North American Cheetah SSP 1996 Master Plan
Jack Grisham, Oklahoma City Zoo

The goals of the Cheetah SSP are:

*to maintain a genetic reservoir for the wild population

*to promote educational programs in both North America and *in situ*

* promote conservation of the species *in situ* through the Cheetah Conservation Fund (CCF) in Namibia and other wildlife organizations

* promote research on this species in the following (but not limited to) areas: assisted reproductive technology as a management tool, infectious disease surveillance, animal health issues, nutrition, behavior and genetics.

At the January 1996 master plan session, the North American cheetah population of 306 animals, which is less than the current carrying capacity of 320 spaces allocated to the Cheetah SSP by the AZA Felid TAG. The population appears demographically viable; breeding must be restricted to genetically valuable animals. All animals were paired based upon the current (1/21/96) Mean Kinship (MK) values for the population. These MK values change as animals die and are born. **Do not** use the current MK list for possible repairing in the event of the death of a recommended paired animal. Contact the Species Coordinator for a new recommendation.

The current master plan covers a two year period (March, 96 until February, 98) and will be reviewed at that time. No breeding recommendation was made for genetically valuable animals under the age of four years. Emphasis was placed on collecting the genetic diversity of the older animals in the population. Recommended animals should only be bred once during this two year period unless otherwise notified by the Species Coordinator. Specific recommendations include both natural breeding recommendations, artificial insemination and a combination of artificial insemination and natural breeding. All offspring produced through recommended breeding may be required to be held at the breeding facility for up to three years. If non-recommended animals are produced, the holding facility should be prepared to hold these animals for the long term. During the last master plan, an additional founder was added to the North American population, but genetic diversity decreased slightly due to non-recommended breeding of high MK animals. This affected the overall effectiveness of the specific recommendations.

During the master planning process Cheetah SSP Advisors (veterinary, infectious disease, pathology) presented updated information on disease and health factors concerning the cheetah (Appendix A). Recommendations were reinforced that **all animals must be tested for FIV and FIP and that no animal may be moved from one facility to another without both having currently tested their animals for these titers. Specific recommendations on disease screening were also discussed and are included in this document.** The Pathology advisor strongly urged, and was endorsed by the Management Group, that we continue to complete the Necropsy Protocol and send tissue samples to **Dr. Linda Munson** (Appendix A).

Assisted reproductive technology (Appendix B), as a management tool, was extensively discussed and endorsed by the Management Group as a method of producing offspring from genetically important females in the population which have not yet produced offspring from natural pairings. The Group also strongly endorsed the development of a Genome Resource Banking Policy for the Cheetah SSP. The Species Coordinator, with the assistance of Drs. David Wildt and JoGayle Howard will develop a draft of the policy prior to the AZA National Conference in September, 1996.

Program Goals

Analysis of the SSP population produced the following results:

Generation length.....6.1 years
Number of Founders.....64
Gene diversity retained to date.....96.9%

