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Which reproductive technologies are most relevant to studying, managing and conserving wildlife?

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Abstract. The advent of *in vitro* fertilisation (IVF) and embryo transfer in the 1970s led to speculation about the potential value of these and other 'reproductive technologies' to conserving endangered species. So far, and for the most part, assisted breeding techniques that are routine in domesticated species are not easily adapted to wildlife. Species differences in reproductive form (anatomy/morphology) and function (mechanisms regulating reproductive success) limit the practical applicability for offspring production. Thus, the limiting factor is the lack of basic knowledge about thousands of unstudied species, the foundation that is essential to allowing reproduction to be enhanced and/or controlled. There now is excellent evidence that reproductive technologies are most useful as tools for studying how different species reproduce, especially defining novel and unique mechanisms. The present paper reviews the status and relevance of various reproductive technologies that are useful or have potential for wildlife. Modern examples of progress are provided indicating how these tools are being used to understand ways that wildlife species reproduce and, in some cases, how such knowledge has been used for successful assisted breeding, improved management and conservation.

Introduction

Impressive progress has been made since the 1950s in the reproductive sciences, largely due to innovative and advanced technologies. Incentive for these developments was largely commercially based and driven by the need to improve livestock production and human reproductive health. Artificial insemination allowed the broadscale distribution of genes (sperm) from outstanding, genetically superior sires, especially in the cattle industry. Embryo transfer (ET) permitted large numbers of young to be produced from dams normally capable of producing only a few offspring during a normal lifetime. *In vitro* fertilisation (IVF), combined with ET, allowed thousands of human couples to combat infertility successfully. In addition, the ability to cryopreserve sperm and embryos permitted both livestock managers and human parents to regulate the timing of offspring production, sometimes spread over generations.

Compared with assisting reproduction in livestock and humans, the management and conservation of wildlife species generally has more complex ideals and logistics. For endangered species maintained in zoos, the aim is not only to produce more young, but offspring of known provenance and appropriate genotype that will preserve the integrity of a species, a subspecies or a population (Hutchins and Wiese 1991). Sophisticated computer programmes packed with pedigree data are used to create detailed genetic management plans that, in turn, dictate those individuals to be mated. Often the first step involves moving animals from one zoo to another for breeding, because most zoos (due to space constraints) hold only a few individuals of many species. Translocating wild animals between zoos presents challenges ranging from the expense of transport to causing stress to the animal (and curator). Perhaps most frustrating is that, even after relocation, placing two conspecifics of the opposite sex together does not ensure reproductive compatibility; animals can refuse to copulate due to sexual partner preferences. There also is the challenge of keeping the population genetically healthy long into the future. Modern zoos rarely remove animals from nature, but there remains a need to occasionally infuse new genetic vigour into captive populations. Issues about how to achieve this goal while contributing to the preservation of the same species in nature are major concerns to zoos worldwide.

All the challenges outlined above have the potential of being resolved through reproductive science and technology (for earlier reviews, see Lasley *et al.* 1994; Wildt and Wemmer 1999; Hodges 2001; Wildt *et al.* 2001, 2003). The most recent compendium on the role of reproductive

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sciences for wildlife conservation, including for mammals, birds, amphibians, reptiles and fish, can be found in the text by Holt *et al.* (2003*a*). Collectively, the literature is clear: the tactical strategies to combat reproductive challenges through technology are complex. Furthermore, some techniques that involve intriguing novelties (e.g. nuclear transfer or cloning), although perhaps being mechanically viable in the future, may not meet real needs of conservationists (for a review, see Critser *et al.* 2003). In addition, too often we associate reproductive technologies with assisted breeding. However, low-technology methods, such as behavioural observation of reproductive activity, also are important for understanding what regulates reproductive success. Another method, that of non-invasive hormonal monitoring (for reviews, see Monfort 2003; Pickard 2003), only contributes indirectly to assisted breeding and offspring production, but is absolutely essential to increasing the breadth of our scholarly knowledge, information that so often has management relevance.

Using species examples (often from our laboratory's experiences), the present paper reviews the status and relevance of various contemporary reproductive technologies as applied to wildlife. There is a disproportionate emphasis on non-invasive endocrine monitoring and processes associated with artificial insemination because these tools are currently most significant for increasing knowledge, managing species *ex situ* and even restoring species to nature. Priorities for the future also are noted.

Non-invasive monitoring of reproductive hormones

Understanding the intricacies of how animals reproduce is fundamental to managing or conserving wildlife. Historically, such information for domestic animals relied on systematically observing social and reproductive behaviours. With the ability to generate specific antisera targeted towards steroid and peptide hormones came the radioimmunoassay. The ability to collect serial blood samples from tractable animals allowed plotting hormonal patterns over time. Substantial information resulted on the hypothalamo–pituitary–gonadal axis in both females and males, knowledge that helped delineate: (*1*) gonadal function on the basis of sex, age and seasonality; (*2*) the timing of spermatogenesis and ovulation; (*3*) the type of ovulation (spontaneous *v.* induced); (*4*) ways to overcome infertility; and (*5*) protocols for consistently successful assisted breeding through aritificial insemination (AI) and ET. These milestones in domestic animals were possible because hormones could be measured in easily collected blood samples, thereby providing the clues as to how reproduction actually worked.

In contrast, it is impossible (and dangerous) to attempt sequential blood sampling in most wildlife species. Beginning in the late 1970s, improvements in wildlife anaesthesia allowed sporadic blood sampling that produced enough data to suggest that hormonal patterns in wildlife were remarkably different from domesticated species. However, the problem remained that too few blood samples were recoverable from most wild animals to plot longitudinal hormonal profiles. In addition, there was evidence that the stress of anaesthesia could alter the normal pattern of hormonal secretion in the blood donor. An alternative tool was needed.

For more than 25 years, independent laboratories have pioneered techniques for measuring hormonal patterns in voided urine and feces (Hodges *et al.* 1979; Bamberg *et al.* 1984; Monfort *et al.* 1990; Lasley and Kirkpatrick 1991; Hodges 1996, 2001; Garniera *et al.* 1998; Schwarzenberger *et al.* 1998) or even saliva (Czekala and Callison 1996). Excreted urinary or fecal hormone metabolites accurately reflect hormonal patterns in blood with, of course, the appropriate delay in passage from the blood into excreta. Whether the hormone is primarily passed into urine or feces is species dependent. For example, most steroids in felids (Brown *et al.* 2001*a*) are voided into feces, whereas the same hormone products are excreted through the kidneys into the urine of cervids (Monfort *et al.* 1990). The feces of elephants contain the majority of the progestagens, but only approximately 10% of the oestrogens (Brown 2000). Therefore, a crucial first step to applying this technology is determining the ratio of steroidal metabolites between urine and feces.

