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DEVELOPING ANIMAL MODEL SYSTEMS FOR EMBRYO TECHNOLOGIES IN RARE AND ENDANGERED WILDLIFE

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INTRODUCTION

The Convention of International Trade in Endangered Species (CITES) has recognized over 200 mammalian species as being threatened by extinction (1). Many vertebrates already are extinct in the wild as a result of an accelerated rate of habitat destruction. As a consequence, it is inevitable that during the next century most wildlife will be managed in captive breeding programs in zoological parks and wildlife preserves. Certain species (i.e., Pere David deer, Mongolian wild horse, European bison) thrive in captive conditions (2); many do not, and failing the development of improved management or husbandry efforts, these species' survival is at even greater risk.

Therefore, it is justifiable to consider alternative methods for assisting wildlife species in producing offspring, especially those few in number and with little success at natural breeding. The obstacles to

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The authors wish to thank L. Stuart, J. Martenson, S. Brown, L. Collins, S. Michie; Drs. M. Miller, L. Simmons, M. Roelke and W. Rall; and the National Zoo's Conservation and Research Center staff for their active participation in various projects discussed in this paper. The authors are grateful to the following organizations for assistance and collaboration: Denver Zoological Gardens, Detroit Zoo, National Zoological Park of South Africa, Henry Doorly Zoo, Johannesburg Zoo, Gladys Porter Zoo, Metro Toronto Zoo, San Diego Zoo, Wildlife Safari Park. Portions of this work were supported by Friends of the National Zoo and a grant from the National Geographic Society. Research projects of M.C.S., K.L.G. and J.G.H. are supported, in part, by the Women's Committee of the Smithsonian Associates.

such an approach are enormous and are considerably accentuated by the sheer number of species requiring assistance. A hypothesis can be formulated that reproductive techniques including embryo collection, storage and transfer improve propagation of rare species. Testing such a hypothesis, particularly in terms of long-range conservation results, is difficult and obscured by a number of factors including the needs for basic research, animal model systems, financial support and trained personnel.

We address here two primary factors associated with developing a successful reproductive physiology program in a zoological collection. First, employing three laboratory animal models, strategies are formulated to adapt and apply embryo and related reproductive technologies to nondomestic species. Secondly, zoological research has inherent characteristics that provide unique concerns and problems for the investigator. The integration of these factors hopefully will provide a basic outline of the challenges to be expected in conducting embryo studies in captive or free-ranging wildlife species.

STRATEGIES

The Use of Animal Models

In a zoological collection containing many rare animals, an important concern is which species (or even which major taxa) should be selected for embryo studies. Research and commercial embryo efforts have been most successful with farm animal ungulates. Thus, it seems most practical to first exploit already existing basic techniques for use in wild artiodactyla and perissodactyla species rather than carnivores or aquatic mammals in which little or no information is available. Likewise, previous efforts in mouse, rat and rabbit embryology suggest that rare zoological rodents and lagamorphs should not be ignored. Embryo collection, storage and transfer could have vital importance for propagating such species, many of which unfortunately are considered less "glamorous" research subjects than larger-sized zoo mammals. Nonetheless, perhaps earlier classical experiments by Adams (3), who successfully produced offspring after transferring fresh embryos from wild rabbits into domesticated rabbit does, should be used as an example of the potential conservation benefits to be realized.

A prerequisite to the detailed study of any nondomestic species is a basic understanding of physiological processes in a suitable domestic animal counterpart. The use of non-endangered animal models permits testing and refining research concepts and techniques before adapting to zoo specimens, frequently available in too few number for reliable, controlled studies. Defining the most appropriate model for any wildlife species is speculative, but more successful if oriented to critical program needs. For example, the National Zoological Park is examining the feasibility of an embryo banking program, primarily for maintaining gen-

etic diversity in captive animal populations. Inbreeding in the conventional management of domestic farm stock generally is a trivial concern. However, limited animal numbers in zoo collections can severely restrict productive, captive breeding. Inbreeding frequently is inevitable and is correlated to a number of calamitous events including severely increased juvenile mortality (4,5), reproductive defects (6) and an increased susceptibility to certain infectious and devastating diseases (2). Embryo banking would permit storing genetic material from founder population females which already have contributed extensively to existing populations. Embryos from these outstanding dams could be reintroduced into captive populations in future generations, thereby conserving genetic diversity and vigor.

