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Abstract: In February 1994, a multi-institutional, scientific team from the NOAHS Center, the Oklahoma City Zoological Park and the Columbus Zoo embarked upon a collaborative study with the Cheetah Conservation Fund (CCF) in Namibia. The project was conducted in full cooperation with Namibian wildlife authorities and a host of Namibian veterinarians, scientists, teachers, students and local farmers. The purpose was to: (1) develop a long-term, positive collaboration with Namibian wildlife officials and researchers; (2) characterize the clinical health, genetics and reproductive physiology of wild-caught Namibian cheetahs maintained in captivity while assisting the CCF in providing husbandry/management advice to managers holding cheetahs in captivity; (3) cryopreserve cheetah spermatozoa for import to be used in the North American Cheetah Species Survival Plan (SSP) propagation program; (4) provide lectures and 'hands-on' training to wildlife veterinarians interested in learning state-of-the-art techniques for inducing anesthesia and monitoring the health, genetics and reproductive status of cheetahs as well as other species; and (5) educate high school undergraduate university student into the importance of the cheetah as a natural resource and the usefulness of biotechnology as a tool in conservation biology. This report briefly summarizes our impressions and scientific findings to date. Obviously, much of the biological material remain in a raw state. Nonetheless, we already have collected and interpreted a substantial amount of information of interest, which is the heart of this report.
Summary Report

Health, Genetics and Reproductive Physiology of Namibian Cheetahs and the Collection and Storage of Spermatozoa, Blood and Tissue

March 28, 1994

by

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Support provided by the Cheetah Conservation Fund, British Airways, Caldwell Zoo, Columbus Zoo, Ft. Worth Zoological Park, Oklahoma City Zoological Park, Rio Grande Zoological Park and White Oak Conservation Center
Major Summary Points

1. The Cheetah Conservation Fund (CCF) is having a profound effect on elevating the profile and importance of cheetahs in Namibia. Attitudes about the species appear to be changing towards an emphasis on value and the importance of conservation.

2. In general, there is enthusiasm and interest by Namibian wildlife authorities and scientists in developing long-term cooperative projects with foreigners. The emphasis, however, needs to be on problem-solving research and providing supplementary training to locals.

3. Most cheetahs held in captivity in Namibia are being maintained as an avocation, and more attention needs given to improved facility design and appropriate diets. There is a direct link in this population between level of care in captivity and health and general appearance.

4. A total of 19 cheetahs (16.3) were anesthetized safely for data collection. Overall health status ranged from marginal to excellent, which may have been related to time in captivity and/or nutrition.

5. Namibian cheetahs (recently wild-caught and long-term in captivity) harbor gastric bacteria and these are observed in the absence of any grossly detected stomach lesions.

6. Namibian cheetahs are largely free of exposure to feline immunodeficiency virus (FIV), based on earlier and present sampling. This provides strong imperative toward cryopreserving semen and blood samples for reproductive and genetic studies from a virus-free population.

7. Cheetahs throughout Namibia produce ejaculate characteristics generally equivalent to those measured in free-ranging and captive cheetahs in other regions of the world. Even free-ranging, recently captured males produce many malformed sperm, but general ejaculate quality is greatly improved in recently trapped males or captive males maintained on high quality (vitamin/mineral supplemented) diets.

8. Five 'marginal' and nine 'good' quality ejaculates were cryopreserved and imported for use in the North American Cheetah Species Survival Plan (SSP) propagation program.

9. There was enthusiastic response to formal lectures about our research mission, the status of captive breeding programs in North America and our practical demonstrations on various technical procedures to veterinarians and to high school and undergraduate university students.

10. Research findings have motivated us to make a series of specific recommendations (see page 8) including considering the importance of supporting CCF and conducting a population/habitat viability assessment (PHVA) for the cheetah within Africa.

Background and Introduction

In February 1994, a multi-institutional, scientific team from the NOAHS Center (National Zoological Park, Smithsonian Institution and the National Cancer Institute), the Oklahoma City Zoological Park and the Columbus Zoo embarked upon a collaborative study with the Cheetah Conservation Fund (CCF) in Namibia. The project was conducted in full cooperation with Namibian wildlife authorities and a host of Namibian veterinarians, scientists, teachers, students and local farmers. The purpose was to:

1. establish a dialogue and to develop a long-term, positive collaboration with Namibian wildlife officials and researchers while serving as ambassadors for our various home institutions, the American Association of Zoos and Aquariums (AZA) and the IUCN/SSC Captive Breeding Specialist Group (CBSG).

