

# Research in Nondomestic Species: Experiences in Reproductive Physiology Research for Conservation of Endangered Felids

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## Abstract

Tremendous strides have been made in recent years to broaden our understanding of reproductive processes in nondomestic felid species and further our capacity to use this basic knowledge to control and manipulate reproduction of endangered cats. Much of that progress has culminated from detailed scientific studies conducted in nontraditional laboratory settings, frequently at collaborating zoological parks but also under more primitive conditions, including in the field. A mobile laboratory approach is described, which incorporates a diverse array of disciplines and research techniques. This approach has been extremely useful, especially for conducting gamete characterization and function studies as well as reproductive surveys, and for facilitating the development of assisted reproductive technology. With continuing advances in assisted reproduction in rare felids, more procedures are being conducted primarily as service-related activities, targeted to increase effectiveness of species propagation and population management. It can be a challenge for both investigators and institutional animal care and use committees (IACUCs) to differentiate these service-based procedures from traditional research studies (that require IACUC oversight). For research with rare cat species, multi-institutional collaboration frequently is necessary to gain access to scientifically meaningful numbers of study subjects. Similarly, for service-based efforts, the ability to perform reproductive procedures across institutions under nonstandard laboratory conditions is critical to applying reproductive sciences for managing and preserving threatened cat populations. Reproductive sciences can most effectively assist population management programs (e.g., Species Survival Plans) in addressing conservation priorities if these research and service-related procedures can be conducted “on the road” at distant national and international locales. This mobile laboratory approach has applications beyond endangered species research, notably for other scientific fields (e.g., studies of hereditary disease in domestic cat models) in which bringing the laboratory to the subject is of value.

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## Introduction

Nondomestic cats are among the most biologically imperiled taxa on earth, and numerous species are believed to be threatened with extinction in their natural environment. All 36 nondomestic felid species are included on Appendix I or II of the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES<sup>1</sup>) (CITES 2003), and 16 species or subspecies are listed as endangered or threatened by the US Fish and Wildlife Service (USFWS 2003). Habitat loss due to human population growth and resource consumption is the main cause of endangerment, and direct killing for predator control is also a factor for some species. The fur trade and poaching for use in traditional Oriental medicine play a lesser role for most species (Nowell and Jackson 1996). Conservation of wild cats is dependent on preserving adequate habitats, populations, and genetic variation in spite of ever-mounting societal pressures. Captive populations of nondomestic felids are valuable as educational, scientific, and genetic resources that can be used to support conservation of wild cats. However, efforts to conserve and manage both captive and wild cat populations frequently are hindered by a poor understanding of felid reproductive biology and the intrinsic and extrinsic factors that may influence reproductive success.

Reproductive research with nondomestic cats can provide insight and direction into establishing more effective genetic and conservation management programs. Reproductive studies in cats typically involve investigation of gamete biology, embryology, endocrinology, and cryobiology. These studies use techniques such as electroejaculation for semen collection, artificial insemination (AI<sup>1</sup>), in vitro fertilization (IVF<sup>1</sup>) and embryo culture, embryo transfer (ET<sup>1</sup>),

<sup>1</sup>Abbreviations used in this article: AI, artificial insemination; AZA, American Zoo and Aquarium Association; BW, body weight; CITES, Convention on International Trade of Endangered Species of Wild Fauna and Flora; ET, embryo transfer; IACUC, institutional animal care and use committee; IVF, in vitro fertilization; MPS, mucopolysaccharidosis; SMA, spinal muscular atrophy; SSP, Species Survival Plan.

reproductive hormone analysis, and cryopreservation of sperm, oocytes, and embryos. In most cases, extrapolation of reproductive research techniques from domestic cats to nondomestic felids has been straightforward from a technical perspective (given similarities in basic cat anatomy), but efficacy has varied considerably due to pronounced species specificities.

Many studies with nondomestic cats cannot be performed at one fixed location in proximity to an established reproductive physiology laboratory but instead, require multi-institutional collaboration on a local, national, or international level. This situation creates major challenges for the various institutions that might be involved to coordinate oversight of the research projects. Uniformity of techniques and technology, which is a key requirement in this type of research, has generated the need to develop a strategy to transport and establish a temporary standardized laboratory at each collaborating institution or, in essence, to create a “mobile laboratory” approach to science. This mobile laboratory strategy is also applicable to other disciplines such as phylogenetics and nutritional and veterinary research, which may be conducted concurrently with reproductive studies and which serve to maximize the acquisition of knowledge from each individual anesthetic event.

