



Application of assisted reproduction for population management in felids: The potential and reality for conservation of small cats

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Abstract

Assisted reproductive technology (ART), using the primary applied tools of AI, ET, and sperm and embryo cryopreservation, has been promoted over the past decades for its potential to conserve endangered wildlife, including felids. However, if the goal is efficient, consistent production of viable offspring for population management, then the ‘potential’ of ART has yet to become ‘reality’ for any non-domestic cat species. For the five small-sized felids (i.e., Brazilian ocelot, fishing cat, Pallas’ cat, Arabian sand cat, black-footed cat) managed by Species Survival Plans (SSPs) in North American zoos, achieving this potential may be an absolute necessity if genetically viable captive populations are to be maintained into the next century. Modeling programs suggest that current SSP populations are not sustainable without periodic introduction of new founders and improved demographic parameters, including longer generation intervals and larger population sizes. ART provides the means to address each of these management challenges. In each small cat SSP species, fecal hormone metabolite assays and seminal analysis have proven useful for characterizing basal reproductive parameters, a necessary prerequisite to developing ART. Of the five SSP species, ART has been used to produce living offspring only in the ocelot, including after AI with frozen-thawed spermatozoa and following transfer of frozen-thawed IVF embryos. The true efficacy of these techniques, however, is still unknown. To improve the applicability of ART for population management, priorities for immediate research include further investigation of ovarian stimulation protocols, sperm and embryo cryopreservation methods, embryo culture systems, and fetal and neonatal viability following ART.

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Keywords: Conservation; Artificial insemination; Embryo transfer; Genetic drift; Genome resource banking

1. Introduction

Assisted reproductive technology (ART), comprised of techniques such as artificial insemination (AI), in vitro fertilization (IVF), embryo transfer (ET), and sperm and embryo cryopreservation, has been promoted over the past 25 years as a potential means to conserve and manage threatened wildlife populations [1–5].

Despite years of research, ART is still touted more for its potential than for any practical applications, and has not yet advanced beyond the rudimentary stages for use in conservation of endangered felids. Undoubtedly, scientific progress has been made, notably in broadening our knowledge of basic felid reproductive biology that forms the basis for improving the efficiency and applicability of ART. However, if the objective is to use this technology to generate viable offspring on a routine and consistent basis for population management, then ART has yet to approach its true potential with any non-domestic cat species.

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The failure of ART as a population management tool has created growing cynicism within the zoo and conservation community about whether this technology has any practical applications for preserving endangered species. This doubt is exacerbated every time a new technology is developed in a laboratory or livestock species and then breathlessly hyped for its conservation potential, creating unrealistic expectations, without due consideration of the practical difficulties involved [6]. For ART to ever play a meaningful role in felid conservation, it is essential that the more fundamental techniques of AI, IVF and ET, in addition to sperm and embryo cryopreservation, first be thoroughly developed via systematic studies, primarily in domestic cat research models. Second, these techniques must be proven to have adequate efficiency for applied usage, since their conservation utility will be directly proportional to procedural efficacy. By any measure, requiring dozens of AI or ET attempts to produce a single viable offspring cannot justify the associated expense, labor, and animal manipulations and stress. Lastly, ART must be applied within established population management programs to have any real conservation impact. The immediate value of ART lies with assisting population managers in the maintenance of viable captive felid populations. Its broader application will require establishment of a global network of trained scientists and veterinarians willing to conduct these procedures as a reproductive service for felid conservation.

2. The potential and necessity of art for population management

The fact remains that ART does have tremendous potential for management of endangered felid populations, especially for some of the small-sized (<20 kg body weight) cat species that are maintained in zoological collections. The American Zoo and Aquarium Association (AZA), comprised of 218 zoos and aquaria, has established Species Survival Plans (SSPs) for 5 of the world's 28 small cat species—the Brazilian ocelot (*Leopardus pardalis mitis*), fishing cat

(*Prionailurus viverrinus*), Pallas' cat (*Otocolobus manul*), black-footed cat (*Felis nigripes*) and Arabian sand cat (*Felis margarita harrisoni*). Each of these species (or subspecies) is included on the IUCN Red List under some degree of endangerment [7] and is listed on Appendix I or II of CITES [8]. These cats represent the priority species for conservation efforts within the North American zoo community. The general mission of SSPs is to maintain genetically viable captive populations, typically retaining $\geq 90\%$ of extant genetic diversity over a 50–100-year period, while also striving to protect each species in the wild. To achieve these goals, curators, keepers, veterinarians, and research scientists at AZA-accredited zoos and affiliated institutions work collaboratively to manage SSP populations and conduct research studies that identify and address critical factors affecting ex situ or in situ species survival.