The availability of numerous species of divergent taxa pose the immediate question of whether differences in genotype among and within species influence the success of embryo freezing. This major concern stimulated our selection of the laboratory mouse and sheep, relatively economical animal models for developmental and comparative zoological research. Both species generally respond favorably to conventional as well as novel superovulatory regimens, thus permitting studies of the effect of exogenous gonadotropins on the number and quality of embryos (7,8). The ease of obtaining large numbers of embryos allows quantitative studies of the influences of cryoprotectant and freeze/thaw techniques in developing optimal banking systems (9,10). Equally important, the mouse, because of its elaborate, research-oriented breeding history, is an ideal model for studying the influence of genotypic variance on reproductive function.

Significance of Genotype on Embryo Survival After Freezing

Collaborative efforts with the National Institutes of Health provide our laboratory with a resource of 148 different mouse genotypes. Embryos from mice of five different genotypes were evaluated for their ability to survive and develop after cryopreservation (7). Ovulation was induced with a standardized pregnant mares' serum gonadotropin (PMSG)/human chorionic gonadotropin (hCG) regimen, after which females were mated with males of the same genotype to produce incrossed embryos. Four- to 8-cell embryos were frozen in 1.5 M dimethyl sulfoxide (DMSO) at a rate of 0.5°C/min to -80°C and stored in liquid nitrogen. After thawing at room temperature, embryos were cultured; in vitro development was evaluated 24 hours later. Not only did the number of embryos produced per female vary among groups, but post-thaw embryo survival based on post-thaw culture ability varied significantly among mice with precisely defined genetic backgrounds (Table I).

The effect of genetics was evaluated further in a second study by examining the influence of mating females of four genotypes (N:NIH[S]-B, N:GP[S], C57BL/6N or C3H/HeN MTV^{-}) with males from a different strain (N:NIH[S]) known to have a reduced embryo survival rate after freezing.

Table I. Effects of incrossed and hybridized mating on post-thaw culture rates of mouse embryos (Schmidt et al. [7])[a]

Genotype	Percent embryos developing to morulae or blastocysts in 24 hours	
	Incross[b]	Hybrid[b]
N:NIH(S)	49	--
N:NIH(S)-B	61[c]	39[d]
N:GP(S)	66[c]	45[d]
C57BL/6N	75	70
C3H/HeN MTV$^-$	56[c]	39[d]

[a]A minimum of 500 embryos were frozen-thawed/ genotype. Rows values with different superscripts differ ($P<0.05$).

[b]Incross indicates pure strain breedings; hybrid indicates N:NIH(S) males mated with females of indicated genotype.

Compared to post-thaw viability of embryos isolated from the incrossed groups, survival was less ($P<0.05$) in three of four hybrid groups by as much as 35% (Table I). This suggests that the genotype of the embryo can exert a dramatic effect on its ability to survive a cryopreservation/thaw stress. The finding that post-thaw embryo survival varies among different genotypes even within the same species has important implications in the formulation of wildlife embryo banks. From this perspective it should not be too surprising when techniques successfully used with mice, cattle or sheep embryos are not directly applicable to wild species. Similarly, optimal freezing techniques for cattle and sheep embryos differ (11,12) and neither are successful when applied to the domestic pig (13). Therefore, natural evolutionary differences in reproductive physiology among zoo species will likely require individual species attention rather than simple deployment of mouse-, cow- or sheep-specific technology. The primary value of animal models, therefore, is in developing basic laboratory procedures and realizing that slight to major modifications may be necessary for each specialized species.

Embryo Studies in the Domestic Sheep and the Scimitar-Horned Oryx

Interest in applying embryo technology to wild ungulates has been preceded by extensive comparative investigations of the domestic sheep. Crossbred ewes are studied to evaluate 1) superovulatory response and embryo recovery rate differences after various estrous synchronization/ exogenous gonadotropin treatments, 2) the feasibility of minor invasive techniques for semen or embryo deposition *in utero*, and 3) comparative evaluation of embryo cryoprotectants. Recent advances for the latter

two purposes have considerable applicability to both nondomestic and laboratory-farm animal embryo programs.