## **Advantages of a Mobile Laboratory Research Approach**

### **Increasing Sample Sizes**

For US zoos (~200 institutions) belonging to the American Zoo and Aquarium Association (AZA<sup>1</sup>), the composition of their felid collections are dictated primarily by a Regional Collection Plan established by the AZA. Due to space limitations in zoos, total population sizes for a species in a given region (e.g., North America) may number from a few up to several hundred individuals. Typically, each zoo exhibits one or two animals of a particular species and very rarely has more than four or five cat species in total. With few exceptions, individual zoos lack enough specimens of a given species to provide adequate sample sizes for intensive empirical research. Even when cat numbers at a given zoo are more robust, limitations regarding the frequency of anesthesia, surgery, and/or exogenous hormone treatment reduce opportunity for hands-on study. Under these circumstances, the ability to conduct research at multiple institutions using a mobile laboratory approach becomes invaluable.

### **Supporting Regional and Global Population Management Programs**

Most felid species maintained within AZA institutions are managed by Species Survival Plans (SSPs<sup>1</sup>), by which staff use standardized computer programs to analyze pedigrees

and demographics, recommend proper pairings to maximize genetic variability, and develop master plans for long-term population survival (Ballou and Foose 1992). SSPs currently exist for the following 11 cat species: tiger, lion, jaguar, snow leopard, clouded leopard, cheetah, ocelot, fishing cat, sand cat, Pallas’ cat, and black-footed cat. These SSP species will likely receive the greatest benefit from the conservation efforts of reproductive sciences, if applied appropriately.

Basic knowledge derived from mobile laboratory studies provides the groundwork to improve natural breeding of captive populations. Characterization of reproductive traits in captive and free-living individuals helps to establish reproductive norms on both the species and population level, pinpointing species differences and similarities and establishing benchmarks for comparative analyses. Similarly, reproductive surveys generate a vast amount of normative data that provide SSP coordinators and curators with a broad overview of the reproductive status of their managed populations and suggest direction for altering management to improve breeding success. Concurrent genetic, nutritional, or veterinary surveys can provide additional information that is of tremendous value to population managers.

Assisted reproductive technology also may play a significant role in population management (Wildt and Roth 1997). Movement of germ plasma as frozen spermatozoa and embryos is preferable to transporting easily stressed nondomestic animals over vast distances. With proven protocols in place for cryopreserving gametes and embryos and conducting artificial insemination or embryo transfer, it becomes feasible to augment natural breeding with assisted reproduction (Ballou 1992). However, the effective use of this technology as a management tool requires that the most genetically valuable individuals be accessible across holding institutions or countries. In US zoos, because SSP coordinators have played a key role in encouraging participation in these studies, significant progress has been made for some cat species in a relatively short time. A logical extension of using assisted reproduction within zoo collections is to establish routine genetic exchange between *ex situ* and *in situ* populations, primarily through the periodic infusion of genetic material (in the form of frozen spermatozoa) from wild cats into the captive population. This approach would support maintenance of adequate genetic variation without the need to remove more wild cats from nature for zoos (Wildt 2000).

A challenging issue regarding the oversight of institutional animal care and use committees (IACUC<sup>1</sup>s) arises as staff of regional and global management programs begin to apply assisted reproductive technology as management tools. Specifically, at what point do these procedures represent traditional research, and when do they become service efforts (equivalent to clinical intervention at the population level)? It appears logical that applying an established technique with a primary goal of producing offspring or improving genetic management of a population should be considered a service activity. In contrast, experimental pro-

cedures performed with a primary goal of generating data to test a hypothesis and possibly improve technique efficiency should remain classified as research studies with continued IACUC oversight.

## Capacity Building in Developing Countries

A critical component of international studies has been establishing collaborations and training programs for resident scientists in developing countries (Swanson and Brown 2002). Generally, these collaborations are initiated through the national universities (because most foreign zoos lack independent research programs), but all of the hands-on research with nondomestic cats still occurs in the zoos. Technology transfer and scientific training are key to expanding the scope of cat research, especially in creating a collaborative network of international scientists. Capacity building in reproductive sciences also influences developing strengths in other disciplines, such as population management. With a compatible worldwide network of trained researchers and managers, it becomes easier to establish global conservation programs for many cat species. The ability to conduct research in nontraditional settings liberates scientists from the laboratory confines, which, in turn, empowers population managers to begin applying these emerging tools between regions, whether in Cincinnati or at a small zoo in Brazil. With appropriate scientific expertise available in each country, building connections between captive and wild populations also becomes more realistic.