2. characterize the clinical health, genetics and reproductive physiology of wild-caught Namibian cheetahs maintained for short versus long times in captivity while assisting the Cheetah Conservation Fund in providing husbandry/management advice to managers holding cheetahs in captivity.
3. cryopreserve cheetah spermatozoa for import to be used in the North American Cheetah Species Survival Plan (SSP) propagation program.

4. provide lectures and 'hands-on' training to wildlife veterinarians interested in learning state-of-the-art techniques for inducing anesthesia and monitoring the health, genetics and reproductive status of cheetahs as well as other species.

5. educate high school and undergraduate university students into the importance of the cheetah as a natural resource and the usefulness of biotechnology as a tool in conservation biology.

The project was supported by the National Zoological Park, the Oklahoma City Zoological Park, the Columbus Zoo and the National Cancer Institute, all of which provided the expertise of the six U.S. team members: David Wildt, Mitchell Bush, JoGayle Howard, Jack Grisham, Lynn Kramer and Stephen O'Brien. Excellent on-site coordination of all activities and financial support for blood analyses (hematology, chemistry and serology) were provided by Laurie and Danny Kraus of the CCF, who also donated their volunteer staff, vehicles and valuable time. Financial support for air and ground travel, equipment shipping, lodging and meals was generously provided by British Airways, Caldwell Zoo, Columbus Zoo, Ft. Worth Zoological Park, Oklahoma City Zoological Park, Rio Grande Zoological Park and the White Oak Plantation. The team also benefited from special friends of the CCF (especially CCF board members Cindy and Gordon Olsen and Wes and Mary Kruger) who magnanimously opened their homes for several evenings of free lodging and meals.

This report briefly summarizes our impressions and scientific findings to date. Obviously, much of the biological material (especially blood and tissues for genetics, blood for hormonal analysis and tissue from gastric biopsies) remain in a raw state. More specific data from these biomaterials will be generated in the coming months. Nonetheless, we already have collected and interpreted a substantial amount of information of interest, which is the heart of this report.

Impressions on the Work of the Cheetah Conservation Fund (CCF)

Namibia has the largest remaining population of free-living cheetahs in the world, but 95% of these live outside conservation areas on farmland where they frequently conflict with livestock and game ranchers. The natural history (including the biology and habits) of this species in Namibia has largely gone unstudied, perhaps because the vast scrub-like and fenced habitat of the country makes the cheetah difficult to monitor. The CCF was established in 1990 to develop and implement long-term monitoring, multi-disciplinary research and conservation efforts for the survival of the free-ranging cheetah and its ecosystem in remaining habitats in Africa. CCF became a registered Namibian trust in 1991, and its primary focus is in these areas outside the protected reserves working with the local farming communities to develop ways to reduce conflict between humans and cheetahs. The Fund also plans to develop and implement a management plan that will benefit both the cheetah and the farmer while securing habitat for long-term species survival.

There is no doubt that the Kraus's presence in Namibia is having a positive impact. Despite a small (all volunteer) staff, CCF has made substantial progress in three areas. First, we were much impressed with the overall high profile of CCF within the country as a whole. People know about this non-governmental organization and understand that its mission is to increase the survival of the cheetah and its ecosystem. Thus, Laurie and Danny's efforts at 'grass-roots' communication is beginning to pay dividends. This was best illustrated to us during our last evening in Namibia when a farmer (very familiar with the CCF) contacted CCF about a freshly-caught cheetah which he then drove (2 hours) to us for collection of valuable information. Before CCF, this farmer let it be known that he destroyed an animal like this first without a second thought. This is all part of a process whereby Namibians (including the ranchers) are beginning to understand that these animals can have value. Second, we expect that more attitudinal shifts will be evident as a result of the CCF's ongoing educational programs. It is apparent that a number of
schools, individual teachers and students have been 'turned-on' to conservation through CCF activities that frequently directly involve local children. The third area of progress is in-country research, including independent studies conducted by CCF staff. For example, CCF has collected biological samples from more than 120 animals to assess the overall health and genetic status of the population. It also is conducting a radio-telemetry project investigating the movement patterns of cheetahs through Namibian farmlands. Although data are incomplete, it appears that activity patterns in free-living Namibian cheetahs are considerably different from those reported by others for the East African counterpart. We find these 'adaptive' kinds of differences intriguing and look forward to a final interpretation of the data. However, CCF plays another critical research role by acting as an invaluable liaison between other researchers and appropriate Namibian officials, scientists and managers. For example, our study would have been difficult, if not impossible, without the preemptive efforts of CCF in coordinating access to animals, gaining official approvals and generating appropriate permits.