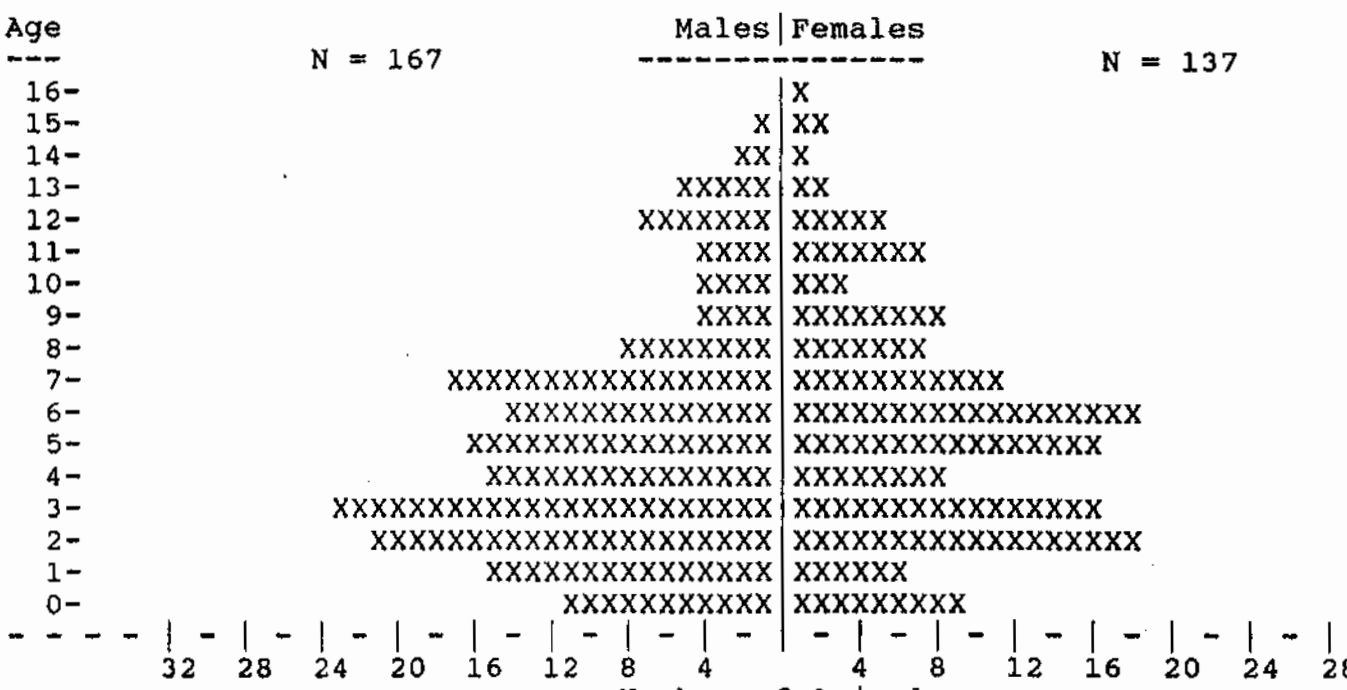
Based on the amount of genetic diversity lost thus far (approximately two generations), it was determined that the effective population size (Ne) was approximately 50, which results in an Ne/N ratio of about 50. This combination of parameters indicates that 90 percent genetic diversity could be maintained in this population for 100 years if the carrying capacity were to increase to approximately 640. It was felt that improved management could decrease the amount of required space substantially.

*Note: Although a new founder was recruited, genetic diversity slightly decreased due to non-recommended breeding of animals with high Mean Kinship.

Age Pyramid Report

Restricted to: CHEETAH Studbook
Association: CHEETAH
Status: Living by 21 Jan 1996

Taxon Name: ACINONYX JUBATUS



X >>> Specimens of known sex...
? >>> Specimens of unknown sex...

Feline Immunodeficiency virus (FIV) tests and their implications in the Cheetah Population
Reported by Peggy Barr, Cornell University

Serological tests for FIV infection in cats include ELISA and Western blot (WB) assays. In our experience, the Western blot is more specific and somewhat more sensitive than the ELISA, especially when testing non-domestic cats. Western blot technology is based on the separation of FIV proteins by electrophoresis so that antibodies against individual proteins in the serum of infected cats can be detected. Binding of serum antibodies to 2 FIV-specific bands (representing 2 separate FIV proteins) is interpreted as a positive test. Development of a single FIV-specific band constitutes an equivocal test. In addition, samples which have a high level of non-specific reactivity as well as reactivity to 1 or more specific proteins produce a "smearing" pattern that also is interpreted as equivocal.

The current FIV ELISA and WB tests may not be sensitive or specific enough to detect all (or even many) lentivirus-infected cheetahs. There is evidence that at least some cheetahs are infected with lentiviruses which are related to, but distinctly different from, other strains of FIV. This virus has been tentatively called "cheetah immunodeficiency virus" (CIV). Unfortunately, the antigen required to test the population for CIV is not available at this time; therefore, we have been testing for lentiviral infections in cheetahs with antigens from an isolate of FIV from a Pallas cat.

During the past 2 years, approximately 220 cheetahs were tested for FIV (by our laboratory) using ELISA and WB procedures. Of the animals tested, 2 cheetahs tested positive by WB (only one of these cheetahs was positive by ELISA). Both of these cats subsequently tested equivocal on later repeat tests. In addition to the 2 positive cheetahs, 16 of the 220 cheetahs tested equivocal. The positive/equivocal animals were housed at 8 facilities. The remaining cats were negative on our tests.