Each of the small cat SSP species presents unique management challenges related to their reproductive biology, disease and stress susceptibility and other evolved traits. Because of species and population specificities, there is no standard strategy that is universally applicable across all taxa. However, small cats do share some common challenges. Most of the captive populations have limited founder sizes (<20 individuals) (Table 1), resulting in low genetic variability and a tendency for inbreeding with related reproductive and pathological consequences [9,10]. Limited genetic variation and inbreeding depression are associated with an increased risk of extinction [11,12]. There also is limited exhibit space available in zoological parks to maintain the world's growing collection of endangered species [13]. For small cats managed in SSP populations, only 80–150 cage spaces have been allocated per species (Table 1). The greatest threat to such small populations is the loss of genetic variation over time through genetic drift—the random changes in allele frequency resulting from each offspring inheriting only half of each parent's genotype [11,14].

SSPs use specialized computer software (Single Population and Animal Record Keeping System,

Table 1
Demographic and genetic status of small cat species managed by Species Survival Plans (SSPs) in North American zoos

Cat species	Current population	Cage space	Number of founders	Genetic diversity (GD)	Inbreeding coefficient (<i>F</i>)
Fishing cat	71	100	8	0.85	0.19
Pallas' cat	67	100	26	0.94	0
Brazilian ocelot	16	150	6	0.90	0
Black-footed cat	22	80	5	0.88	0.02
Sand cat	23	80	8	0.90	0.08

References [19–24].

SPARKS [15]; Population Management 2000, PM2000 [16]) to collate studbook data and conduct pedigree analysis to determine genetic interrelatedness (i.e., mean kinship), make breeding recommendations and maximize genetic variability [14,17]. One aspect of the PM2000 software includes population modeling, allowing integration of demographic and genetic data to project population trends over variable time periods into the future. Although this ‘genetic drift’ modeling program may generate overly pessimistic projections for some species (based on direct comparisons to pedigree analysis data), it does provide a reasonable starting point for examining the effect of changes in various population parameters [18]. Using updated information from small cat studbooks and SSP reports [19–24], this modeling program allows us to examine the projected impact of proposed alterations in founder number, generation intervals, population size and other management factors on the genetic diversity of small cat SSP species, as shown in the following three examples.

2.1. Fishing cats

The current fishing cat SSP population of 71 animals was derived from 8 original founders and, until 2001, was not managed to optimize genetic variability and limit inbreeding [23]. The recent importation of nine captive-born founders from Southeast Asia has increased genetic variability slightly but few of these have yet to reproduce. Assuming that most of these new founders eventually produce offspring, a target genetic diversity (GD) value of 90% should be attainable within the next few years. When fishing cat population parameters are evaluated in the PM2000 modeling program, we find that GD is projected to decrease sharply from 90% to 73% over a 50-year time period (Fig. 1a). To counter the loss of diversity resulting from genetic drift, new founders may be periodically introduced into the SSP population [14]. The fishing cat SSP is working with colleagues in Thailand to improve management of fishing cats in Thai zoos, with one goal being to generate surplus captive-born offspring that can serve as founders for the SSP [23]. If one new fishing cat founder is added to the SSP population every 5 years, GD after 50 years improves from 73% to 81% (Fig. 1b). However, to obtain our genetic goal of 90% GD, without other demographic changes, the SSP would require the introduction of one new founder each year over the entire 50-year period (Fig. 1c).