The obvious advantage of assessing urinary or fecal hormone concentrations is that gonadal function can be determined without even touching the animal: the waste is simply recovered from the ground or enclosure floor and analysed in the laboratory. Resulting hormonal profiles are generally less 'noisy' than those observed after analysing blood because the excretory patterns represent a pool of metabolites rather than reflecting the often hour-to-hour fluctuating dynamism quantified in blood. The urinary/fecal approach often is limited in analysing pituitary hormones because these proteins are degraded rapidly during metabolism. However, there are exceptions where it has been possible to detect the ovulatory luteinizing hormone (LH) surge in giant panda and killer whale urine using radioimmunoassay or enzyme immunoassay techniques (Monfort and Brown, unpublished observations). Furthermore, there has been considerable recent interest in measuring adrenal corticoid metabolites in urine and feces as an index of the relatedness of 'stress' and reproductive success in both zoo-maintained and free-living wildlife (for reviews, see Monfort 2003; Pickard 2003).

Non-invasive hormonal monitoring has been used extensively since the early 1980s to characterise the reproductive biology of many non-human primate, antelope, equid, felid, canid, marsupial and bird species (Lasley and Kirkpatrick 1991; Hodges 1996; Hirschenhauser *et al.* 1999; Brown *et al.* 2001*a*, 2001*b*; Paris *et al.* 2002; Monfort 2003; Pickard 2003). From a management perspective, this technology has been used to identify acyclicity, pregnancy and impending birth. New phenomena have also been discovered. For example, urinary hormone monitoring has revealed that

the endangered Eld's deer from South-east Asia has a long-day photoperiodically cued onset of ovarian cyclicity in contrast with the short-day cued cycle of the North American white-tailed deer (Monfort *et al.* 1990). Longitudinal studies of the cheetah indicate that more than 25% of the captive population fails to demonstrate ovarian cyclicity at any given time (for a review, see Brown *et al.* 2001*a*). More recent studies have shown that this failure is due to reproductive suppression by conspecifics within the same enclosure (Wielebnowski *et al.* 2002*a*). The findings make sense. On the plains of Africa, cheetahs are generally solitary. When placed in zoos in groups, ovarian activity shuts down and returns only under solitary conditions without the presence of other females in the same enclosure. Fecal monitoring of felids also has revealed that some species are very 'un-cat like' in how ovulation occurs. In the domestic cat, tiger and lion, ovulation occurs typically after vaginal/cervical stimulation as a result of mating (induced ovulation). However, there are some species of cats, such as the rare clouded leopard from South-east Asia, that have a relatively high incidence of spontaneous ovulation for reasons that remain unknown (Brown *et al.* 1995, 2001*a*).

Perhaps most important is the usefulness of this technology for understanding why species fail to reproduce in captivity. Fecal hormone monitoring has revealed major differences in reproductive patterns among rhinoceros species (Heisterman *et al.* 1998; Schwarzenberger *et al.* 1998; Brown *et al.* 2001*b*) as well as birds-of-prey (raptors; Staley *et al.* 2003). Are there discernible variations in patterns among animals that are reproductively successful compared with those that are not? Could the findings be mediated by stress responses? Studies have correlated fecal glucocorticoid excretion patterns with potential stress in the face of less-than-ideal reproduction and husbandry conditions. For example, fecal corticoids are higher in clouded leopards housed adjacent to predator species or located near the public in contrast with counterparts that are maintained in more remote and vertically spacious enclosures (Wielebnowski *et al.* 2002*b*). Thus, there are significant animal welfare applications to using such technologies to guide 'enrichment' of captive environments, potentially improving both reproductive success and animal well-being.

There also is significant potential for applying non-invasive hormonal profiles to animals living in nature (Monfort 2003). This is an especially rich area for new discoveries by those interested in combining interests in behavioural ecology with reproductive physiology. For example, years of studies of African wild dogs in nature questioned the significance of a mating system where only a single dominant female reproduced. Did the subordinates fail to become pregnant due to stress imposed by the alpha female? On the contrary, researchers studying wild dog packs in East Africa carefully collected fecal samples for subsequent hormonal analysis. Their findings revealed that the dominant pup-producing dam also excreted the highest glucocorticoid concentrations over time, indicating the 'adrenal expense' to being the reproductively successful female (Creel *et al.* 1997). Similar endocrine studies have been conducted in other free-living species, including the dwarf mongoose (Creel *et al.* 1992), Yellowstone bison (Kirkpatrick *et al.* 1996), moose (Berger *et al.* 1999) and greylag goose (Hirschenhauser *et al.* 1999), among others.

Although urinary or fecal hormone monitoring is currently quite popular for wildlife studies, it is worth noting that blood, when available, still offers the fastest turnaround for data production in the laboratory. Some species in captivity, notably whales, dolphins, the giant panda and elephant, have been conditioned for frequent blood draws. In cases where urine and faecal production is vast (i.e. the elephant), blood sampling is far more practical and traditional hormonal analysis has revealed that as many as 25% of adult females fail to show any ovarian activity (a phenomenon known as 'flatlining'; Brown 2000). Repeated blood sampling/hormonal analysis also is being used to time ultrasound-guided AI. In a given cycle, an elephant will produce two distinctive LH surges at a precise 3-week intervals (Brown *et al.* 1999). Knowing the timing of the first surge allows prediction of the second ovulatory surge and preparation for AI that follows shortly thereafter (Brown 2000). To date, eight elephant calves have been produced (from 12 pregnancies) when hormonal monitoring has been used to coordinate seminal deposition with precise time of ovulation (Brown, unpublished observations; also see the section on AI below).

What are the current limitations of this technology? So far, there are amazingly few. For example, recent studies from our laboratory have shown that it is possible to generate accurate profiles of gonadal steroid metabolites by analysing the feces of the bamboo-eating giant panda (Kersey *et al.* 2003). The fact that hormonal profiles can be produced from such fibrous material (the feces often still containing whole leaves and stems) proves the growing power of this technology. Nonetheless, improvements are needed in the extraction of metabolites from feces, which can be extremely labour intensive and time consuming. Research also is needed to develop sensitive antibodies that cross-react across diverse species (similar to what is now possible for diagnosing ovulation and pregnancy in great apes using human LH and chorionic gonadotropin kits, respectively). In addition, having rapid assays for gonadal and pituitary hormones would permit monitoring to be more manager friendly, allowing immediate decision making (e.g. for timed matings, assisted breeding or to predict impending parturition). A related priority is a more detailed examination of the value of adrenal hormone excretory patterns as an index of animal well-being. The result could be new objective data for enriching zoo habitats to increase both health and reproductive fitness. Finally, a technique that allows the instantaneous assessment of the endocrine status of an animal living in nature would offer

exciting opportunities to interrelate the physiology of an animal with its natural environment.