A need exists for minor invasive procedures for artificial insemination (AI) and embryo transfer. Many zoo mammals are too small for nonsurgical AI or embryo transfer. Additionally, many appear highly susceptible to stress, pointing to the need for atraumatic, manipulative procedures. The technique of laparoscopy was used to transabdominally cannulate the uterine horn of the ewe (Fig. I), either for AI after superovulation or transfer of fresh or thawed embryos into recipients (14). Embryo transfer results were comparable (14) or even superior (10) to that from ewes receiving embryos by a conventional laparotomy approach. Therefore, laparoscopic embryo transfer may have important application in similar studies involving rare, nondomestic species in which less intrusive, atraumatic surgical procedures are highly desirable.

The sheep model has become valuable in analyzing alternatives to traditionally used cryoprotectants such as DMSO or glycerol. Because propylene glycol (PPG) recently was found suitable for freeze-preserving mouse embryos (15,16), its effectiveness also was studied in sheep. Late-stage morulae and early-stage sheep blastocysts were equilibrated (21°C) in modified phosphate buffered saline (PBl) for serial 10-min intervals in .75 M and 1.5 M solutions of PPG or glycerol (GLYC). After transfer into 6 X 50 mm ampules and a programmable freezer unit, embryos were cooled at 1°C/min to -5°C, seeded and then frozen at .3°C/min before plunging into liquid nitrogen. Embryos were thawed (37°C) and diluted in .75 M sucrose-PBl before being cultured in a 5% CO_2 in air atmosphere in Whitten's medium (+.15% bovine serum albumin). After 24 hours, embryos were assessed for quality grade (QG: 1=excellent to 4= poor) and viability grade (VG, based on a fluorescein diacetate assay, 0=no staining to 5=very bright, uniform fluorescence). Overall, post-thaw survival (based on VG $\geq$ 3.0) was not different in PPG-(19/25) and GLYC-(18/25) treated groups. Cryoprotectant had no effect on QG ratings but the proportion of embryos with freeze-thaw induced damage to the zona pellucida was less ($P<0.01$) using PPG compared to GLYC (Table II, Fig. II). A cracked or lysed zona pellucida could be of critical importance with respect to viral contamination, a particularly salient issue when considering freeze-preserving wildlife embryos for import/export purposes.

Embryos cryopreserved in PPG, thawed and transferred to recipient ewes have resulted in live-born offspring. Of particular interest was a recipient ewe giving birth to a pair of phenotypically dissimilar lambs (Fig. III). The superovulated donor ewe was naturally mated with one ram (Suffolk) and laparoscopically inseminated with frozen-thawed spermatozoa from a second male of a different breed (Corriedale). The recovered embryos, frozen in PPG and later thawed, were laparoscopically