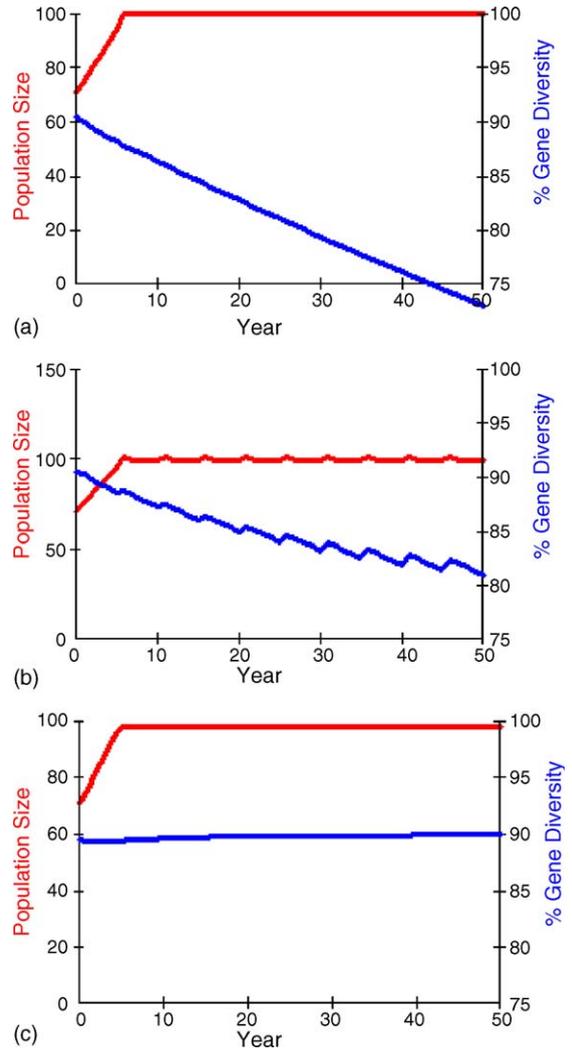


Fig. 1. Fishing cat population model depicting changes in SSP population size and genetic diversity over a 50-year time period with (a) no introduction of new founder genes, (b) introduction of one new founder every 5 years or (c) introduction of one new founder each year.

2.2. Pallas' cats

Even populations that are more robust genetically and intensively managed may experience some degree of genetic loss. For example, the Pallas' cat SSP population was derived from 26 founders, primarily wild-born cats, imported from Russia and Mongolia in the late 1990s [25]. Compared to fishing cats, current genetic variability is much higher (94%) and known inbreeding is non-existent ($F = 0$) (Table 1). Because capture and importation of additional wild-born cats is undesirable, the present founder population is unlikely to be supplemented in the near future. Population modeling, however, suggests that genetic viability of the

current SSP population is not sustainable with GD decreasing from 94% to 73% over 50 years (Fig. 2a). With improved management and expansion of available cage space (from 100 to 125 spaces), it may be possible to double the effective population size (N_e) and lengthen the generation interval by 50%. However, even under this optimized scenario, genetic variation after 50 years is still below target values at 88% (Fig. 2b).

2.3. Ocelots

The Brazilian ocelot population in North American zoos, consisting of just 16 animals representing the genetic contribution of 6 founders and their offspring, will lose substantial genetic variation (GD decrease from 90% to 72%) over then next 50 years (Fig. 3a). The Brazilian Ocelot Consortium (BOC), a partnership involving the Ocelot SSP, 10 AZA-accredited zoos and a Brazilian non-governmental conservation organization (the Associação Mata Ciliar), has proposed to import 20 additional founders over the next 5 years to augment the North American population [21,26]. Even