Artificial insemination

Artificial insemination was perceived initially as a technique that would help zoo curators quickly solve the many challenges to managing captive wildlife. Using AI, the problem of sexual incompatibility between designated breeding pairs could be circumvented, as well as the expense of shipping animals from one zoo to another (Wildt and Wemmer 1999; Hodges 2001). However, early studies (largely in wild ungulates and the great cats) soon revealed that AI technology developed for cattle was of little value to other species, even wild bovids. Again, species-specific reproductive mechanisms inhibited the rapid deployment of AI to wildlife. Especially important was identifying the duration of the reproductive cycle and the timing and type of ovulation (i.e. spontaneous *v.* induced). Perhaps most underestimated was the sheer difference among species in reproductive tract morphology. Some species had simple tracts that allowed sperm to be deposited *in utero* (gaur, banteng, addax, gerenuk). However, the reproductive tracts of others species (scimitar-horned oryx, rhinoceros, elephant) were so complicated (usually by a tortuous or bifurcated cervix or excessively long vaginal vault) that there was little hope of placing sperm transcervically or even adjacent to the cervical os.

Although the challenges have been substantial, AI has been used with some effectiveness for managing and conserving a few wildlife species. For example, AI with fresh or thawed semen has been used to produce offspring in approximately 20 non-domestic bird species, most prominently certain wild cranes and birds-of-prey (Gee 1995; Blanco *et al.* 2002; Donoghue *et al.* 2003). A few zoo-based genetic management plans have relied upon AI. Two well-known examples are the cheetah and black-footed ferret. Assisted breeding was first attempted in the cheetah in 1980 using cow AI protocols and, thus, no cheetahs ever became pregnant with this quick-fix approach (for a review, see Wildt *et al.* 2001). Only after extensive studies of zoo and free-living cheetahs that included investigation of the reproductive cycle, time and type of ovulation, methods for inducing ovulation and sperm collection/processing protocols was there consistent success. In one study, 10 litters were produced by AI (41.2% incidence of success), including three pregnancies using frozen semen imported from Africa (Wildt *et al.* 1997; Howard, unpublished observations). In the case of the black-footed ferret, a small mustelid that once ranged throughout the Great Plains, AI was used initially to help avoid species extinction and later as a strategy for improving a ferret reintroduction programme into nature (for a review, see Howard *et al.* 2003). Because of loss of prey and disease, black-footed ferrets had declined to a total of 18 individuals. Owing to limited founder numbers, it was imperative that all animals be equally represented in subsequent generations to retain genetic heterozygosity. Because of the sexual aggressiveness of males upon females and because some males failed to properly mount an oestrual female, AI was necessary. Again, initial efforts focused on basic studies of sperm biology, ovulation induction and methods to process and artificially deposit sperm *in utero* to efficiently produce offspring. From 1991 to 2003, more than 100 black-footed ferret kits were produced by AI (Howard, unpublished observations) with the most valuable offspring maintained as breeding stock, but with some also released into western states.

A more contemporary success story involves AI for circumventing poor natural breeding in giant pandas living in zoos and breeding centres in China. This species, notorious for sexual incompatibility between conspecifics, has been studied intensively in our laboratory and that of our Chinese colleagues since 1998 (Wildt *et al.* 2003). Initially, a multidisciplinary approach was used to examine behavioural, physiological, genetic, health and nutritional factors that may be limiting reproductive success. However, physiological fertility was insignificant; reproductive traits were similar between proven and unproven breeders. In the current *ex situ* population of approximately 130 giant pandas in China, fewer than 10 males have mated naturally and sired young (Huang *et al.* 2002), largely because of male aggression on females. 'Good behavioural' breeders were rare, with most breeding centres or zoos having only one male capable of mating, thus making it difficult to maintain maximal genetic diversity in the population. Artificial insemination has long been a valued concept by Chinese managers of giant pandas, with the first confirmed pregnancy occurring in 1978 at the Beijing Zoo. Since then, progress in developing AI has been slow, but has accelerated recently due to detailed studies of sperm biology *in vitro*, including evaluating the impact of culture, cooling and cryopreservation media on sperm viability and function (Huang *et al.* 2002). In one study at the China Research and Conservation Center for the Giant Panda (in the famous Wolong Nature Reserve), AI was found to be as effective as natural breeding (pregnancy success in four of seven females (57.1%) *v.* in 14 of 21 females (66.7%), respectively). Offspring have also been produced with cooled as well as thawed sperm, including from males suffering terminal disease (Huang *et al.* 2002). Furthermore, success has been adequate to now consider the transport of semen for AI among giant panda holding facilities, thereby allowing the development of a metapopulation management strategy.

In addition to overcoming behavioural incompatibilities and the challenges of translocating animals, AI provides a potential means of importing new genes from wild populations into zoos, thereby maintaining the genetic vigour of captive populations (Holt *et al.* 1996*a*, 2003*b*; Wildt *et al.* 1997). The potential of the reverse action has also been discussed; that is, using sperm from zoo animals to bolster the genetic heterozygosity of wild animals living in small habitats.

A major challenge to conserving species in nature is the fragmentation of wild places (i.e. the breaking apart of landscapes into small segments that have no corridors to allow animals to move), thereby preventing appropriate genetic exchange. The result can be non-viable populations that eventually die out due to inbreeding depression (for a review, see Taylor 2003). For some species living in such conditions, it may be feasible to capture females for short periods (up to several weeks) for AI with sperm from zoo-maintained, healthy males. The females could then be returned to the original habitat to give birth to a new generation of genetically healthy young.