However, these broad collaborations can present a very real bureaucratic challenge to the scientist involved and for the IACUC responsible for overseeing the project. IACUC oversight would normally be the responsibility of the principal scientist's home institution; however, on-site inspections, among other aspects, would be beyond the reasonable purview of most institutions. In such a situation, the use of a mobile laboratory system provides another obvious advantage. Although on-site inspection may be impractical across multiple institutions, examination of the portable laboratory setup itself can serve to help IACUC members appreciate the quality and care of research that will be conducted at very remote sites.

## Managing Domestic Cat Models of Hereditary Disease

The domestic cat serves as a research model for systematic, laboratory-based reproductive studies that have contributed significantly to our general understanding of felid reproduction and assisted reproductive technology. Without the knowledge derived from domestic cat studies, it is unlikely that substantial progress would be possible in studying reproduction of nondomestic felids. In turn, the mobile laboratory approach developed for nondomestic cat research can benefit the management of laboratory cat colonies. In this

regard, nondomestic felids might be considered reproductive research models for some laboratory cat populations, most notably for cat models of hereditary disease.

Domestic cats represent research models for the study of at least 18 heritable metabolic defects that are homologous to human inborn errors (Migaki 1982). Most of these diseases involve single gene mutations and are inherited as autosomal recessive traits. Clinical symptoms and disease progression in affected cats frequently are markedly similar to those factors in affected humans, and these disease models have proven invaluable for investigating the molecular basis of disease, pathogenesis, and the use of ameliorative or corrective therapy (Haskins and Giger 1997). For feline models of human genetic disease, the applied advantages of IVF, ET, and embryo cryopreservation for propagating, managing, and preserving populations are immense. With embryo cryopreservation, it is easier to move models between institutions or even countries, to maintain them indefinitely within liquid nitrogen tanks thereby reducing costs for colony maintenance and allowing financial resources to be redirected toward other aspects of model research. Scientists also gain greater control over experimental design, because they are able to schedule timing of pregnancies more precisely and to produce litters consisting entirely of individuals heterozygous or homozygous for the genetic defect.

Application of these techniques already has proven feasible in studies with cat models for mucopolysaccharidosis (MPS<sup>1</sup>) and spinal muscular atrophy (SMA<sup>1</sup>). Both MPS-homozygous and MPS-heterozygous kittens have been produced after transfer of frozen-thawed embryos created using a mobile laboratory approach with the MPS model cat colony at the University of Pennsylvania (Swanson et al. 2000). Similarly, the mobile laboratory was essential in establishing a new cat model for SMA at Michigan State University, allowing sperm collection and cryopreservation from a SMA-homozygous male, generation of SMA-heterozygous IVF embryos, and subsequent transfer to produce viable SMA-heterozygous offspring (W. F. Swanson, unpublished data).

## Examples of Basic Research and Applied Service Intervention Outside the Laboratory

### Assessing Reproductive Traits and Sperm Function

Electroejaculation of anesthetized males for collection and evaluation of semen is one of the most commonly used reproductive techniques with nondomestic felids. The same basic approach used in the domestic cat, applying low-voltage transrectal stimulation with a three-electrode rectal probe, is effective for collecting semen from most, if not all, cat species (Figure 1). Because equipment requirements are minimal (Figure 2a), electroejaculation represents a basic



**Figure 1** Electroejaculation of an ocelot for semen collection and evaluation at a Texas zoo.

component of many collaborative research studies conducted outside the laboratory, including under field conditions. Recovered semen may be evaluated for volume, presence or absence of sperm, and sperm concentration, motility, and morphology. These semen parameters may be compared among individuals and species to assess the basic reproductive physiology and reproductive status of different species or populations. For example, electroejaculation of cheetahs, lions, pumas, and jaguars has allowed comparison of basic seminal traits in captive individuals with traits of their free-living wild counterparts.

Among other results, these studies have established that cheetah sperm quality (which includes a very high incidence of teratospermia) is similar among wild and captive cats, a finding coincident with limited genetic variation characteristic of both populations (O'Brien et al. 1983; Wildt et al. 1987a). Similarly, lion populations in Asia and Africa and puma populations in North and South America display pronounced differences in ejaculate quality, also possibly linked to limited genetic variability among certain isolated populations (Barone et al. 1994; Wildt et al. 1987b). Finally, captive jaguars in Brazil were shown to exhibit inferior reproductive parameters compared with wild jaguars, a difference possibly attributable to captive husbandry conditions, although genetic factors cannot be ruled out (Morato et al. 2001).