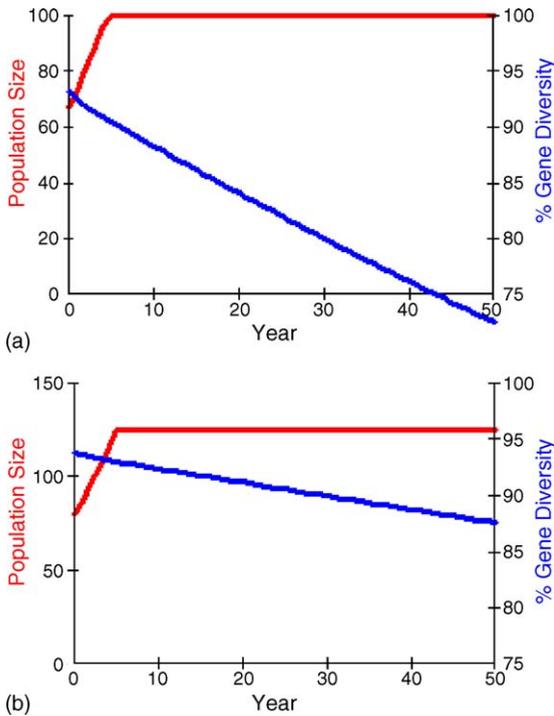


Fig. 2. Pallas' cat population model showing changes in SSP population size and genetic diversity over the next 50 years with (a) no changes in demographic parameters or (b) improved management resulting in longer generation intervals (i.e., from 4 to 6 years), increased effective population size (i.e., N_e/N ratio from 0.24 to 0.5) and more cage space (i.e., from 100 to 125 spaces).

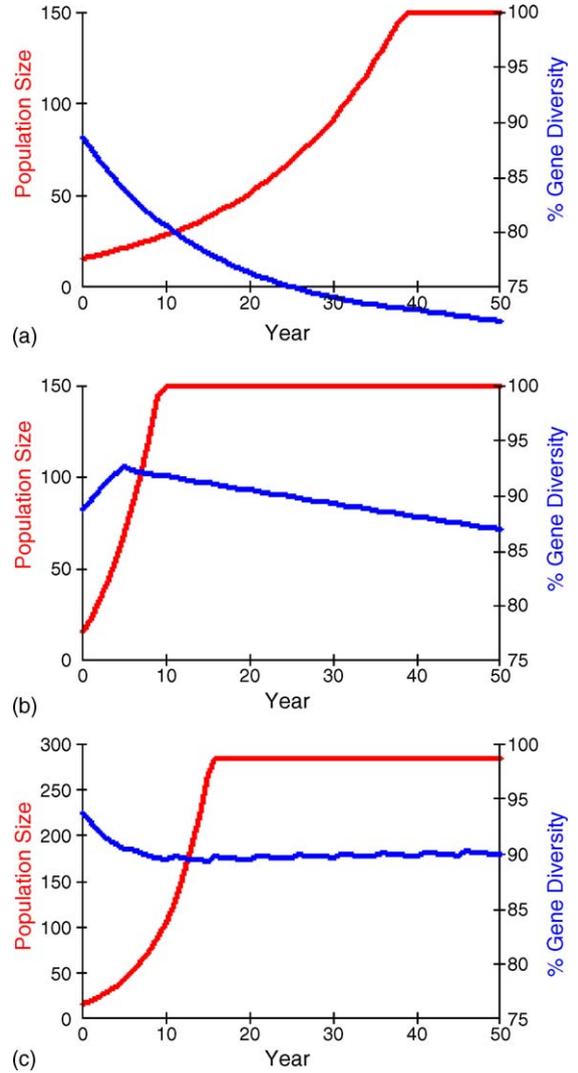


Fig. 3. Brazilian ocelot population model depicting changes in SSP population size and genetic diversity over 50 years with (a) no introduction of new founder genes, (b) introduction of 20 new founders in the first 5 years with rapid population expansion and (c) after initial founder introduction, periodic founder supplementation (one every 5 years) combined with expansion of population size from 150 to 300 individuals via cooperative management with Brazilian zoos.

with this significant genetic boost (to a projected GD of 95%), variation still is projected to decrease to 87% over the 50-year time period without the introduction of additional founders (Fig. 3b). However, if Brazilian ocelots in U.S. and Brazilian zoos are managed, as proposed by the BOC, as a larger metapopulation, available cage space will double from 150 to 300 spaces and the periodic incorporation of new founders (i.e., one every 5 years) from the wild population in Brazil might be expected. Under these circumstances, the SSP's

genetic goal (i.e., 90% GD over 50 years) can be achieved (Fig. 3c).

For all five small cat SSP species, modeling suggests that current populations are not genetically viable even over a relatively short time period of 50 years. Periodic introduction (or re-introduction) of genetic variability, longer generation intervals and increased population sizes will be required to maximize the survival prospects of these captive populations into the distant future. For all of these species, the importation of new founders from range countries has become problematic from both conservation and regulatory perspectives. Because each of these species is threatened to some degree with extinction in the wild, capturing wild-born animals as founders for zoo populations is no longer feasible nor desirable. For some cat species, wildlife laws and regulatory agencies now prohibit any international trade of wild-born individuals. As one alternative, the Fishing Cat and Ocelot SSPs have focused on developing captive-breeding programs in range countries to produce captive-born founders of each species as one component of their broader conservation programs [21,23,26]. Although this approach has merit, it does require substantial resource commitment that may be prohibitively expensive for all SSPs to duplicate and the long-term availability of new founders is still not assured.