There also are significant limitations to AI technology for wildlife (for a review, see Pope and Loskutoff 1999). Although it is generally possible to collect sperm from most wild mammals by electroejaculation (under anaesthesia), this process remains difficult for some species, including the rhinoceros, wild equids, certain great apes, canids and marsupials. This challenge needs more study, especially the impact of anaesthesia on seminal release, as well as developing more appropriate tools for eliciting ejaculation (Hildebrandt *et al.* 2000). Furthermore, species specificities are significant across taxa and even within families in terms of sperm quality and processing required to maintain viability *in vitro*. For example, the giant panda generally produces billions of sperm per ejaculate (Huang *et al.* 2002) compared with wild cats, which usually produce only a few million sperm (Howard 1999). Is this because the giant panda breeds over only a few days each year, whereas most male felids mate year round? Or, does this mean that cats are more reproductively efficient (due to lower sperm concentration) at the gamete (sperm–ovum) interface in the oviduct than the giant panda? Not all interesting scholarly questions involve species so evolutionarily distant from one another. For example, sperm from the snow leopard only survive *in vitro* in medium that has a vastly different pH from optimal medium for the tiger and cheetah (Roth *et al.* 1994). The question why these fascinating differences among species within the same taxonomic family exist is common when studying wildlife.

For females, there is little information available on the dynamics of the reproductive cycle, including seasonality and the timing of ovulation. If AI is to be successful, then sperm need to be placed *in utero* in the periovulatory period. Although information on female cyclicity can be collected using non-invasive hormonal and behavioural monitoring, the most consistent AI protocols will still require the induction and/or synchronisation of ovulation using exogenous gonadotropins (Howard 1999). Contemporary, commercially available hormones (e.g. equine chorionic gonadotropin (eCG), human chorionic gonadotropin (hCG) or gonadotropin-releasing hormone) give notoriously erratic performances when applied to wildlife. In our experience, there is no relationship between body mass and dose of eCG/hCG required to induce optimal ovulatory responses among felids. For example, a large-sized species such as the African cheetah (approximately 35 kg) actually requires a lower eCG dose to elicit eventual ovulation than the smaller-sized Latin American ocelot (approximately 9 kg) (Howard 1999). What is the origin and significance of this variance in ovarian sensitivity? There is also frequent concern about immunogenic responses triggered by repeated administration of chorionic gonadotropins, as has been demonstrated in the cow (Jainudeen *et al.* 1996), rabbit (Greenwald 1970), goat (Roy *et al.* 1999), non-human primates (Ottobre and Stouffer 1985) and felids (Swanson *et al.* 1995). In fact, it has been established that conventional gonadotropin protocols can result in aberrant endocrine profiles and abnormal oocyte/embryo morphology (e.g. in the tiger; Crichton *et al.* 2003). Thus, there is a need to pursue alternative sources of gonadotropins for diverse wildlife species.

Synchronising ovarian activity by inducing ovarian luteolysis, either using prostaglandin $F_{2\alpha}$ or vaginal progesterone pessaries, has resulted in highly variable success in wild ungulates, including the scimitar-horned oryx, bongo, eland, addax, Mohor gazelle and gerenuk (Schiewe *et al.* 1991; Holt *et al.* 1996*b*; Pope and Loskutoff 1999; Penfold, personal communication). Other species, for instance most felids, have internal synchrony mechanisms: ovulation does not occur without mating. Thus, ovulation can be controlled solely through the administration of eCG/hCG because active luteal tissue is usually absent on the ovaries (Wildt *et al.* 1998). However, it is not uncommon to encounter spontaneous ovulations in felids (e.g. clouded leopard, lion, margay and fishing cat, among others) and the presence of corpora lutea markedly blunts ovarian responses to eCG/hCG (Pelican *et al.* 2003). As a result, our laboratory has explored the usefulness of ovarian inhibition before exogenous gonadotropin treatment for producing more predictable ovulatory responses. Pretreatment with the progestin levonorgestrel (Norplant, Wyeth Pharmaceuticals, Philadelphia, PA, USA), but not the gonadotropin-releasing hormone antagonist antide, before eCG/hCG stimulation more than doubled embryo yield after IVF and resulted in a uniform excellent response for AI in the domestic cat (Pelican *et al.* 2003). Similar responses have been produced from levonorgestrel therapy in the cheetah and fishing cat but, oddly, not in the clouded leopard, again illustrating the clout of species specificities.

A final significant challenge is sperm deposition itself. For mammals, the variation in tract morphology and the intractability of the species has been generally overcome using sedation and/or anaesthesia. However, such drugs can slow or stop normal uterine contractions that propel the sperm through the reproductive tract and into the oviducts. For this reason, sperm are often deposited directly into the uterus transabdominally by endoscopy (Howard 1999; Pope and Loskutoff 1999). For ungulates, this may or may not be necessary (Pope and Loskutoff 1999). Acceptable pregnancy success has been achieved in sedated scimitar-horned oryx using rectal palpation and the insertion of an insemination

pipette vaginally into the cervix or uterus for sperm deposition (Morrow *et al.* 2000). In contrast, smaller hoofed species, such as the Eld's deer, have required endoscopic intrauterine insemination to achieve consistent pregnancy success (Monfort *et al.* 1993). A few species have tolerated AI manipulations while fully conscious, including the gaur and banteng (wild cattle species; Wolfe, personal communication).

However, clearly the most significant recent advancements in AI for wildlife have involved the Asian and African elephants (for reviews, see the special issue of *Zoo Biology* (2000; 19: 297–484)). Neither species has a self-sustaining population in North America (Olson and Wiese 2000; Wiese 2000). If mortality and fecundity rates are not improved, the populations will continue to be in decline, becoming 'reproductively extinct' within 50 years (Olson and Wiese 2000; Wiese 2000). The challenges to reversing this trend are as massive as the species itself. Most zoos prefer not to house bull elephants due to their unpredictable and dangerous temperament during aggressive, testosterone-driven periods of musth and bulls tend to suffer from low libido and questionable sperm quality. Furthermore, in addition to a female with massive bulk, there is the challenge of passing semen through a long and oddly oriented reproductive tract, with the vaginal opening located ventrally between the hind legs. All these challenges hardly make the elephant a promising candidate for AI. Nonetheless, through a highly coordinated, interinstitutional collaboration across zoos and research laboratories, 12 pregnancies have been produced, resulting in the birth of eight live calves. Success would have been unlikely without the combined use of hormonal monitoring (to identify the double LH surge and, thus, predict ovulation) combined with both endoscopic and state-of-the-art ultrasonography (to examine ovaries and to guide the insemination catheter; for a review, see Hildebrandt *et al.* 2003). This has been highly significant because it gives a chance of reversing a downward slide in fecundity for two species beloved in zoos while demonstrating that 'size does not necessarily matter' in designing AI protocols. The multidisciplinary approach to achieving consistent AI success in the elephant should be considered a model for other megavertebrates. For example, concurrent studies are in place using poolside endocrine assessments, ultrasonography and AI to begin producing offspring in marine mammals. At the time of writing, AI conceptions had been achieved in three killer whales, three Pacific white-side dolphins and two bottlenose dolphins (Steinman *et al.* 2003).