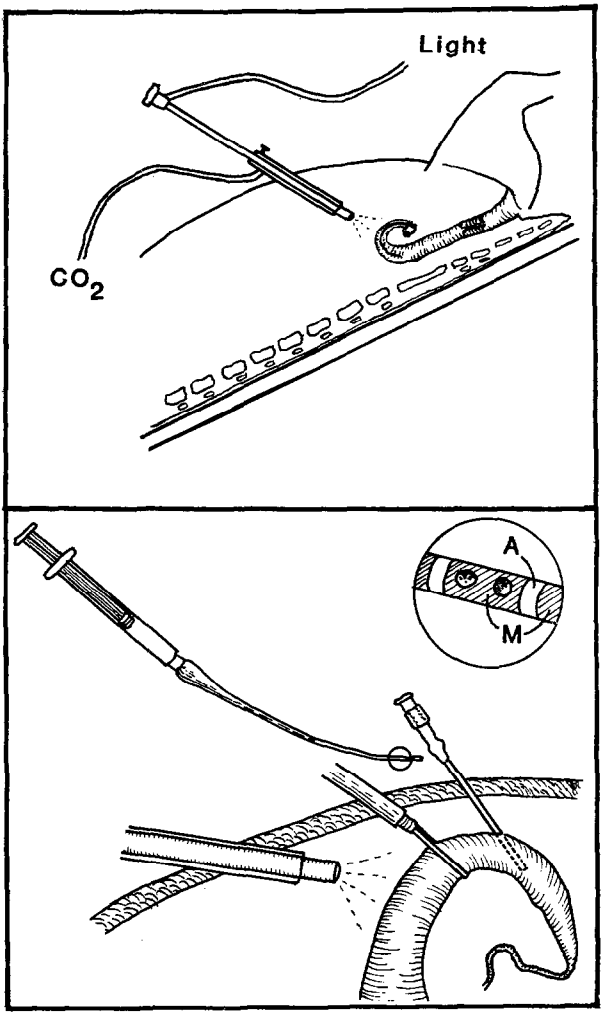

Figure I. Laparoscopic transabdominal approach for AI or embryo transfer. In the top panel the anesthetized female is placed in a head-down, supine position and the laparoscope used to identify the reproductive tract. In the bottom panel, an ancillary forcep is used to elevate and secure the uterine horn. A catheter is inserted through the abdominal wall and into the uterine lumen and the stylet replaced with a tom-cat catheter containing semen or embryos (with a medium [M] and air [A] interface) (Schiewe et al. [14]).

Table II. Evaluation of thawed, ovine embryos frozen in propylene glycol or glycerol (Schiewe et al. [10])

Assessment	Cryoprotectant	
	Propylene glycol	Glycerol
No. of embryos	25	25
Quality grade[a]:		
Pre-freeze	1.6 ± 0.1	1.5 ± 0.1
Post-thaw	2.0 ± 0.2	2.1 ± 0.2
Culture	2.4 ± 0.2	2.5 ± 0.2
FDA viability grade[b]:		
After culture	2.9 ± 0.2	3.1 ± 0.3
Zona pellucida damage, no. (%)	1 (4)[c]	10 (40)[d]

[a]QG: 1=excellent, 2=good, 3=fair, 4=poor; means ±SEM.

[b]VG: 5=very bright, uniform fluorescence to 0= no fluorescence; means ±SEM.

[c,d]Row values with different superscripts differ (P<0.01).

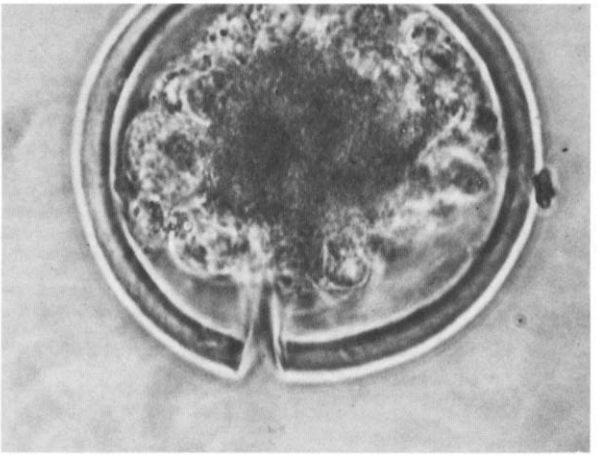

Figure II. Zona pellucida damage in a thawed sheep morula originally frozen in glycerol.

transferred to the uterine horn of the recipient ewe. The phenotypic features of the resulting lambs correlated with breed characteristics of the natural mating sire as well as the AI semen donor. Of significance was the observation that one lamb resulted from a frozen embryo originally conceived with frozen-thawed spermatozoa. Such findings, also reported in the cattle industry (17,18), offer encouraging prospects for wildlife sperm/embryo banks, because it is now apparent that the genetic component of frozen-thawed spermatozoa can withstand a second cryostress after integration into the embryo.