Some non-domestic cats infected with FIV apparently have a poor or delayed antibody response to FIV infection, or have fluctuations in their antibody levels. Such a fluctuation may be responsible for the 2 cheetahs mentioned above going from a positive to equivocal status. The cats need to be retested over time to determine their true FIV status. One hypothesis for the poor delayed or variable antibody response to FIV in non-domestic cats is that the non-domestic cat strains of FIV have a strong tropism for monocytes/macrophages and/or are highly cell-associated, thus allowing the virus to escape immune recognition in some infected cats. This hypothesis is based on our research on the Pallas cat FIV isolate, but must be tested with additional research.

Summary of Research Report from Suzanne Kennedy-Stoskopf (North Carolina State University College of Veterinary Medicine)

Samples from 48 cheetahs representing six zoos have been submitted for flow cytometry to evaluate lymphocyte subsets. Seventy data points were available because of repeat testing and repeat sampling of the same animals. The intent of this study was to determine if FIV+ cheetahs had inverted CD4:CD8 ratios as seen in FIV seropositive lions. CD4+ lymphocytes are helper T cells and CD8+ lymphocytes are cytotoxic/suppressor T cells. The ratio between these two T lymphocyte populations is normally greater than 1.0. With FIV infections in domestic cats and some lions, this ratio drops below 1.0 suggesting that the virus is having an adverse impact on the animal's immune system. Because of the unknown status of the SD-WAP cheetahs, it is not possible to correlate CD4:CD8 ratios with Western blot results, although four of the animals did have CD:CD8 ratios below 1.0.

A more interesting observation was that many cheetahs sampled had what are probably elevated B lymphocytes as these cells were both positive for immunoglobulin (Ig+) as well as a B cell marker. Sixty-five percent of the 48 cheetahs had 5-20% Ig+ cells while 35% had Ig+ cells ranging between 23-70%. The significance of this observation is not known. Although the domestic cat has 30-40% Ig+ cells in peripheral circulation, this data and work previously performed by Michelle Miller-Edge and Mike Worley suggest that cheetahs have fewer Ig+ cells. The same has been observed in African lions. A questionnaire has been prepared requesting health status in these 48 cheetahs to determine if there is any correlation between elevated Ig+ cells and disease states frequently associated with cheetahs. In addition, relationships between these animals will be evaluated to determine if there is a familial incidence.

Update on the pathology survey in cheetahs
Reported by Linda Munson, University of Tennessee, College of Veterinary Medicine

Since 1988, we have surveyed the tissues of 118 cheetahs from United States Zoos, 78 cheetahs from southern African zoos and breeding facilities, and 16 free-ranging African cheetahs, in addition to slides from several Australian cheetahs, that died, to determine the significant diseases in the population. Twenty-four cheetahs were surveyed during 1995, and several notable diseases have increased in prevalence in the population. These include:

1. **Severe gastritis.** Of the 21 stomach samples analyzed from cheetahs that died, 48% had severe gastritis, in contrast to only 21% of 56 samples analyzed from cheetahs that died before 1995. Although the overall prevalence of gastritis in the population has not changed significantly, the shift has been from mild to severe gastritis. No wild (free-ranging) cheetahs tested to date have had gastritis, whereas cheetahs in African zoos and breeding facilities have gastritis similar in prevalence and severity to captive-held U.S. cheetahs.

The gastritis is associated with spiral bacteria (*Helicobacter spp.*) in the cells of gastric glands, although the bacteria alone cannot be considered directly causative, because wild cheetahs harbor bacteria in gastric glands without accompanying inflammation. The lack of correlation between bacteria and gastritis in wild cheetahs suggests that some factor in captive management predisposes cheetahs to develop a gastric inflammatory response. The types of *Helicobacter spp.* and other microorganisms in the stomach may affect the inflammatory response. For example, *Mycoplasma spp* was isolated from some affected cheetahs. A genetic predisposition also is possible, because *Helicobacter*-associated gastritis in humans can have a familial basis. Stress and/or total bacterial numbers also may affect disease emergence in cheetahs as has been noted in humans with gastritis.

Antibiotic treatment trials conducted at Columbus Zoo and White Oak Conservation Center have shown that bacterial burdens can be temporarily reduced, but not eliminated and re-infection appears to occur over time. White Oak Conservation Center also hopes to conduct a vaccine trial in 1996-7 to prevent or reduce infection.