Embryo transfer and *in vitro* fertilisation

The challenges of applying embryo technologies, including ET or IVF/ET, to wildlife have been reviewed recently by Loskutoff (2003). Transferring embryos from one female to another is a powerful tool in the cattle industry, especially when multiple embryos can be collected from a superior dam and gestated in common surrogate females to exponentially increase the donor's lifetime reproductive output. Embryo transfer has not found application in the genetic management of *ex situ* wildlife populations. Originally, there was much excitement about interspecies ET. In this scenario, embryos from a wildlife (i.e. endangered) species would be transferred to a more common surrogate of a different species. The first success, a gaur calf born to a Holstein cow in 1981, was followed by years of failures and disappointments (Pope and Loskutoff 1999). There were occasional reports of the embryos of one species surviving to term in another, most prominently a bongo calf born to an eland cow (Pope and Loskutoff 1999). In addition, there were other sporadic successes involving interspecies ET, including a banteng born to a cow, a zebra to a domesticated horse and an Indian desert cat to a domestic cat (Pope 2000; Loskutoff 2003). However, none of these accomplishments was consistently repeatable. There now appears to be limited interest in interspecies ET, at least using current technologies. It is clear that the biological mechanisms that ensure survival of transplanted embryos into a foreign species uterus are more sophisticated and fragile than once appreciated. There have also been legitimate concerns about the behavioural and social competence of offspring produced by this means. For example, does a bongo born to, and raised by, an eland act like a bongo or an eland, or perhaps it makes absolutely no difference? Most likely this factor is taxon-specific and particularly important for species where reproductive and parenting behaviours are learned, rather than purely instinctive (e.g. primates).

In vitro fertilisation followed by ET has also been sporadically successful in a few zoo-held wildlife species, including the baboon, rhesus macaque, marmoset, gorilla, Indian desert cat, ocelot, tiger, African wild cat, Armenian red sheep, water buffalo, gaur, red deer, llama and caracal (Pope and Loskutoff 1999; Pope 2000; Pope *et al.* 2000; Loskutoff 2003). Here, again, no species is being managed genetically using IVF/ET, largely because sheer lack of fundamental biological information on the species of interest and the complexities of conducting even intraspecies ET. Perhaps the most significant potential of IVF for wildlife involves *in vitro* oocyte maturation (IVM) to rescue germplasm from genetically valuable females that die unexpectedly or undergo ovariohysterectomy for medical reasons. Sufficient studies have been conducted to reveal that the general culture methods developed for livestock and humans can often promote maturation of oocytes recovered from within the ovaries of wild mammals (Loskutoff 2003). To date, IVM followed by IVF/ET has resulted in live offspring in the Armenian red sheep, water buffalo, gaur, red deer and llama (Pope and Loskutoff 1999). However, success is highly dependent on culture supplements, as well as the age and health of the ovarian donor. *In vitro* oocyte maturation combined with IVF/embryo cryopreservation is a worthwhile research tool, especially because there are many genetically valuable founders representing

hundreds of species living in zoos that may never naturally reproduce. This may be the only means of retaining the genes of these individuals.

The use of ET in combination with IVF and/or IVM has the same challenges as outlined for AI; that is, a lack of basic knowledge about fundamental reproduction and an inability to synchronise the reproductive cycle artificially. Embryo transfer is also unlikely to become a wildlife management tool because of the need for significant numbers of surrogate dams, even conspecific females. Herein lies one of the major conundrums for modern zoos: lack of space to maintain sufficient animals to meet genetic goals. For example, how many hungry tigers would a zoo need to maintain to have adequate surrogates for a few genetically valued donors? There are now also well-known challenges associated with *in vitro*-produced embryos, including: (*1*) an increased incidence of ultrastructural and cytogenetic problems; (*2*) more spontaneous abortions, perinatal loss and fetal abnormalities; (*3*) an increased incidence of dystocias in recipients due to increased offspring size; and (*4*) a potential skewing of sex ratios to favour males (Loskutoff 2003). All these limitations emphasise the importance of pre-emptive basic research on a species-by-species basis before even remotely considering these approaches for management or conservation initiatives.

It is worth concluding this section with a positive comment on the comparative value of IVF studies for wildlife. Compared with straightforward AI testing, IVF offers some significant advantages. For example, often the only quantifiable result in AI trials is number of offspring produced. In contrast, the advantages to scholarship are significantly enhanced during IVF trails because of the ability to examine both sperm and oocytes, their interaction *in vitro* and the number and quality of resulting embryos. Such information is fundamental to eventually predicting reproductive success using a host of assisted breeding technologies, including AI.

Cryopreservation of spermatozoa

The value of cryopreserving germplasm and embryos from wildlife species has been reviewed extensively (Holt *et al.* 1996*a*, 2003*b*; Wildt *et al.* 1997; Watson and Holt 2001). The need for, and the advantages of, transferring sperm between zoos (rather than moving living, stress-susceptible animals) to maintain genetic diversity are readily apparent. Infusing new genetic material across zoos or from wild populations into isolated zoos via AI would be facilitated by using frozen–thawed spermatozoa. Organised repositories of cryopreserved sperm from every genetically valuable male (frequently referred to as genome or genetic resource banks (GRBs)) would also serve as insurance. Entire wild animal populations in zoos have been lost forever from unexpected catastrophes (such as fire). The ability to use frozen sperm increases the generation interval indefinitely and requires fewer males in captivity because some of the genetic diversity is maintained strictly as frozen sperm in liquid nitrogen. Having sperm samples from representative males living in natural habitats protects the existing genetic diversity from unforeseen dangers (e.g. canine distemper epidemic and Serengeti lions; Roelke-Parker *et al.* 1996) and eliminates the need to ever remove more males from the wild to support zoo breeding programmes. Meanwhile, stored sperm from wild and captive individuals could be introduced back into the contemporary population immediately, a decade from now or even centuries into the future. Thus, the advantages of sperm cryopreservation are profound.