Semen recovered using a mobile laboratory approach also may be used to investigate sperm function and gamete interaction. In the most simple form, semen is diluted with culture medium, centrifuged, and then resuspended in alternative media formulations. Sperm parameters (motility, viability, acrosome status) may be assessed over time in samples suspended in buffered medium on a slide warmer or in a portable CO<sub>2</sub> incubator (Figure 2c). Variation in sperm motility or viability among species may be indicative of specificities in physiology that may affect sperm processing methods. For example, in snow leopards, studies revealed that spermatozoa were hypersensitive to the alkaline

pH of the standard culture medium, a trait not observed with any other cat species (Roth et al. 1996).

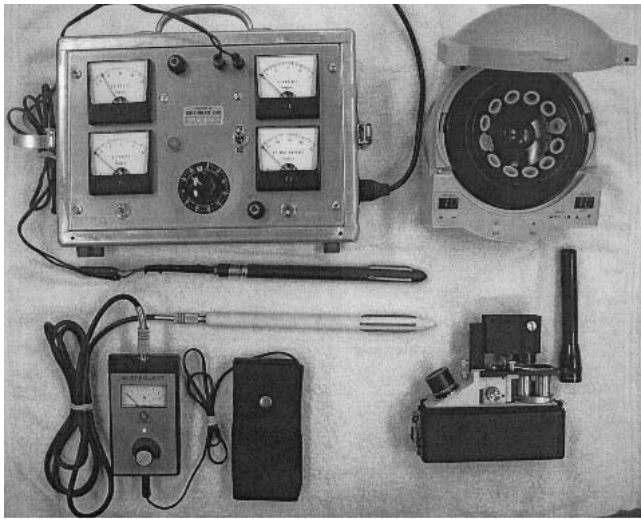
Additional information may be gained by the evaluation of sperm capacitation (assessed via calcium-ionophore-induced acrosome reactions or changes in tyrosine phosphorylation) and gamete interaction (using salt-stored domestic cat oocytes or viable conspecific oocytes). Fixation of incubated spermatozoa at set time points allows later assessment of acrosome status, protein phosphorylation, or sperm penetration at a full-service laboratory. This approach has provided insight into the impact of teratospermia on sperm function in several species, including the cheetah and clouded leopard (Pukazhenthil et al. 1998, 2001).

## Reproductive Surveys

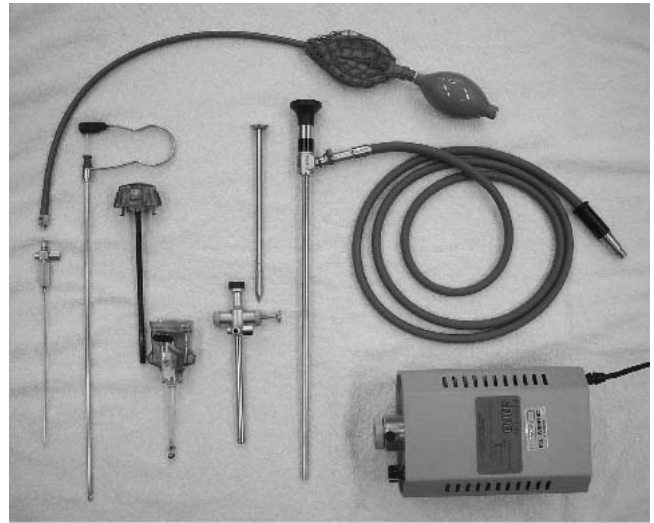
Reproductive surveys of captive populations that are maintained at multiple institutions allow one-time assessments of large numbers of individuals to clarify reproductive potential, identify causes of subfertility, and help correct underlying management problems. Unlike research studies conducted to assess basal reproductive traits and sperm function, these broader surveys are usually more aligned with clinical service efforts that are designed to provide guidance in improving reproductive efficiency in the targeted species. In these surveys, reproductive assessments may include gathering of historical data from zoo records on reproductive success and husbandry, measurement of testicular volume, collection and analysis of semen (including evaluation of sperm concentration, motility, morphology, acrosomal status, and viability), collection of blood for subsequent analysis of serum hormone concentrations (testosterone, estradiol, progesterone, cortisol), and laparoscopy to evaluate ovarian activity and reproductive anatomy. In many instances, blood and skin samples also are collected and processed for veterinary and phylogenetic studies.