2. **Amyloidosis of the kidney and liver.**

The prevalence of renal and hepatic amyloidosis has risen abruptly from 21% (of 75 samples) to 54% (of 24 samples) during 1995. Amyloid is a partially degraded serum protein that is elevated during chronic inflammatory diseases and deposits in tissues, blocking normal blood circulation. As a result, the tissues gradually lose normal function, or (as is true in renal interstitial amyloidosis) abruptly die. During the last year, most kidney failures in cheetahs have been caused by amyloidosis, and 85% of these cases have been associated with chronic gastritis. Therefore, the notable increase in amyloidosis in the last year could be attributed to the increase in gastritis.

3. **Severe veno-occlusive disease** (VOD)

Four cases of severe veno-occlusive disease with abrupt decompensation and hemorrhage occurred in yearling cheetahs during 1995. These cases contrast with the previous pattern of VOD in cheetahs, that of a slowly progressive obstruction of the blood supply through the liver resulting in gradual liver failure in cheetahs 5 or more years old. Similar severe hemorrhagic VOD cases have been noted in captive-held cheetahs from Australia, South Africa, and Zimbabwe. Wild cheetahs from southern Africa that have been evaluated during the SSP survey have not had VOD. However, some recently wild-caught, captive-held cheetahs in Namibia have had elevated liver enzymes similar to those noted in these recent severe cases. The similarity of diets in wild and captive-held cheetahs in Africa suggest that the disease does not have a nutritional basis. Furthermore, tissue analyses from 2 of these severe cases did not disclose a causative toxin (Dr. Dalen Agnew, personal communication).

A history common to most of these rapidly decompensating VOD cases is a recent translocation of the affected cheetah from an off-exhibit facility to an on-exhibit enclosure in a zoo, 2 to 12 months before death. Because stress can reduce circulation to the liver and lead to regional scarring similar to that noted in these cases, translocations may have resulted in this severe VOD.

RECOMMENDATIONS:

1.Gastritis:

A. Continued surveillance of cheetahs through gastric biopsies of live cheetahs and participation in the SSP Pathology Survey for cheetahs that die. Formalin-fixed biopsies should be sent to Dr. Linda Munson (the Cheetah SSP Pathologist) and results will be included in ongoing epidemiological studies for the SSP as well as communicated to the participating institution. Ongoing studies include 1) the relatedness of cheetahs with severe gastritis, 2) enclosure characteristics in zoos with high and low prevalences of severe gastritis, 3) the bacterial

types present in cheetahs with severe gastritis, and 4) relatedness of cheetah movements between zoos versus patterns of gastritis in that shipping and receiving populations.

B. Biopsy cheetahs to be translocated and treat those cheetahs with tetracycline, metronidazole, and pepto-bismol for 2 to 3 weeks prior to shipping to reduce bacterial levels.

2. Amyloidosis:

A. Complete the characterization of cheetah amyloid to determine if some unique quality of amyloid in this species is the basis for this disease. This project is being conducted by Dr. Ken Johnson, University of Minnesota.

B. Reduce chronic inflammatory diseases, such as gastritis, in the population, thereby reducing circulating amyloidogenic proteins.

3. Veno-occlusive disease:

A. Initiate studies monitoring fecal cortisol levels in cheetahs during translocations between institutions and from facilities with high prevalences of VOD.

B. Initiate studies evaluating changes in circulation to the liver during epinephrine and corticosteroid challenges.

4. Continue comprehensive disease surveillance:

A. Surveillance for significant diseases in the population should continue for ongoing epidemiological and pathologic studies aimed at preventing these diseases. Disease surveillance also will provide the data that is essential to determine the pathogenicity of FIV or CIV in cheetahs.

B. All cheetahs that die should be necropsied and tissues harvested according to the Cheetah SSP Necropsy Protocol. The Necropsy Protocol is on the following pages. The complete set of fixed tissues should be sent to Dr. Linda Munson (the Cheetah SSP Pathologist) for ongoing disease surveillance in the cheetah population. Ship tissues to:

Current Feline Immunodeficiency virus (FIV) status and recommendations: January 20, 1996
Reported by Peggy Barr, Suzanne Kennedy-Stoskopf, Randy Junge, and Linda Munson

1. Current status in the population

220 cheetah have been tested in Peggy Barr's laboratory at Cornell University since 1994 by Western blot (WB) and ELISA

2 positive cheetahs (by WB) later changed to equivocal; one of these 2 cheetahs was also ELISA +: These cheetahs do not have clinical signs.