Concepts developed and expanded in domestic sheep currently are being applied to a nondomestic bovid, the scimitar-horned oryx (Oryx tao). The mature female of this species weighs approximately 160 kg. To date, efforts have concentrated on applying estrous synchronization/superovulation treatments as developed for the domestic cow and sheep to the oryx. Serial rectal palpations of natural cycling, untreated oryx have indicated that ovarian follicular and luteal activity generally is restricted to one ovary, the contralateral gonad being small, firm and inactive. Although this ovarian pattern deviates from that of the domestic cow, the oryx corpus luteum (CL) lyses in response to a domestic cow dose of prostaglandin $F_{2\alpha}$[a] ($PGF_{2\alpha}$, 25 mg, i.m.). Table III provides a summary of ovarian response and embryo recovery results from donor oryx given serial injections (i.m.) of follicle stimulating hormone-pituitary[b] (FSH-P®) or a one-or two-dose PMSG[c] injection (i.m.) treatment following estrous synchronization with vaginal medroxyprogesterone acetate[a] (Depo-Provera®, 60 mg) pessaries or dual $PGF_{2\alpha}$ (25 mg) injections given 16 days apart. Gonadotropin dosages and treatment schedules have been comparable to previous reports in domestic cattle (19-21). Administering FSH-P or PMSG at the described levels to a species less than half the size of the domestic cow generally has produced highly variable results but has been modestly effective in stimulating ovulation. However, certain females have been relatively refractory to conventional superovulatory therapies, which is in agreement with Durrant's (22) ovulation induction attempts in four oryx females. Even so, in our studies multiple ovulatory responses (11-16 CL/female) have been achieved on three occasions, twice with FSH-P and once with PMSG. Using routine, nonsurgical flush procedures, high-quality embryos have been recovered (Table III, Fig. IV), but only from donors with distinct, prominently raised CL (>3 mm diameter). Compared to that of the domestic cow, the smaller-sized cervical canal and virtual absence of a uterine body have increased the difficulty of nonsurgical embryo collection. Also, the size of the reproductive tract permits only 15-30 ml of medium to be passed in each flush. Embryo recovery efficiency has increased with technician experience; a recent attempt

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[a]Upjohn Co., Kalamazoo, MI
[b]Schering Veterinary Supply, Omaha, NE
[c]Equitech, Atlanta, GA

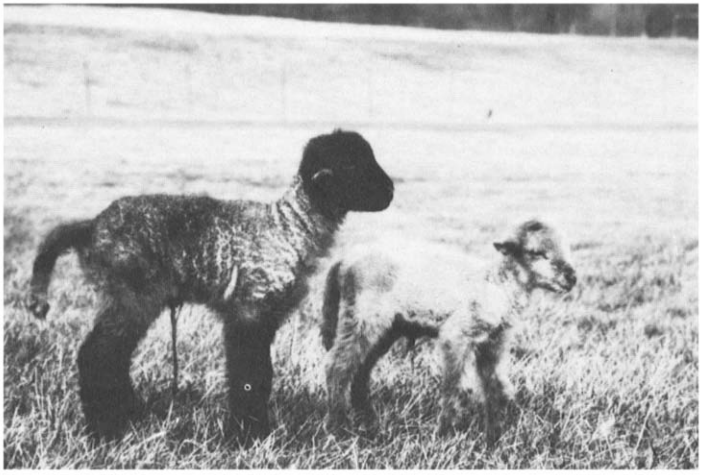

Figure III. Phenotypically dissimilar twin lambs born from frozen-thawed embryos that were laparoscopically transferred to a recipient ewe. The donor was naturally mated with a Suffolk ram and also laparoscopically inseminated with frozen-thawed Corriedale ram semen.

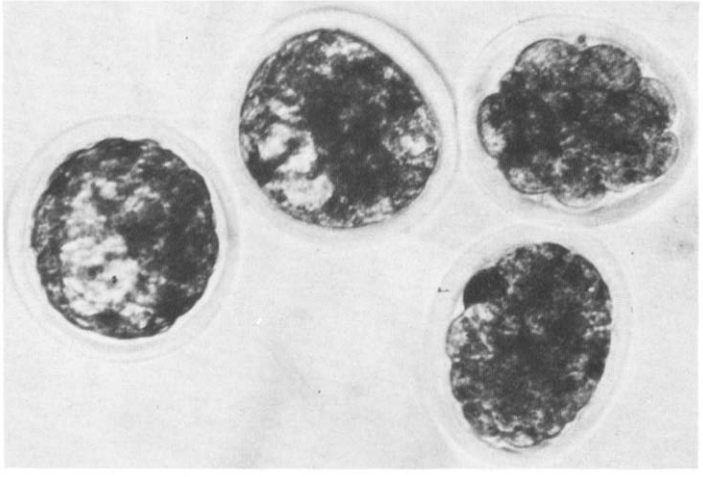

Figure IV. Scimitar-horned oryx embryos (two blastocysts, two morulae).

