this can be rectified by those of us who are motivated to entice (and provoke) young scientists to become involved in integrative conservation biology.