16 other cheetahs have had equivocal results

The meaning of an equivocal test in cheetahs is still uncertain. It may indicate non-specific reactivity or an incomplete specific cross-reactivity.

These FIV+/equivocal cheetahs are housed at 8 facilities.

Of the 50 females and 50 males with lowest mean kinship ratios (recommended breeders), 25 males and 25 females have not yet been tested.

Correlation of Western blot test results run by three different laboratories on the 24 cheetahs from the SD-WAP is still not known.

2. Current assessment of FIV and FIV testing in cheetahs

1. The current tests for FIV may not be sensitive or specific in cheetahs.
2. The WB test for cheetah immunodeficiency virus (CIV) is not yet available to test the population.

3. Because CIV (or FIV) cannot be reliably detected in cheetahs, it is possible that positive animals are not being recognized.
4. The pathogenicity of either FIV or CIV in cheetahs is currently not known.

3. RECOMMENDATIONS: (These recommendations supersede the March 1995 Cheetah SSP FIV recommendations)

There appear to be many species-specific strains of lentivirus and it is unknown if lentiviruses such as FIV cross species. The pathogenicity of lentiviruses in cheetahs and the risk of viral transmission from FIV+ or equivocal cheetahs is unknown. Therefore, these recommendations are based on current knowledge of the transmission and pathogenesis of FIV in domestic cats. As more information on lentiviral infection in cheetahs is acquired, these recommendations may be modified in the future.

1. Untested cats should still be tested within the next 60 days. All samples should be accompanied by the **STUDBOOK NUMBER.** All tests will be run in Peggy Barr's laboratory and test results will be sent directly to the Species Coordinator. Serum samples also will be archived for future testing when more reliable methods are available.
2. Cubs less than 6 months do not need to be tested. Test cubs at 6 months or later.
3. The Cheetah SSP **does not endorse** the movement of FIV+/equivocal cheetahs between facilities. In situations where the shipping and receiving institutions both wish to transfer a FIV+/equivocal cheetah, then both institutions' veterinary and curatorial staff and the Species Coordinator **must be appraised** of the FIV status of the animal to be transferred and the status of both shipping and receiving collections. If a FIV+/equivocal cheetah is placed in contact (not quarantined) with the receiving institution's collection, then the receiving institution's collection is considered positive.
4. All cheetahs should be tested by Peggy Barr's laboratory by WB within 30 days before shipment. FIV test results from other labs **WILL NOT BE ACCEPTED** for preshipment.
5. Because FIV is known to contaminate semen in domestic cats, FIV+/equivocal cheetahs should not be bred, nor should their semen be used in AI. If a FIV+/equivocal cheetah is genetically valuable, then the Cheetah SSP and involved institutions should evaluate these recommendations on a case by case basis. Offspring of FIV+/equivocal cheetahs should be considered at risk for infection and should be monitored for seroconversion for at least 3 years. Negative cheetahs in "positive collections" (cheetah collections with one or more FIV+ or equivocal cheetahs) can be included in SSP breeding recommendations.
6. Because of the ambiguity of the current tests, annual testing of previously-negative cheetahs is not required, unless those cheetahs were in contact with a FIV+/equivocal cheetah within the last 12 months. FIV+/equivocal cheetahs should be tested at least annually.
7. FIV+/equivocal and negative cheetahs should not be kept in the same or adjacent enclosures and should have no direct body or bodily fluid contact.
8. All institutions should maintain records of animal movements from area to area within that facility.
9. More sensitive and specific tests should be developed (eg. PCR-based tests, WB using CIV).
10. Disease surveillance should be continued to facilitate correlation with FIV and CIV status.

FELINE INFECTIOUS PERITONITIS (FIP)

The Cheetah SSP recommended that all institutions continue to monitor their collections annually for FIP by sending serum samples to DR. JIM EVERMANN. Test results from other laboratories **WILL NOT BE ACCEPTED** for interzoo shipment of cheetahs. Test results should be sent directly to the Species Coordinator.