Table III. Ovarian response and embryo recovery rates in scimitar-horned oryx (Oryx tao) receiving FSH-P or PMSG treatment[a]

	Gonadotropin treatment	
	FSH-P[b]	PMSG[c]
Proportion of females ovulating	7/10	6/11
Mean no. (±SEM) of CL/females ovulating	5.0±2.3 (1-16)	3.7±1.5 (1-11)
Mean no. (±SEM) of unovulated follicles in all females	1.5±0.5 (0-4)	1.7±0.4 (0-5)
Proportion of ovulating females with embryos	2/7	4/6
Range in embryo no. in females with embryos	2-16	1-4

[a]Numbers in parentheses are ranges. Before gonadotropin treatment, estrous cycles were synchronized by either vaginal pessaries (containing 60 mg medroxyprogesterone acetate) in situ for 16 days or by two injections of 25 mg $PGF_{2\alpha}$ given 12 days apart.

[b]FSH-P injections (5 mg each) were given twice daily (i.m.) beginning 72 hours before pessary removal or second $PGF_{2\alpha}$ injection.

[c]PMSG (2000 IU) was given (i.m.) beginning 48 hours before pessary removal or second $PGF_{2\alpha}$ injection; if follicular activity was negligible 48 hours later, a supplemental PMSG dose (500-2000 IU, i.m.) was injected.

resulted in 16 morulae from a female with 16 palpable CL. Current studies emphasize the comparative efficacy of freeze-preserving oryx embryos in PPG vs GLYC, a natural extension of sheep embryo efforts.

Studies of the Domestic Cat and Cheetah

Program interests extend to the cat family (Felidae), which consists of 37 species; all are considered threatened or endangered with the

exception of the domestic cat. Although some felids (tiger, lion, puma, jaguar) breed easily and indiscriminately in zoo collections, others (including the cheetah, snow leopard, clouded leopard and many small cats) often reproduce poorly under captive conditions. The reason for success or failure within any given species is obscure and often confounded by a total lack of physiological information for the species. On this basis we began studies of the reproductive physiology of Felidae, using the domestic cat as a model and the cheetah as a targeted endangered species. While the initial objective was to propagate an endangered species by artificial breeding, the result has been the realization of the extreme biological complexities inherent to certain wildlife species. Such specialized phenonema are having provocative effects not only on the success or failure of artificial breeding attempts, but also on general health, management and natural breeding efforts of selected captive populations.

Early studies in our laboratory demonstrated the efficacy of inducing behavioral estrus and ovarian activity in domestic cats with serial injections of FSH-P (2 mg/day, i.m. for 5 days) (23). Additionally, electroejaculation was determined to be an effective method for collecting semen from male domestic cats (24). Subsequent studies concentrated on freeze-thawing cat spermatozoa in pellet form in an egg yolk (20%)-lactose (11%)-glycerolated (4%) diluent (25). Eventually, artificial insemination of naturally estrual or FSH-P treated females with fresh or thawed spermatozoa resulted in births of live kittens (25).

These results served as both an incentive and a biological basis for similar artificial breeding attempts in the cheetah. Thirty mature female cheetahs from nine zoological parks were restrained manually for gonadotropin injections. A dose of 10 mg FSH-P was given i.m. daily for 5 days (Days 1-5). During treatment each cheetah was examined daily for visual evidence of sexual behavior. On Day 6 and again on Day 7 hCG was injected (500 IU, i.m.). Laparoscopy (26), under surgical anesthesia, was used on Day 1 (pre-treatment) and on Day 6 to monitor ovarian response in all females. Occasionally, additional laparoscopies were performed on Days 5, 7 and/or 10 to confirm ovarian follicle number and the presence of corpora hemorrhagica or CL.

Electroejaculation was used to collect semen from adult cheetah males. Sperm concentration and % motility ratings were assessed immediately. Ejaculates were either treated with tissue culture medium and maintained at 37°C until insemination or mixed with the egg yolk-lactose-glycerol diluent (2:1), cooled (5°C) for 30 min, pellet frozen on dry ice and stored in liquid nitrogen. Frozen pellets were rapid-thawed (37°C) in physiological saline and maintained at this temperature until insemination. Cheetah females were anesthetized for AI usually on the afternoon of Day 5 and the morning of Day 6. Either semen was extended with additional diluent or ejaculates were mixed to allow inseminates to con

tain approximately 30 X 10^6 motile spermatozoa. Nonsurgical inseminations were made intracervically or in utero, immediately adjacent to the proximal cervical os.

A high proportion of cheetahs treated with FSH-P ovulated (23/30, Table IV). Laparoscopic photographs of ovarian morphology have been presented in previous reports (27,28). Only one female displayed behavioral evidence of sexual receptivity and none of the females became pregnant.