One management strategy that is recommended to delay the loss of variation via genetic drift is to prolong the generation interval by postponing the age of first reproduction [14]. However, practical application of this approach is limited for many small felids because of their short life spans (<15 years), low reproductive output (1–4 kittens/litter) and early onset of reproductive senescence (~7 to 10 years of age). Similarly, expanding the number of cage spaces for small cats is difficult, although less than one-third of AZA-accredited institutions presently house even one of the five SSP species. The misperception that small cats make poor exhibit animals combined with competition for cage space with other charismatic small mammals likely will preclude any substantial growth in population sizes.

ART offers the potential means to address each of these management challenges [3,27]. Given the genetic realities of small population management, the application of ART might even be considered an absolute necessity if the small cat SSP species are to survive beyond the next 50–100 years in captivity. For some species, frozen sperm samples, from unrelated captive animals or wild populations, could serve as the source of new founder genes, provided that effective semen

collection, sperm cryopreservation and AI (or IVF/ET) protocols have been established [3,27,28]. The banking and periodic infusion of genetic material from new founders can have immediate and long-term benefits for maintaining genetic diversity [29,30].

With most small cats, the pedigrees are relatively shallow since captive populations were either just recently established (i.e., Pallas' cats), or are currently being created (i.e., Brazilian ocelots) or presently incorporating new founders into small existing populations (i.e., fishing cats and sand cats). In this situation, collecting and banking semen from all living male founders could help protect against genetic loss, especially if sperm donors should die before reproducing naturally [29,30]. Because founder females would not be banked in this scenario, their first generation male offspring also would need inclusion in the genome resource bank [29]. After banking, ART using frozen spermatozoa could ensure that all founders produce offspring and founder representation is equalized in future generations. Subsequent analysis of genetic interrelatedness would dictate which founder samples would require re-infusion into the captive population at later time points [29]. In our fishing cat example, ART would allow each of the nine new founder's genetic contribution to be balanced within the SSP population and permit the future introduction of new founders more frequently than is possible through captive-breeding programs in range countries alone. Sperm use frequency would depend on the amount of banked spermatozoa available from each founder balanced with the SSP's genetic needs [30], placing a premium on the economical use of this limiting resource. The greatest value of ART for improving gene flow may be in allowing semen to be collected and frozen from free-living founders for use with captive populations without requiring the removal of additional cats from the wild [27,30].

ART also has value for optimizing demographic parameters in species with small population sizes, such as Pallas' cats. In particular, generation interval may be extended indefinitely by sperm freezing, effectively allowing retention of higher GD levels with smaller population sizes [31]. For example, generation intervals for male Pallas' cats could be increased to 15 years by using their frozen spermatozoa while still allowing females to reproduce before the onset of reproductive senescence at 8 years of age. In our Pallas' cat model, a boost in the average generation interval to 10 years, along with other proposed demographic improvements, would be adequate to maintain 90% GD over 50 years without introduction of any new founders.

Lastly, ART could provide another avenue for establishing a genetically defined population for some species, such as Brazilian ocelots, as well as preserving the genetic contribution of female founders. In our ocelot example, generation of Brazilian ocelot IVF embryos in Brazil followed by cryopreservation and subsequent transfer into generic female ocelots in the U.S. would facilitate creation of a subspecific population of founders. This approach would reduce the need for extensive captive breeding in Brazil and the international transport of living ocelots between countries. An added benefit of embryo cryopreservation would be the capacity to bank down the female's genetic contribution, balance out female founder representation in the SSP population and routinely exchange genetic material with the Brazilian captive population through cooperative management between countries.