ADDRESSES OF COLLABORATORS IN DISEASE SURVEILLANCE

FIV tests:

Dr. Peggy Barr
Dept. of Microbiology
NYS College of Veterinary Medicine
Cornell University
Ithaca, NY 14853

FIP tests:

Dr. Jim Evermann
Washington State University
Washington Animal Disease Laboratory
College of Veterinary Medicine
Pullman, WA 99164
509-335-9696

Fixed tissues (necropsy or biopsy samples) and disease surveillance questions:

Dr. Linda Munson
Department of Pathology
College of Veterinary Medicine
University of Tennessee
2407 River Dr.
Knoxville, TN 37996
423-974-5616 FAX
423-974-8215

Clinical veterinary questions:

Dr. Randy Junge
St. Louis Zoo
Forest Park
St. Louis, MO

Assisted Reproduction for Managing Cheetahs
Reported by JoGayle Howard, NOAHS

Assisted reproduction refers to propagating animals using methods other than natural mating. The most conventional methods include artificial insemination (AI; manually depositing collected sperm into a female), in vitro fertilization (IVF; sperm and egg interaction outside the body that results in an embryo) and embryo transfer (ET, translocation of an embryo produced by in vitro or in vivo fertilization into a surrogate host). Laboratory analyses of semen and circulating reproductive hormones or their excreted (urinary or fecal) metabolites also should be considered 'assisting' tools, because resulting information provides the fundamental knowledge that allows techniques like AI, IVF and ET to be effective.

Advantages of assisted reproduction are enhanced further in the context of cryopreservation (Wildt et al., 1993b). For example, genome resource banks (frozen repositories of sperm, eggs and embryos) could preserve existing genetic diversity for generations, to be re-derived when necessary for 'gene infusions' into current living populations. Cryopreservation also provides 'insurance' from catastrophes, ranging from natural disasters to disease epidemics. Most importantly, cryopreservation technology combined with assisted breeding has enormous potential for collecting, moving and infusing genetic material from truly wild individuals into genetically stagnant populations. The result is profound - eliminating the need to take animals from the wild for zoo breeding programs while leaving these individuals in nature to protect native habitat. Such an ambition already is becoming reality in the cheetah (see below). Furthermore, assisted reproduction has the potential of restoring genetic vigor to wild populations that may become highly fragmented.

Semen cryopreservation techniques also offer a feasible approach for transferring genetic material among zoos without the health risks associated with transporting living animals. Translocation poses a risk of disease transmission among individuals or populations. With the current concern about susceptibility of felids to certain infectious viruses including feline immunodeficiency virus (FIV), canine distemper virus (CDV) and feline infectious peritonitis (FIP) (O'Brien et al., 1985; Roelke et al., 1993), assisted reproduction may be one of the few available means for propagating genetically-valuable individuals infected with a certain pathogen. However, it is essential that these assisted breeding techniques not serve as disease vectors. In domestic cats, FIV is present in semen and can be transmitted to females by AI (Jordan et al., 1994, 1996a,b; Howard et al., 1995). Therefore, high priority studies need to be continued for assessing transmission potential when using fresh or cryopreserved semen, and developing methods to ameliorate the possibility of disease distribution.

Artificial reproduction techniques are available for assisting in the management of felids. Although the objective is not to manage the population by assisted breeding, these techniques could allow more rapid production of offspring from valuable individuals that have failed to reproduce naturally in captivity. To-date, AI has been used to produce offspring in the leopard cat (Wildt et al., 1992), cheetah (Howard et al., 1992b), tiger (Donoghue et al., 1993), puma (Moore et al., 1981; Barone et al., 1994b), ocelot (Swanson et al., 1996), clouded leopard (Howard et al., 1996a) and snow leopard (Roth et al., 1996). In all cases, the ovaries of sperm recipients were stimulated to develop follicles and ovulate using the exogenous hormones, equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG). Additionally, all sperm samples were deposited in utero by a transabdominal laparoscopic intrauterine AI technique (Howard et al., 1992a).

Development of AI in Cheetahs

During the last ~15 years, the cheetah has been the focus of intensive, multi-disciplinary, cooperative research that has included genetic analyses, reproductive/endocrine studies of both captive and free-living individuals. A large-scale biomedical survey also was conducted to assess the reproduction status of captive cheetahs in North America (Wildt et al., 1993a; Howard et al., 1993). This systematic approach determined that a large proportion of female, zoo-maintained cheetahs are not consistently reproductively active. The condition of teratospermia also is the norm, not the exception for the species (Wildt et al., 1993a). Together, these results laid a foundation of basic knowledge for developing the actual AI procedure.