Table IV. Summary results of adult female cheetahs treated with FSH-P/hCG injections and then artificially inseminated with fresh (n=19) or frozen-thawed (n=4) spermatozoa

Total number of females treated with gonadotropins	30
Number of females responding by ovulating	23 (76.7%)
Mean number CL (±SEM) post-treatment	5.6 ± 0.6
Mean number unovulated follicles (±SEM) post-treatment	2.8 ± 0.7
Number of females in behavioral estrus	1 (3.3%)
Number of females pregnant	0

The negative pregnancy data were important in illustrating the need for more basic research as a prerequisite to artificial breeding attempts. This resulted in a series of detailed examinations of reproductive and genetic factors in the domestic cat (29-32) and cheetah (6,33-35). The findings have been intriguing, indicating that the family Felidae demonstrates a fascinating array of novel reproductive, endocrine and genetic characteristics.

In 1981, 40 electroejaculates were collected and analyzed from a captive cheetah population in southern Africa and then compared to semen quality of domestic cats (6). Spermatozoal concentrations in cheetah

ejaculates were ten-fold less than in domestic cats and an average of 71% of all cheetah spermatozoa were morphologically abnormal, compared to 29% in cats. Comparable numbers of abnormal spermatozoa were observed in cheetah ejaculates collected from zoos in the United States as well as males free-ranging in natural habitats in East Africa. A parallel study which analyzed over 200 isozyme and cellular protein loci of over 50 cheetahs found the captive population to contain 10 to 100 fold less genetic variation than other mammalian species including the domestic cat (33). Further investigation of genetic uniformity examined the variability of the cheetah's major histocompatibility complex (MHC), a locus which encodes a group of cell surface antigens responsible for cell-mediated rejection of allogeneic tissue grafts (2). Simultaneous studies in domestic cats indicated that unrelated individuals reject reciprocol skin grafts within 7 to 13 days after surgery. Similar skin grafting procedures were performed among 6 pairs of unrelated cheetahs and one pair of siblings. None of the 14 animals demonstrated acute graft rejection, suggesting that cheetahs were genetically monomorphic for a single haplotype, an unprecedented observation in a wild species. Skin from a domestic cat grafted to the cheetah was rapidly rejected.

Together these data indicate a remarkable genetic uniformity for this very specialized felid. Combining the reproductive and genetic findings, the cheetah's status becomes reminiscent of that observed in highly inbred strains of laboratory animals or farm stock. The practical consequences may be more serious based on analyses of captive breeding records demonstrating consistently greater juvenile mortality rates in cheetahs compared to other noninbred zoo mammals (2). More importantly, at least one captive cheetah population has demonstrated extreme vulnerability to a corona virus (feline infectious peritonitis) which drastically afflicted a cheetah compound in Oregon and which may be related to the monomorphic characteristics of the MHC (2). Together, the genetic findings graphically illustrate the potential consequences of inbreeding in captive wildlife and re-emphasize the significance of genetics in making artificial breeding programs effective. For the domestic cat and cheetah, without a major shift in research strategy from artificial insemination to an organized, basic research approach, little would have been learned or accomplished.

Current efforts concentrate on comparative aspects of semen quality among various felid species (36) with emphasis on sperm cell morphology and methods for improving viability in vitro. Artificial insemination attempts have been curtailed while studies evaluating the influence of exogenous gonadotropins on embryo quality have been initiated. Here too, it appears that the cat may be expressing an altered sensitivity compared to results reported for other nonfelid species. The reproductive tracts of domestic cats in natural estrus or after FSH-P treatment were flushed 8 to 9 days after onset of multiple matings. Cats in natural estrus produced fewer unfertilized ova and a greater proportion of high-quality embryos (QG, 1-3) than FSH-P treated

counterparts (Table V). Embryos transferred to synchronized recipients resulted in a total of three pregnancies (determined by abdominal palpation at 35 days) and two litters live born (one from natural estrus and one from induced-estrus embryos). Although the data indicate that FSH-P induced oocytes eventually can result in offspring after embryo transfer, it also appears that embryo quality in the cat may be compromised by the exogenous gonadotropin treatment.