In the initial application of the survey approach in US zoos, reproductive assessments (including electroejaculation) were performed on 95 males representing 28 nondomestic cat species (Howard et al. 1984). Pronounced variation was observed in reproductive traits across species, especially in average percentages of pleiomorphic spermatozoa. These results helped to establish the first substantive database of "normal" reproductive parameters in multiple cat species (Howard 1993). In another survey, the reproductive status of 128 cheetahs in 18 North American zoos was evaluated as part of an investigation of low reproductive efficiency among captive cheetah populations (Wildt et al. 1993).

In addition to semen collection of 60 males, laparoscopic evaluation of 68 females was conducted during the survey. Findings established a broad database of physiological information about this species and revealed the existence of uniform teratospermia among males and minimal ovarian activity among many females, helping to determine priority areas for future investigation. However, the broader goal of the survey was to diagnose the cause of poor reproductive



a



b



c



d



e

**Figure 2** Basic equipment of a mobile laboratory. (a) Semen collection, evaluation, and processing (left to right): AC-powered and DC-powered electroejaculators, microcentrifuge, hand-held phase-contrast microscope. (b) Laparoscopy (left to right): bellows with Veress needle, grasping forceps, trocar/cannula units, laparoscope, fiberoptic cable, light source. (c) In vitro fertilization and embryo culture (left to right): gas flow meter, gas washing bottle with slide warmer, portable incubator, compressed gas (5% CO<sub>2</sub> in air) cylinder. (d) Embryo cryopreservation (left to right): DC-power source, controlled-rate freezer, liquid nitrogen bath with heating unit. (e) Gas anesthesia (left to right): nonbreathing circuit with face mask and gas scavenger canister, oxygen flow meter, isoflurane vaporizer, compressed oxygen tank with regulator.

efficiency in the species and to identify appropriate corrective measures.

As another example of a reproductive survey that could be classified as a clinical service effort, reproductive evaluations were conducted on 185 male cats representing eight endemic Latin American species housed at 44 zoos in 12 Latin American countries (Swanson et al. 2003). Almost all of these cats were wild-born individuals, representing an extremely valuable population genetically. However, the survey revealed that most of these males (~80%) had never reproduced in captivity, and the majority had very low sperm counts in their ejaculates. Two factors—nutrition and exhibitry—were determined to be the most important affecting reproductive status and to represent deficiencies that are easily corrected by zoos intent on improving breeding success. In subsequent studies with ocelots, margays, and tigrinas in Brazilian zoos, provision of adequate diets and appropriate exhibits resulted in pronounced improvements in sperm recovery, percentage of normal sperm morphology, ovarian responsiveness to exogenous gonadotropins, and fertilization of recovered oocytes (Morais et al. 2002; Swanson et al. 2002).

### Developing Assisted Reproductive Technology

The application of assisted reproduction, such as AI using frozen-thawed spermatozoa, can provide significant advantages for managing the genetic composition of populations. In cats, AI typically is conducted using laparoscopy, a minimally invasive surgical technique that allows the sperm to be deposited in the cranial uterus near the oviducts while ensuring that the female has ovulated after hormone treatment. This technique has been used extensively in collaborative studies at multiple institutions, in part because minimal equipment is required (Figure 2b) and the surgery is minimally traumatic, reducing infection risk and recovery time. Insufflation of the abdominal cavity with room air using a bellows eliminates the need for a bulky mechanical insufflator and gas cylinder, and no evidence of increased infection risk has been reported (based on hundreds of laparoscopic procedures). Since this AI technique was developed in the domestic cat (Howard et al. 1992), it has been applied successfully to produce offspring in the following eight nondomestic cat species: tiger, cheetah, snow leopard, puma, clouded leopard, ocelot, tigrina, and leopard cat. Three species (leopard cat, ocelot, and cheetah) were produced after insemination with frozen-thawed spermatozoa (Wildt and Roth 1997). Developing AI protocols in each species involves conducting traditional research studies. However, as these techniques become well established, their use will become more prevalent in clinical situations as a service provided for propagation and population management.