Early AI attempts in the domestic cat relied upon vaginal sperm deposition which proved relatively inefficient (Platz et al., 1978). Further, no pregnancies were established in sedated cheetahs or tigers vaginally inseminated near the time of ovulation (Wildt et al., 1986, 1987). Further studies determined that even highly motile spermatozoa deposited intra- or transcervically into anesthetized tigers fail to enter the proximal aspect of the uterine cornua (Wildt et al., 1987). These findings prompted the adaptation of the laparoscopic intrauterine insemination technique for depositing spermatozoa directly into the uterine horns. Pregnancy rates in domestic cats improved markedly (Howard et al., 1992a), but only when AI was conducted after confirmed ovulation. Therefore, it appears that anesthesia inhibits both sperm transport and ovulation, and, ideally, AI should be conducted soon after ovulation. To-date, all pregnancies in non-domestic felids resulting from laparoscopic AI have been in females that were post-ovulatory at the time of AI.

Another factor influencing successful assisted reproduction in felids is species ovarian sensitivity to exogenous gonadotrophins, specifically eCG and hCG. For example, there is no relationship between body mass of a given cat species and the dose of eCG and hCG required to induce ovarian follicle development and ovulation, respectively. The extent of variability in hormone sensitivity was first realized

while developing an AI regimen for the cheetah. Despite the cheetah being ~8-10 times the size, the optimal eCG dosage (200 IU) was determined to be only twice the effective dosage of the common domestic cat (100 IU) (Howard et al., 1992a,b, 1996b). In the most recent study, 16 female cheetahs in 27 ovulation induction trials were used to assess either 100, 200 or 400 IU eCG and either 100 or 250 IU hCG 80 h later. All gonadotropin dosages stimulated ovarian activity in cheetahs, however, ovulation rates and the morphology of ovulation sites (i.e., corpora lutea; CL) varied among treatments. Although dosage did not influence the number or size of follicles, the number of CL was related directly to eCG dosage. Most CL in cheetahs were detected in females treated with 400 IU eCG (~10 CL/female), followed by 200 IU eCG (~7 CL/female) and 100 IU eCG (~1 CL/female). The eCG dosage had an effect on CL development with 2 sizes of CL observed: 1) small CL (2-4 mm diameter) that were associated with the 400 and 100 IU eCG dosages; and 2) large CL (5-8 mm diameter) that were associated with the 200 IU eCG dosage. Thirteen AI procedures in females receiving the optimum eCG dosage (200 IU) were conducted after ovulation by laparoscopically depositing washed sperm into the uterine lumen. Six of the 13 inseminations (46%) resulted in a pregnancy following intrauterine AI with 3 to 28 x 10^6 motile spermatozoa. These results indicated that both the excessive and insufficient eCG dosages are associated with under-developed small-sized CL; whereas, optimum eCG dosages result in large-sized CL of normal appearance (similar to that observed in non-stimulated estrous cycles). Based on ovarian response and CL development, the optimum gonadotropin dosages for AI in the cheetah appears to be 200 IU eCG and 100 IU hCG.

Artificial insemination with cryopreserved sperm from Namibian cheetahs

In 1994, a collaborative project between the National Zoo's NOAHS Center, Cheetah Conservation Fund, Caldwell Zoo, Columbus Zoo, Ft. Worth Zoological Park, Oklahoma City Zoological Park, Rio Grande Zoological Park and the White Oak Conservation Center was initiated to cryopreserve Namibian cheetah spermatozoa for importation to be used in the North American Cheetah SSP program. Namibian cheetahs are largely free of exposure to feline immunodeficiency virus (FIV), providing a strong imperative toward cryopreserving semen samples for artificial insemination (AI) studies from a virus-free population. Spermatozoa were cryopreserved from 10 Namibian cheetahs for transportation to the United States. To date, 7 females have been inseminated with frozen-thawed Namibian sperm (from 4 different males) using the laparoscopic intrauterine AI technique. Of these, one female (SB# 2124) located at White Oak Conservation Center was diagnosed as pregnant by ultrasonography and delivered one live cub on May 26, 1995. Maternal care was excellent and the cub was observed nursing, however, the cub was found dead five days later. Another female (SB#1903) located at the Rio Grande Zoo was diagnosed as pregnant by radiography and delivered 3 cubs on November 3, 1995. The female cannibalized 1 of the 3 cubs, and the 2 remaining cubs were removed for hand-rearing. One of these died, but the third cub (a female) survived and is in excellent health. The births of these cubs represent the first successful AIs with cryopreserved cheetah sperm. Interestingly, the 2 wild-caught males that produced both litters were subsequently found dead in their natural southern African environment. The male that produced the cub at White Oak Conservation Center was killed in a poacher's snare. The other male that produced the cub that survived at Rio Grande Zoo was killed by another male cheetah during an attempted territory take-over. So AI combined with cryopreservation allowed these genes to become immortalized into the captive management program. This achievement demonstrates, in striking fashion, the power of the technology for moving 'wild' genes and infusing new genetic material into zoo-maintained populations.