Table V. Embryo number/quality results from mated, domestic cats in natural estrus or after an FSH-P induced estrus[a]

	Natural estrus	FSH-P induced estrus
Total number of embryos/ unfertilized oocytes	42/1 (2.3%)	62/20 (24.4%)
Embryos receiving quality grades of 1-3 (%)	78.5	48.3
Number of pregnancies/ total transfers	2/7	1/8
Number of litters with live offspring	1	1

[a]Uterine horns flushed 8 or 9 days after first day of mating.

Other Considerations and Concerns

Influence of stress and the potential value of field studies. This paper has emphasized the need for basic research, a requirement originating as a result of unique speciation. These specializations exist either as biological norms or potentially as a result of the captive environment. Therefore, it is prudent to recognize and appreciate temperamental sensitivities of wildlife animals used for research purposes. Also, because of variations in susceptibility to stress, physiological norms measured in captivity may not correlate with values found in free-ranging species. Therefore, studies to evaluate adrenal-reproductive relationships, improved chemical and physical restraint and noninvasive techniques for physiological monitoring are of high priority. A most exciting recent concept in our program has been the collection of reproductive, endocrine and genetic data from free-ranging species in natural, ecologically dynamic habitats (37). Findings from

these efforts may provide clues to the effects of the captive environment and physiological explanations to the increasing infertility observed in zoo populations.

Animal availability. Scientifically sound, physiological research is impossible without access to animals. Species requiring the most study usually are available in the fewest numbers. Additionally, physiological studies generally require animal restraint and/or immobilization, frequently a concern and point of contention between the curatorial/keeper staff and researcher. The lack or unavailability of a certain species at any one zoo may be compensated for by close cooperation among different zoos. Therefore, a clear and open line of communication and collaboration among the administrative, curatorial and research staffs within and among zoos assures species accessibility and optimal research productivity.

Financial support. Artificial breeding technology and related basic/applied research programs are costly to initiate and maintain. Federal grant support for zoological research is minimal and most efforts at major parks are financed by private support groups for the individual zoo. The primary obstacle to rapid growth and improvement of zoo research is the lack of resources to provide salaries for highly qualified research staff. The actual laboratory effort (requiring equipment and supplies) is a secondary problem which in many instances could be alleviated by the closer collaboration with local universities with existing research programs. Nonetheless, new mechanisms for financial support require exploitation. The popularity of preserving endangered species should not be neglected. Financial needs should be publicized to the private sector and commercial enterprise.

Trained personnel. Graduate-student education programs are critical to future needs for good investigators well trained in research concepts, but with a background and appreciation for the management skills associated with studying, maintaining and handling wild species. Reproductive physiologists in animal science, biology and veterinary departments at local universities also offer an important resource for zoo research needs.

Image of physiological research. Frequently the concept of physiological research in a zoo is misunderstood by nonresearchers and the public. At one extreme animals are perceived as being excessively manipulated to the point of being treated inhumanely; at the other, these novel research techniques offer a "quick fix" to the endangered or infertility status of many species. The justification for such research, of course, exists at an intermediate level. Therefore, increased communication and awareness within the zoo community is needed, not only

for applauding the benefits of basic research but for stressing the limitations of current technology toward practical artificial breeding.

Presentation and publication of data. Research data from zoological studies should meet the same strict review criteria as information disseminated by the laboratory/domestic animal research community. anecdotal announcements of reproductive successes which have neither the benefit nor sanction of scientific review. These reports, which are common in zoo research, offer no benefit to other scientists and, in fact, may impede legitimate progress by distracting or discouraging funding sources. Such publicized information should be considered rumor and precluded from reviews of advances in the field.

CONCLUSION

Reproductive technologies including embryo collection, storage and transfer provide incentive for solving management problems and improving the endangered status of many zoological species. Achieving these objectives will be neither simple nor immediate. The currently conventional use of semen and embryo procedures for breeding cattle became "routine" only after many years of basic and applied research by hundreds of scientists supported by commercial grants and unlimited animal numbers. Similar resources are unavailable to zoo researchers, and time constraints for extremely endangered species further complicate the task. Nevertheless, zoological researchers should now concentrate on establishing physiological norms for selected species of interest. Comparative studies in domesticated animals will accelerate progress. The immediate goal should be publication of sound scientific data, including information concerning negative results. Only then can organized, composite strategies be formulated to consider artificial propagation as a viable alternative for preserving species.

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