The current challenge, of course, is creating more empirical evidence. From a theoretical perspective, we have recently conducted computer simulations to determine the efficacy of different GRB strategies for maintaining genetic diversity (Harnal *et al.* 2002). First, using historical data on the captive populations of three species with varied pedigrees (Eld's deer, Przewalski's horse and Sumatran tiger), retrospective analyses were conducted to determine whether GRBs, established years in the past, would have increased genetic diversity in the current populations. Second, the effectiveness of different semen banking and use strategies was tested in these species to determine which approaches would be most effective for maintaining genetically viable managed populations. A 'wild bank', consisting of sperm from five to 10 males unrelated to the managed population and to each other, was compared to a 'best male' bank containing sperm from only the most genetically valuable males alive in the *ex situ* population. Overall, a sperm usage frequency of five times per year was most efficient and 'wild banks' were highly successful at enhancing genetic diversity. Nonetheless, the simulations revealed that different species (or populations) require different GRB management scenarios; in this case, the greatest benefits of sperm banking/AI were realised for the Eld's deer and Przewalski's horse over the tiger. Thus, a GRB strategy that is efficient for one species is not necessarily optimal for another. Population pedigree clearly dictates the best GRB plan to use. Furthermore, the ultimate value of a GRB depends on the genetic relationships between the GRB semen donors and the females alive in the extant population at the time when the semen is used. For example, modelling demonstrated that GRB effectiveness is significantly diminished if all living females are descended from the banked males. This argues for establishing banks using genetically underrepresented males or males from unrelated (e.g. wild) populations.

In terms of real life research, there appears to be substantial ongoing investigations involving the cryobiology of sperm from wildlife species. Studies are also becoming more sophisticated. Initially, efforts focused on adapting cattle technology and then conducting simple post-thaw assessments (e.g. of sperm motility after thawing). When more detailed examinations have been made, more cell damage is evident, especially to sperm membranes. Nonetheless, cattle sperm cryopreservation protocols are fairly reliable for many wild bovids,

antelope and cervids (Monfort *et al.* 1993; Holt *et al.* 1996*b*; Pope and Loskutoff 1999; Roth *et al.* 1999; Morrow *et al.* 2000; Solti *et al.* 2000). For example, offspring have been produced with consistency in the scimitar-horned oryx (Morrow *et al.* 2000), Eld's deer (Monfort *et al.* 1993), Mohor gazelle (Holt *et al.* 1996*b*, 2003*a*), gaur and banteng (Solti *et al.* 2000; Wolfe, personal communication) using AI and sperm frozen and thawed using methods minimally modified from original cattle techniques.

In contrast, the sperm of carnivores (from wild mustelids, felids and canids) do not cryopreserve well using standard livestock methodologies. This should not be surprising given that researchers still tussle with identifying the ideal methods for freezing domestic dog sperm, despite decades of research by many prominent researchers (for a review, see Farstad 2000). Nonetheless, there has been slow progress in using cryopreserved sperm and AI in wild carnivores, although overall results remain unsatisfactory. A few pregnancies have been produced in the fox, wolf, Siberian ferret, giant panda, leopard cat, ocelot and cheetah (Howard 1999; Pope 2000), including the production of three cheetah litters from frozen sperm imported from wild males living in Africa (Wildt *et al.* 1997). However, fascinating species differences remain. For example, recent data from our laboratory demonstrate a remarkable tolerance of giant panda sperm for a variety of cryopreservation insults, from cold shock to varying cryoprotectants of extreme concentrations (Howard and Spindler, unpublished observations). In contrast, sperm from the endangered mustelid, the black-footed ferret, consistently resists freeze–thawing. Testing a host of cryoprotectants, freezing and thawing rates have failed to identify any method to avoid almost 100% post-thaw mortality, including severe membrane and acrosomal damage (Howard and Moreland, unpublished observations). The fact that we have produced offspring in genetically related mustelids, including the European ferret and Siberian polecat (Howard *et al.* 2003) using AI with thawed sperm simply re-emphasises that even taxonomically related species can experience a full spectrum of sperm sensitivities to cryopreservation.

Dealing with this challenge certainly argues the need to generate knowledge about the complicated membrane structure of the spermatozoon and the varying species sensitivities to osmotic stress and cryoprotectant and water influx/efflux (Watson and Fuller 2001). For example, sperm of the African and Asian elephants are significantly different in membrane fatty acid composition (Swain and Miller 2000), which no doubt partially explains the extreme sensitivity of the latter species to freeze–thawing. Additional factors are also relevant to measuring success, including sperm number and morphology. Recall that the cats, as a taxon, generally ejaculate comparatively few spermatozoa. These sperm also experience an exquisite sensitivity to simple cooling (before freezing) that can cause massive acrosomal damage, which, in turn, erodes fertilising capacity further (Pukazhenthi *et al.* 1999). Thus, for the felids, multiple lines of research attack are required to (*1*) determine ways to reduce acrosome damage from cooling while (*2*) considering the numbers of additional sperm needed to assure fertilisation. Carnivores, in general, also appear susceptible to losses in genetic heterozygosity (inbreeding) by producing increased numbers of malformed spermatozoa. Although it is well established that these sperm do not participate in fertilisation, they also are known to be more susceptible to freeze–thawing, especially osmotic stresses (Watson and Fuller 2001).

Together, contemporary evidence suggests that advancing sperm cryopreservation success in wildlife species will depend on more classical cryobiological investigations that focus on the biophysical properties (sensitivity to cold shock, membrane composition, permeability to water and cryoprotectants) of sperm within and across species. If these properties can be characterised and understood, then cryopreservation protocols can be more efficiently designed to speed practical application of the technology. Given that (*1*) AI success with fresh sperm continues to advance and (*2*) more detailed basic cryobiological studies are conducted that focus on biophysical uniqueness and post-thaw functionality, it will be only a matter of time before sperm cryopreservation becomes more routine for genetic management and conservation.

Cryopreservation of embryos and oocytes

Embryo cryopreservation allows storing the full genetic complement of the sire and dam and, thus, has enormous potential for protecting and managing species and population integrity and heterozygosity. As with other methods described above, the success of applying this technology to wildlife will be dictated by the uniqueness of the embryo of each species. Virtually none of this characterisation has been studied beyond a few domesticated species and the human (Paynter *et al.* 1997). Furthermore, the differences among embryos in cryosensitivity are substantial, as demonstrated by the variance between the 'freezable' cow compared with the 'difficult-to-freeze' pig embryo.

Regardless, cattle embryo freeze–thaw protocols have been fairly effective in the few studies conducted in wild Bovidae (gaur, banteng, bongo, eland), including the production of living young in each of these species (Loskutoff *et al.* 1995). In contrast, data for non-ungulates is rudimentary, with evidence that the hurdles ahead will be substantial. Carnivores also present the usual set of challenges that have been overcome, in part, by conducting pre-emptive studies in domesticated counterparts. For example, the domestic cat has been a model for identifying embryo cryopreservation protocols for wild felids (Pope 2000). After domestic cat kittens were produced using frozen–thawed embryos and ET, the same techniques were applied successfully to the African wild cat (Pope *et al.* 2000). A similar strategy

has been used recently to produce an ocelot kitten through IVF followed by embryo cryopreservation and transfer to a conspecific recipient (Swanson 2001). Even with these milestones, embryo cryopreservation has not been used as a management tool and there are no large-scale embryo banks for any endangered species.