Results of Previous SSP Recommendations for AI in Cheetahs

The Cheetah SSP previously recommended that AI be used to enhance reproduction in 9 specific females with low mean kinship values (i.e., underrepresented in the North American SSP population). Females designated for AI were: SB# 249, 330, 359, 387, 429, 430, 513, 1834, 1907. Six of the 9 (66.7%) females (SB# 249, 330, 359, 387, 429, 430) were 10 to 12 years of age at the time of AI. All recommended AIs were conducted by a team of reproductive physiologists from the National Zoological Park's NOAHS Mobile Laboratory. All 9 females demonstrated ovarian activity following ovulation induction with gonadotropins (200 IU eCG/100 IU hCG), however, ovarian response varied among females. Six females (SB# 249, 330, 359, 387, 513, 1834) demonstrated a high number of unovulated follicles (5 to 16 follicles/female) and a low number of CL (0 to 4 CL/female), resulting in a sub-optimal ovarian response. Each of the 3 remaining females (SB# 429, 430, 1907) produced excellent ovarian activity with a high number of CL (6 to 9 CL/female) and low number of follicles (0 to 2 follicles/female). Pregnancies were produced following AI with fresh sperm in 2 of the females (SB# 429 and 430). In fact, two pregnancies were achieved in each female, demonstrating the repeatability of the AI technique. Female #430 conceived twice on consecutive AI attempts; whereas, female #429 produced 2 litters following 4 AI attempts. Both of these females are wild-caught cheetahs that had never displayed interest in breeding naturally in captivity. Since cubs survived, AI now has captured these valuable wild genes to contribute further to the zoo-managed species program.

Current SSP Recommendations for Assisted Reproduction in Cheetahs

The North American Cheetah SSP recognizes the value of assisted reproduction, specifically artificial insemination with fresh or

cryopreserved sperm, as a useful management tool. The Cheetah SSP is the first to ever successfully use cryopreserved sperm transported internationally for producing genetically valuable young. This strategy is allowing the incorporation of new genes into North America without removing additional animals from the wild. For this reason, the SSP continues to endorse and support this strategy as a model that will be applicable to other SSP species.

The Cheetah SSP recommends the following actions:

1. When necessary to facilitate management of the SSP population, AI should be used as per SSP recommendations.
2. No cryopreserved sperm from Namibian cheetahs should be used (with the exceptions described below), because sufficient founder genetic diversity currently exists in the North America.
3. The cryopreservation program should be continued by:

a. further development of the cooperative program with the Cheetah Conservation Fund and the Namibian Ministry of the Environment.

b. the systematic collection, storage and use of cryopreserved sperm from genetically valuable males in North America.

The priority is to first use sperm from living males presently in North America and to maintain the cryopreserved Namibian sperm as a reservoir of genetic material. The cooperative program with Namibia also will help preserve and protect the extant genetic diversity of the in situ population. There is a need to develop formal methods for obtaining germ plasm in exchange for conservation assistance in Namibia.

When assisted reproduction is necessary, the first priority for selection of the sperm donor is to use fresh sperm from the living male in North America. The second priority is to use cryopreserved sperm from designated males in North America. The third priority is to use the cryopreserved sperm from Namibian donors. The exception to the latter are the AI's using cryopreserved Namibian sperm previously approved.

4. There is a need to develop a formal genome resource banking (GRB) Action Plan for the cheetah to help address issues related to collection, tracking, storage, ownership, accessibility and use. A Tiger GRB prototype Action Plan already is available and this plan should be modified to fit the management needs for cheetahs in North America.
5. A high priority relevant to the Action Plan is the issue of germ plasm ownership. The SSP coordinator will develop a draft statement that will serve as a framework for developing a regional policy final recommendation.

REFERENCES

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