IVF and ET, especially in conjunction with embryo cryopreservation, offer other potentially valuable tools for felid conservation. With a laparoscopic technique, oocytes



**Figure 3** Ocelot kitten produced after laparoscopic oviductal transfer of frozen-thawed embryos.

may be recovered for IVF after direct aspiration of ovarian follicles (Goodrowe et al. 1988). A mobile laboratory approach, using laparoscopic follicular aspiration and IVF in a portable incubator system, has allowed production of embryos in seven species (tigers, cheetahs, pumas, ocelots, tigrinas, Geoffroy's cats, Pallas' cats) (Swanson and Brown 2002; Swanson et al. 2002; Wildt and Roth 1997; unpublished data). Earlier ET procedures relied on a more invasive laparotomy to deposit the embryos into the oviduct or uterus, which reduced applicability across institutions and increased risk of abdominal adhesions. Recently, a laparoscopic technique for oviductal ET developed in domestic cats (Swanson et al. 2001) has been used successfully to produce two litters of ocelot kittens from frozen-thawed embryos (Figure 3). As with AI, IVF and ET techniques eventually will make the transition from research applications to service procedures with more routine clinical use.

Cryopreservation methods for spermatozoa have been readily adaptable to a mobile laboratory approach. Cat sperm cryopreservation typically involves diluting the sample in a cryoprotectant solution (containing egg yolk and glycerol), cooling it in a refrigerator or ice chest, and then "pelleting" the sperm into indentations on dry ice

(Howard 1993). Alternatively, sperm may be placed in plastic freezing straws, sealed, and frozen suspended over liquid nitrogen vapor. Frozen samples may then be placed into liquid nitrogen dry shippers for transport. After thawing, spermatozoa may exhibit substantial acrosome damage but still have adequate viability to fertilize oocytes *in vitro* (as shown in the domestic cat, tiger, caracal, ocelot, Geoffroy's cat, and Pallas' cat) (Donoghue et al. 1992; Pope 2000; unpublished data) or to produce offspring with AI (as shown in the domestic cat, ocelot, leopard cat, and cheetah) (Wildt and Roth 1997).

Routine cryopreservation of spermatozoa is easily incorporated into mobile laboratory studies. For example, in the reproductive survey of Latin American felids, 63 ejaculates were of adequate quality for cryopreservation and potentially provided enough sperm for 100 AI or several thousand IVF procedures (Swanson et al. 2003). In studies in cheetahs, semen collected from wild males temporarily housed in captivity in Namibia was cryopreserved, transported to the United States, and then used to inseminate and produce offspring in captive cheetahs in US zoos (Howard et al. 1997). Ongoing studies with jaguars in Brazil (R. Morato, University of São Paulo, personal communication, 2002) and ocelots in Mexico (W. F. Swanson, unpublished data) are testing the feasibility of capturing free-ranging cats in remote habitat areas (in conjunction with radiotelemetry studies) and cryopreserving recovered semen, followed by the immediate release of the sperm donor after anesthetic recovery. In these studies, blood samples from most cats are screened for feline viral exposure (e.g., immunodeficiency virus, leukemia virus, corona virus) to reduce the low probability of disease transmission in frozen-thawed samples further.

Embryo cryopreservation, like sperm freezing, requires minimal equipment (Figure 2d) as a component of a mobile laboratory, and a source of liquid nitrogen is easily located in most countries. To date, offspring have been produced after transfer of frozen-thawed embryos in only a few cat species (wild cat, caracal, and ocelot) (Pope et al. 2000; C. E. Pope, Audubon Zoo, New Orleans, Louisiana, personal communication, 2002; Swanson 2003). Currently, application of embryo cryopreservation and transfer as a management tool is being explored in cooperation with the Ocelot SSP. US zoos are attempting to establish a genetically defined ocelot population in captivity and are focusing on the ocelot subspecies (*Leopardus pardalis mitis*) native to southern Brazil. In collaborative studies in Brazilian zoos, ocelot embryos have been generated using mobile laboratory techniques and cryopreserved for later transport to the United States. To date, more than 80 embryos have been frozen, and this number represents 15 founders for the US population (Swanson 2003). Importation into the United States and transfer of these frozen embryos to produce viable kittens will assist the Ocelot SSP in addressing one of its most pressing conservation needs. As with other assisted reproductive techniques, the classification of cryopreservation procedures as primarily research or service oriented

will change as the technology becomes more efficient and is applied routinely as a population management tool.