A similar situation exists for oocyte cryopreservation; few recent studies have been conducted, despite considerable advantages, similar to those potentially achievable from using cryopreserved sperm. There has been significant progress in oocyte cryopreservation for livestock and laboratory animals, as well as the human, in part, by evaluating ultrarapid cooling as a means of maintaining cytoskeleton stability (Tucker *et al.* 1998; Shaw *et al.* 2000; Vajta 2000). Some of these advances have been made using the oocytes of non-human primates (certainly, a type of wildlife; Younis *et al.* 1996). A particularly relevant area of research need involves the cryopreservation of immature oocytes, because these cells (harvested at the germinal vesicle or germinal vesicle breakdown stage) do not have a temperature-sensitive meiotic spindle. Genetically valuable animals that die abruptly or those that are subjected to ovariohysterectomy for medical reasons could be a source of oocytes for such studies. Oocytes could be cryopreserved rather than matured immediately, thereby: (*1*) allowing more flexibility of use; and (*2*) giving time to collect and/or locate genetically appropriate sperm for IVF. However, before any of the above strategies become practical, there is a need to understand the fundamental cryobiological factors that determine embryo and oocyte viability and functionality before and after cryopreservation for virtually every individual species.

Cryopreservation of gonadal tissue

Although little research has been directed towards wildlife species, the cryopreservation and subsequent use of gonadal tissue offers intriguing opportunities (Honaramooz *et al.* 2002; Snow *et al.* 2002). Recent developments in the xenografting of ovaries and testes clearly demonstrate the potential value of cryopreserving gonadal tissue (Shaw *et al.* 2000). Thawed ovarian tissue has been transplanted into conspecific recipients in the mouse and sheep, resulting in living young (for a review, see Paynter *et al.* 1997). Xenografting thawed ovarian tissue from the marmoset monkey (Candy *et al.* 1995) and African elephant (Gunasena *et al.* 1998) to the immunodeficient (or nude) mouse has resulted in antral follicle development. A similar phenomenon has occurred in nude rats receiving transplants of thawed wombat ovarian tissue (Wolvecamp *et al.* 2000).

There also has been growing interest in cryopreserving testicular tissue, with much progress made on the basis of pioneering studies involving human cancer patients undergoing chemotherapy. Multiple births have been reported following fertilisation by microinjection of sperm, or even spermatids, isolated from thawed testicular tissue (Gianaroli *et al.* 1999). More recently, it has been shown that spermatogonial cells are viable after transplantation to a conspecific testis, provided the recipient is treated to destroy endogenous germ cells (Russell and Griswold 2000). Alternatively, spermatogonial cells from the rat have been xenografted to immunodeficient mice that, in turn, have produced viable sperm (Russell and Griswold 2000). So far, cryopreserving spermatogonia appears to be comparatively easy because these cells have less specialised morphology compared with mature spermatozoa. Although spermatogonial stem cell transfer from a donor rat or hamster to a recipient mouse results in rat and hamster spermatogenesis, transplantation of germ cells from phylogenetically more distant species (e.g. rabbit, dog, pig, bull, horse, primate) fails to establish spermatogenesis in the mouse testis (Honaramooz *et al.* 2002).

Although practical application must be relegated to the future, this gonadal technology should be a target for contemporary studies. The cryopreservation of ovarian and testicular tissue could be an attractive tool for wildlife programmes, especially in situations where population numbers are critically low, other options have failed and managers are faced with the need to rescue all extant genetic diversity, including from dying neonates.

Sperm sexing

Unbalanced sex ratios, especially excessive male births, can play havoc with small population management for wildlife species, especially for large-sized mammals in captivity. A classic example is the preponderance of male calves (approximately 70%) produced by two rhinoceros species in North American zoos (Wildt and Wemmer 1999), but other examples are available (Glatston 1995). Thus, recent advances in sexing mammalian sperm on the basis of well-known differences in DNA content in X- compared with Y-bearing sperm (for reviews, see Johnson 2000; Garner 2001) deserve consideration. Modern differentiation technology relies on a flow cytometer/cell sorter with a modified, high-speed configuration specifically targeted for sperm. Although most effort has been directed towards commercially viable uses, especially for livestock (Garner 2001), preliminary studies have revealed some differences in DNA content for X- and Y-bearing sperm in the elk, elephant, camel (Johnson 2000), marmoset, baboon, gorilla, hippopotamus and giraffe (for a review, see O'Brien *et al.* 2002). An AI study of sexed and thawed elk sperm has been conducted that produced 11 offspring, nine of which (82%) were of the predicted sex based on the use of predominately X- or Y-bearing sperm in the inseminates (Schenk and DeGrofft 2003).

A significant challenge in using this technique for controlling sex in wildlife breeding programmes will be the often low sperm densities encountered and/or a tendency for males to produce pleiomorphic spermatozoa. Ideally, initial ejaculate

quality, including sperm concentration, motility and morphology, would be in the range that would allow adequate numbers of cells to be sorted over a few hours to achieve fertilisation via a standard AI dose (i.e. multiple millions of viable cells). In real life, this may be problematic, so that the alternative is using IVF or intracytoplasmic sperm injection (ICSI) to produce embryos using the sexed sperm. Such is the case with a contemporary study of gorillas in North American zoos (O'Brien *et al.* 2002). Gorillas typically live in harems with a dominant male, so any 'surplus' production of males presents a problem to managers. This investigation is examining the potential of sexing fresh and thawed gorilla spermatozoa and then using X-bearing sperm to produce embryos by IVF or ICSI for subsequent transfer to genetically appropriate females. This type of study has ramifications not only for improving management efficiency for this high profile species, but also as a model for how this approach could find wider application in zoos.

Nuclear transfer and somatic cell cloning

Nuclear transfer (or cloning) 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 of the original donor, except for the mitochondrial DNA, which is derived from the recipient. Nuclear transfer has received widespread attention in the livestock industry because of the potential of rapidly expanding the genes of outstanding individuals and the production of unique genotypes benefiting biotechnologies, including the production of human pharmaceuticals. Most progress has derived from embryo micromanipulations that involve placing extracted DNA from an embryonic blastomere into an empty zona pellucida. Even more remarkable has been the production of offspring from DNA removed from a cumulus, fibroblast or somatic cell (Campbell 1999).