3. The reality of ART in small felids

Basic reproductive studies, using fecal hormone and semen analysis, have been initiated in each small cat SSP species to broaden our knowledge of species-specific reproductive physiology and help to improve captive-breeding success (ocelot [32,33], fishing cat [34], Pallas' cat [25,35], sand cat and black-footed cat; Herrick and Swanson, unpublished). A fundamental understanding of basic reproductive biology of each species is a mandatory prerequisite before applying ART. Although extrapolation from domestic cat studies provides some initial starting points, species differences are to be expected and can have a profound impact on the success of ART. For example, basic research, using fecal hormone analysis and periodic semen evaluation, demonstrated that Pallas' cats have an extreme reproductive seasonality controlled primarily by photoperiod and that altering light exposure during the winter months can disrupt the normal breeding season [25,35]. Accordingly, attempting semen collection, AI or ET in Pallas' cats during the non-breeding season is unlikely to be successful on a routine basis. Similarly, basic research studies have shown that, unlike most felids, fishing cats and margays are spontaneous ovulators [21,24], a phenomenon that can affect both the timing and nature of ovarian stimulation protocols. Lastly, basic research has shown that many small cats have high percentages of abnormal sperm forms and low sperm numbers per ejaculate [36,37]. Teratospermia adversely affects multiple aspects of sperm function and the recovery of small ejaculates limits sperm availability for use in assisted reproductive procedures [37,38].

For population management, the consistent, efficient production of viable offspring via AI or ET procedures is a necessity. Among felids, AI has been more widely applied than ET, with offspring being produced in nine cat species to date [4,39]. Improved success with AI followed development of a laparoscopic insemination technique that permitted visualization of the ovarian response following gonadotropin treatment and direct deposition of spermatozoa into the cranial uterine horns [4,39]. Efficacy in most species, however, has been low with pregnancy percentages following AI typically less than 10% and survivorship of offspring frequently poor due to intrinsic health problems, or maternal aggression and neglect.

Among small cats, viable offspring have been produced after AI with freshly collected and/or frozen-thawed spermatozoa in the ocelot, leopard cat and tigrina, but sample sizes have been too small to determine technique efficacy [39–41]. Genetic management of all five small cat SSP species could benefit from effective AI procedures, but immediate application is probably most relevant for three species: the fishing cat, sand cat and black-footed cat. These species have small founder and population sizes but, based on projected cage space and demographics, have the capacity for fairly rapid expansion. The recent importation of captive-born fishing cat and sand cat founders from range countries should boost genetic diversity over the next few years [20,41], but AI may be necessary to ensure that each founder contributes multiple offspring to the next generation and to provide continual genetic supplementation over subsequent decades.

AI research is progressing in the fishing cat [42] but there are no published studies of AI in sand cats or black-footed cats. A significant barrier to applying AI effectively in small cats is the poor survivability of cat spermatozoa following cryopreservation and thawing [43], especially given the low sperm numbers typically recovered in small cat ejaculates. Although AI may be preferable for its relative simplicity [28], the availability of viable spermatozoa as a limiting resource may favor application of IVF and ET as an alternative.

Embryo transfer, using *in vitro* derived embryos, has been investigated to a limited extent in non-domestic cats, with pregnancies reported in three small cat species—the ocelot, wild cat and caracal [5,26,44]. Small sample sizes, to date, preclude making any conclusions about ET efficacy. Standard IVF techniques appear to have broad cross-species applicability with embryos being produced using

freshly collected or frozen-thawed spermatozoa in all five small cat SSP species ([5,26]; Swanson, unpublished data). Among the SSP species, most research to date has been conducted in the ocelot [26], including two pregnancies established following laparoscopic transfer of frozen-thawed ocelot embryos and the cryopreservation of ~80 Brazilian ocelot IVF embryos, representing 13 founders for the SSP population. As one component of the BOC, half of these embryos are to be imported into the U.S. to help create a Brazilian ocelot founder base for the SSP population. For ocelots and sand cats, the existence of surplus generic or sub-specific hybrid females within zoos provides a ready source of conspecific recipients for ET. The three other small cat SSPs all manage fertile females that are not recommended for breeding due to genetic over-representation but are fully capable of serving as ET recipients.

4. Research priorities for applying ART

Advancing ART from potential to reality for small cats still requires significant advances in our knowledge and understanding of felid reproduction. The following four areas represent priorities for immediate research.