## Logistics of Going Mobile

### Travel

Only a few US zoos have state-of-the-art reproductive physiology laboratories, therefore research at most collaborating zoos must be conducted in alternative venues (usually the institution's veterinary hospital). Although these facilities typically provide adequate resources for anesthesia, animal monitoring, and surgical procedures, this arrangement does require transport and assembly of most research equipment to establish an auxiliary laboratory. For many zoos in developing countries, veterinary facilities are frequently rudimentary and lack basic anesthetic equipment, which necessitates transport of both necessary veterinary and research resources. For institutions in close proximity (i.e., driving distance) to an established laboratory, this situation does not present an undue burden. However, for more distant institutions, air travel is required and a premium is placed on research equipment that is small, light-weight, and durable (Figure 2a-e).

For most air travel, equipment may be transported without damage if it is first wrapped in protective padding, (e.g., bubble wrap), placed in hard-sided cargo containers, and checked onto airlines as personal baggage. Most airlines regulate the number, size, and weight of containers; however, in some countries (e.g., Brazil), the airlines consider only weight. Because of recent incidents of domestic and international terrorism, heightened airline security has resulted in stricter limitations on carry-on luggage and routine screening of checked baggage. As a consequence, transporting complex electronic equipment in checked baggage or carry-on luggage has become more challenging.

Before scientists travel to foreign destinations, they should consider registering research equipment with the US Customs Service. Registration reduces the likelihood of import tariffs or confiscation upon return, and it documents for foreign customs officials the intent to return the equipment to the United States. A certificate of registration and a letter of invitation/explanation from collaborators in the native language are frequently sufficient to obtain entry into foreign countries, although exceptions exist. If in doubt, travelers should contact the foreign customs service before arriving in a country with crates of valuable research equipment.

### Building Trust with Capacity

The success of efforts to expand scientific capacity in foreign countries and conduct collaborative studies abroad through a mobile laboratory approach ultimately depends on two factors: identifying highly motivated, focused collabor-

rators, and building trust with those individuals. In many developing countries, limited financial resources and other societal factors may represent nearly insurmountable barriers to conducting scientific research. Accordingly, it becomes critical that scientific trainees and collaborators have an irrepensible drive to learn and apply new skills in completing their research studies, especially when their North American or European colleagues may reside thousands of miles away. The importance of building trust with foreign colleagues cannot be overemphasized. The era of “extractive” science thankfully has passed. In the past, North American or European scientists traveled to foreign countries, collected their samples with little local involvement, and returned home to analyze and publish their data. In the 21st century, sensitivity and respect for different cultures, sharing of resources and data, and working collaboratively and collectively in establishing global scientific networks are of paramount importance, especially in the field of conservation.

## Veterinary Considerations

Felids can be safely anesthetized with a wide variety of agents, which facilitates routine anesthesia as a standard component of research studies or service activities conducted at other institutions or in the field. Depending on the situation, nondomestic felids can be anesthetized using injectable anesthetics alone, induced with an injectable anesthetic and maintained with inhalant anesthesia, or induced and maintained with inhalant anesthesia alone. With any anesthetic procedure, there is an inherent risk of fatality; however, mortalities are extremely rare in healthy domestic and nondomestic cats with proper anesthetic monitoring. A properly trained veterinarian must be available to initiate and monitor anesthetic procedures. If the attending veterinarian at a collaborating institution has limited or an unknown level of expertise, it is preferable to communicate with the attending veterinarians carefully and to involve a skilled veterinarian who has had considerable experience anesthetizing the species as part of the research or service team.

Although most interspecies differences are minimal, it may be difficult to select an appropriate anesthetic agent for certain species. For semen collection procedures in most felids, tiletamine-zolazepam (2-7 mg/kg body weight [BW]<sup>1</sup>, depending on the species) has been the agent of choice in providing adequate relaxation and analgesia while maintaining laryngeal reflexes and smooth muscle tone. Exceptions exist for three species: the tiger, which may display grand mal seizures, re sedation, and hind limb paresis after apparent anesthetic recovery; and the jaguarundi and puma, which typically do not reach an adequate plane of anesthesia with tiletamine-zolazepam alone (Swanson et al. 2003). In some species (e.g., serval and Pallas' cat), a combined regimen of ketamine (2-4 mg/kg BW) and medetomidine (0.02-0.04 mg/kg BW), with or without butorphanol (0.2-0.4 mg/

kg BW), provides adequate anesthetic depth for electroejaculation, although supplementation with low amounts of isoflurane (0.5-1%) often is necessary. This regimen is rapidly reversible with atipamezole (0.2 mg/kg BW), which provides an added margin of safety and avoids the prolonged recovery that may occur with other anesthetics.