It was natural that these reproductive feats would attract attention as a potential means of propagating endangered species. Interest in this subject (of seemingly unending fascination by the media) has been fueled by the birth of both a gaur (Lanza *et al.* 2000) and banteng calf after nuclear transfer and gestational surrogacy in domestic cows. The relevance (and irrelevance) of nuclear transfer for wildlife has been thoughtfully addressed by Critser *et al.* (2003). Of particular interest was the approach of these authors to consider the value of cloning in a bilateral fashion, both in terms of 'technological reality' as well as 'conceptual and practical challenges'. In a way, their evaluation addressed the array and complexity of factors limiting the technical feasibility of nuclear transfer for becoming routine in any species, domesticated or wild.

From a technological limitations perspective, the variables considered by Critser *et al.* (2003) included nuclear reprogramming, recipient oocyte function, cytoplasmic inheritance, DNA structure, oocyte activation, gestational surrogacy and embryo/fetal development. Five areas of particular concern for wildlife have been identified. The first is the availability of appropriate recipient oocytes: is it important whether the recipient oocyte is vastly different phylogenetically from the donor DNA? The authors suggest that perhaps this issue is not too self-limiting because there is sufficient cross-species evidence to indicate that certain embryogenic mechanisms are conserved across mammalian taxa. However, the second factor relating to cytoplasmic inheritance is a technological challenge because it is essential that the recipient oocyte for the donor nucleus be from the same (or physiologically similar) species due to the need to mimic non-nuclear (or mitochondrial) DNA. A third (and yet another species-dependent) DNA factor is telomere length. In some species (i.e. sheep), telomere length becomes abnormally shortened following nuclear transfer, resulting in unstable DNA and failed embryo development. The incidence of this phenomenon is unknown for most species and, thus, may well impact success. The fourth factor relates to the already discussed challenge of requiring ready availability of an appropriate surrogate species, preferably closely related taxonomically to the species of the donor nucleus. The 'what species to use' question for embryo transfer involving wildlife always will be problematic. And the fifth technological factor is the growing database on developmental anomalies in cloned offspring, including high pre- and perinatal death rates, placental abnormalities, neonatal respiratory distress, chronic pulmonary hypertension, cardiopulmonary deformities and 'large calf syndrome'. Some of these conditions were observed in both the gaur (Lanza *et al.* 2000) and banteng studies, including an unusually high prenatal mortality and the production of abnormally large-sized offspring.

Critser *et al.* (2003) also have addressed conceptual and practical challenges associated with nuclear transfer and *ex situ* breeding programmes for wildlife. This included a prerequisite for balancing the need to maintain adequate genetic diversity in captive populations (a weakness of cloning) with avoiding species extinction (perhaps a strength; see below). Second is the realisation that preserving biodiversity is more than the propagation of any single individual. Effective conservation entails dealing with enormous biocomplexity to sustain diverse and genetically viable wildlife populations through habitat protection and the involvement of a vast array of stakeholders, including local communities. Producing an occasional offspring by nuclear transfer (or any other technology) is irrelevant under this definition of conservation. In fact, it has been argued that a focus on reproductive technology may reduce attention towards higher priorities for wildlife, such as preserving habitats. After all, why bother saving wild places and wildlife if reproductive specialists can avoid extinctions through last-ditch heroics via technology? Although this question may appear extreme, it is a real concern of some conservationists and must be countered by

continuous emphasis on the role of reproductive science in practical problem solving through basic and applied research (Wildt and Wemmer 1999; Wildt *et al.* 2003). The issue of cloning, due to its extreme technical ineffectiveness and hot-button sensitivity, tends to negate the value of reproductive technologies to wildlife management and conservation. These concerns extend to the interest of fringe groups in rederivation of long-extinct species and the ethical, disease, proprietary and commercial issues that inevitably will emerge from such activities.

Despite the negatives, we would endorse the suggestion of Critser *et al.* (2003) that, rather than simply ask 'is nuclear transfer applicable to wildlife', it is more prudent to ask 'as the technology evolves, how can nuclear transfer become a useful tool in a repertoire of assisted breeding technology'? In this context, it may well be that there are at least four contemporary values for nuclear transfer, the obvious being to enhance basic research to better understand species-specific mechanisms. In cases of near (or even recent) extinction, nuclear transfer could be the last available approach for saving a species, especially in instances where viable tissue is stored, one gender is missing or the sex ratio is severely skewed. Third, if nuclear transfer ever became a viable technology, then somatic cell cloning would be particularly advantageous because it essentially eliminates genetic drift by avoiding recombination. And fourth, biotechnology is changing rapidly, making it impossible to predict future options or opportunities. Because cloning has been successful in a few species, it may be possible to rescue DNA from stored tissues in the future, using it for consistently producing living young of the original donor. Given these potential, long-term benefits, it would be wise to freeze-store not only gonadal, but also somatic cells from valuable individuals living in zoos and in nature, thereby further supporting the value of GRBs.

Conclusion

The application of reproductive technologies to wildlife will ride the coat-tails of the livestock and human biomedical fields unless more resources are allocated to this fascinating area of science. Progress will continue to be gradual due to limited resources, but also because the management and conservation of rare species are biologically and politically complex. In a world of rapidly growing numbers of humans (all with needs), it becomes more difficult to save wild places, animals and plants. Feeding and caring for people are always the first priorities of society.

However, ignoring biodiversity and all the wondrous unknown data yet to be gleaned from wildlife is shortsighted, especially because such information may be crucial to meeting the needs of people. For convincing (and/or assurance), the reader is referred to two texts on the values of bioprospecting from nature (Wilson 1992; Perlman *et al.* 1997). If there is agreement that preserving natural ecosystems is good, then stable wild environments need to be filled with healthy, reproducing wildlife populations. Thus, for scholarship and for making the right management decisions, there is need to study reproduction, the foundation on which a species survives, thrives or becomes extinct. But, of course, it is not reproductive technologies *per se* that should drive our research. Rather, it is the interesting mechanistic questions that need to be addressed that, in turn, influence which technique could be useful. Therefore, reproductive technologies are only a set of tools in a virtual toolbox to be used to assist in developing knowledge. When sufficient intellectual capital is generated, the data and selected tools may find practical use in managing and conserving wildlife. As illustrated throughout this paper, there is a growing list of such success stories. Meanwhile, these techniques will continue to have most value in simply understanding the reproductive biology of more than 40 000 vertebrate species on the planet that have gone unstudied.

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