4.1. Ovarian stimulation and recipient synchronization

AI, oocyte recovery and recipient synchronization procedures require exogenous gonadotropin treatment of females to induce follicular growth, oocyte maturation and/or ovulation. These exogenous gonadotropins, as large foreign glycoproteins, may persist for days in circulation, inducing neutralizing antibody responses and aberrant maternal environments [45–47]. Isolation, characterization and production of recombinant felid-specific gonadotropins could eliminate these complications [48]. In addition, gonadotropin treatments often are applied without regard to ovarian cyclicity status, resulting in inconsistent responses, especially in spontaneously ovulating species. Further studies into down-regulation of ovarian activity prior to gonadotropin treatment are needed [49,50].

4.2. Sperm and embryo cryopreservation

The routine use of AI or IVF/ET procedures for population management requires freezing of spermatozoa and embryos for transport and storage [27]. Very

few detailed studies of sperm cryopreservation have been conducted in any cat species and sperm viability and acrosome integrity after thawing remain low [43]. Similarly, there have been no systematic studies of embryo cryopreservation in any non-domestic cat species. Given the multiple variables that can affect viability of frozen spermatozoa and embryos [51–53], investigation of sperm and embryo cryopreservation in small felids should receive greater emphasis.

4.3. Embryo culture systems

Cat embryos frequently are cultured in media that have been developed for embryos of other species or other cell types [5,54], possibly compromising their *in vivo* viability. The ability to transfer IVF-derived embryos more successfully may depend on systematically refining media and culture systems to support felid-specific embryo metabolic needs [55,56]. Development of a defined, optimized culture medium for felid embryos should be a priority.

4.4. Fetal and neonatal viability

One unknown factor associated with AI or ET in felids is the potential impact on fetal and neonatal survival [57]. Efficient application of ART requires that pregnant females carry fetuses to term, give birth naturally and then raise healthy offspring with minimal human intervention. Non-invasive methods for early pregnancy diagnosis and fetal monitoring are needed in cats in addition to studies assessing neonatal health at parturition and during post-partum growth.

5. Conservation challenges

Beyond these research priorities, there are several other challenges that must be addressed for ART to play a meaningful role in small felid conservation. First, development and application of ART can be an expensive proposition, considering the costs of research staff salaries, laboratory facilities, scientific equipment and supplies, animal colony maintenance and travel. Government and university-based funding typically has been scarce for any reproductive research involving non-agricultural or non-traditional laboratory animal species. Adequate funding must be available to support both basic and applied reproductive studies in felids. Second, the relatively long time frame needed for improving the efficiency of ART, a by-product of limited research funding and few laboratories studying these species, is a growing concern. With wild felid

populations disappearing and importation of new founders becoming unattainable, technology development must take on a greater sense of urgency.

Third, the routine application of ART on an international basis will require substantial improvement in the basic infrastructure, husbandry and management of zoos in most of the small cat range countries. Capacity building in zoo biology and reproductive sciences is of critical importance in integrating ART into global species management programs [58]. Lastly, international conservation programs incorporating ART depend on the exchange of genetic resources among countries and can be derailed quickly by politics, provincialism and short-sighted government regulations. Developing conservation partnerships, such as the BOC, ensures that all parties benefit from program activities and that the preservation of the species in question remains the central focus of all participants.

In conclusion, ART represents a peripheral but critically important component of the broad-based, multi-disciplinary conservation efforts that will be necessary to ensure the long-term survival of small-sized felids in captivity and the wild. In the near term, ART does offer the potential to substantially benefit the captive management of SSP populations, provided that research priorities and other challenges are adequately addressed and technique efficacy is improved within realistic time frames. In reality, our prospects for ensuring the survival of small felids into the future would be significantly enhanced by the effective use of ART, but conversely, would be greatly diminished by our failure to achieve that goal.

Acknowledgements

The author thanks the studbook keepers and SSP coordinators for each of the small cat SSP species (ocelot: Nanette Bragin and Ken Kaemmerer; fishing cat: Lynda Roberts; Pallas' cat: Dr. Martha Caron; black-footed cat: Liz Harmon and Steve Wing; sand cat: Kim Clark and Kara Akers) for their efforts and dedication. My appreciation also is extended to Dr. Victor Shille for his pioneering reproductive studies in domestic cats that laid the foundation for much of the ongoing research with endangered felids.

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