Inhalation anesthesia is generally safe across cat species, although some procedures (e.g., laparoscopy) may compromise respiration when the patient is placed in an inclined position to improve visualization of intra-abdominal organs. For example, snow leopards frequently become apneic under inhalation anesthesia and laparoscopy, which necessitates positive pressure ventilation throughout the procedure. For small-sized cats, inhalation anesthesia may be provided conveniently using a nonbreathing system. This technique simplifies the equipment requirements and requires only a vaporizer, oxygen flow meter, regulator, and disposable gas scavenger canister (Figure 2e). Compressed oxygen cylinders are readily available in most cities and countries through gas supply companies and can be preordered for delivery to the collaborating institution. More detailed information on anesthesia of felids is available in the literature (Fowler 1995; Nielsen 1999).

## Regulatory Oversight

Several regulatory authorities have jurisdiction over activities conducted with endangered cat species (Garbe 1990). In the United States, the Fish and Wildlife Service (USFWS<sup>1</sup>) and/or the various state wildlife departments regulate research access to endemic wild cat populations. With captive populations in US zoos, scientific studies typically do not require approval or individual permits from federal or state authorities. Internationally, regulatory authorities may vary or even be nonexistent, yet it is the responsibility of the researcher to identify the relevant agencies and regulations. Note that violation of any foreign wildlife law is illegal under US federal statutes (the Lacey Act). Among national and international regulations, the Endangered Species Act and CITES are of primary relevance for cat research. Importation into the United States of any biological samples from cat species listed in Appendix I or II of CITES (i.e., all nondomestic cats) requires a CITES import permit (CITES I) and/or export permit from country of origin (CITES I and II). Processing the permit application and publishing the permit request in the Federal Registrar for public comment, as required, may delay issuing of the permit for several months. More specific information about CITES and USFWS regulations is available online ([www.cites.org](http://www.cites.org) and [www.fws.gov](http://www.fws.gov)).

At the institutional level, many zoological institutions have in-house IACUCs that review any proposed research studies with the collection of a zoo. Very few zoos receive funding from the US Public Health Service, so there has been no regulatory requirement for most zoos to establish



formal IACUCs. In some cases, informal review of proposals by the zoo veterinarian, research coordinator, and/or curatorial/keeper staff is considered adequate by the institution; however, the trend is for this process to become more formalized within the IACUC format. Because the nature of research with zoo animals may be quite different from research with laboratory or domestic species, zoo-based IACUCs may use different criteria in judging the appropriateness of proposed studies. Standardization of IACUC review procedures among zoos would ease the regulatory burden associated with cross-institutional collaboration, therefore the establishment of such a policy should be a priority for zoo research coordinators. In the absence of a standardized IACUC review process, zoos should consider accepting IACUC review findings from other established zoological institutions as valid appraisals without requiring additional review from their own in-house IACUCs.

As mentioned above, one important consideration in the IACUC review process with nondomestic species is differentiating between traditional research (i.e., hypothesis-driven, controlled prospective studies) and service-based clinical interventions on the individual or population level. For example, as assisted reproductive technology becomes more refined, these techniques will be incorporated increasingly into population management programs as service tools to optimize genetic variation. Similarly, clinical intervention with wild populations may entail capturing or anesthetizing individual animals, but with the primary objective of performing a service for maintaining population health and not for conducting traditional empirical research. In these situations, it is unclear whether review and approval of the IACUC should be required before the procedures may be conducted.

## Summary

A mobile laboratory research strategy, as described in this article, clearly has benefited conservation efforts for endangered felids through the acquisition of basic physiological information and application of that knowledge to natural breeding and assisted reproduction. Key to this progress have been collaboration across institutions and countries and a common sense of mission focused on preserving endangered wildlife. If these efforts are to have a meaningful impact on the decline of felid populations, it is imperative to develop basic and applied reproductive sciences further and to incorporate the knowledge into genetic management programs for cats maintained in captivity. On a global scale and of more relevance to wild populations, this knowledge and technology must be accessible to our scientific colleagues in developing countries, which encompass the natural ranges and habitats of most endangered felid species. As advances in reproductive sciences evolve into functional management tools, their application becomes less focused on primary research and more oriented toward providing a management service. From a regulatory standpoint, a distinction should

be made between conducting research studies in the traditional sense and using established methods for genetic or clinical intervention at the population level.

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