Establishing a Cooperative Rapport with Namibian Colleagues

The North American delegation made considerable progress in beginning to understand the biopolitics of wildlife, and specifically cheetah conservation in Namibia. Detailed discussions on this issue and the common missions of zoos and wildlife researchers were met with substantial enthusiasm by most local colleagues. CCF arranged two very important meetings which involved discussions with authorities in the Namibian Ministry of Wildlife, Conservation and Tourism (including with Ben Ulanga, Deputy Minister, and staff) and with the Research Institute of the Etosha National Park (including Milan Lindique, Head of Research, and staff). All discussions stressed the importance of networking and global/regional cooperation and the willingness of North America to assist by (a) coordinating or participating in projects, when invited, (b) providing timely reports on research findings and (c) offering technical advice and hands-on training. Some of the discussions were quite frank because in certain arenas, it is apparent there is anti-foreigner bias. This has been precipitated by the traditional assumption that Americans (especially those associated with zoos) are only wildlife 'users' with little interest in the developing country itself or formulating practical solutions to management problems. There also is the assumption that Americans want to 'dictate' wildlife management/ use. Therefore, much of our dialogue focused upon the role of zoos in active field conservation research and the development of integrated in situ ex situ management plans. We found that many recent examples (like the black-footed ferret and Florida panther) were useful in illustrating our points effectively. We also stressed that, although basic research frequently is the focus in captive populations, this information is key to finding practical solutions. For example, we emphasized how 15 years of basic research in the domestic cat now was allowing assisted reproduction to be incorporated into captive management plans. Finally, we emphasized that our only interest in becoming involved was at the total discretion of the local host country. We believe these discussions alleviated some of the concerns, and we were assured that Namibian researchers would actively endorse and encourage assistance and participation by U.S. colleagues in the future. Additionally, these meetings seem to have benefited the CCF directly; for example, there was considerable interest by Etosha National Park staff in the CCF cheetah radiotracking and health surveys and mutual commitment to exchange information including blood samples for disease tracking.

We also developed a strong rapport with various U.S. Embassy staff including Ambassador McCallie who was keen to learn about American cooperative research in Namibia and is very supportive of CCF efforts. He enthusiastically questioned us about our respective backgrounds, home institutions and research programs. He and his wife also hosted a dinner party at his home for the team to meet the CCF's local Namibian Board of Trustees as well as many Namibian wildlife, scientific and academic authorities. This gave us the opportunity to meet, among many others, the Minister of Wildlife, Conservation and Tourism who is keenly interested in the viability of cheetahs and leopards in Namibia. As a final example of the Embassy's endorsement, we were delighted that Ambassador McCallie spared an entire afternoon to observe
one cheetah evaluation, including anesthesia, semen collection/cryopreservation and gastric biopsy procedure at a farm near Windhoek.

The Health and Biology of Cheetahs

**Overall data collection strategy.** Raw materials were collected from living cheetahs that were scattered across the country. We took advantage of our Mobile Laboratory Team approach, moving boxes of equipment and supplies from one location to another. During the 16 day expedition, the team logged more than 4,500 highway kilometers. Generally, we radiated out from a local town, usually driving 1 to 3 hours, arriving at a farm, discussing strategy and gaining final approval from the owner, collecting data and then leaving late the same evening after the animals had recovered safely from anesthesia. During the day, we spent considerable time having discussions with the family, their associates, friends and local veterinarians. At the end of each visit, management advice was provided that usually involved recommendations on animal housing and especially improving diets (see below). At the end of the project, all data for a specific farm (including health and reproductive findings) were summarized into a standardized computer format and mailed to the farmer with a thank you cover letter from the CCF.

**Housing, anesthesia, general health and diet.** Most Namibian farmers holding cheetahs do it as an avocation, not as an actively managed population for the purpose of captive propagation. All captive cheetahs in Namibia are registered in the International Cheetah Studbook. Our general impression was that the level of care reflected the attitude that cheetahs are 'abundant' in the country, so the species is considered an 'easy keeper' requiring no specialized housing or dietary care. We believe that one major benefit of our study will be this and further written reports that will emphasize the need to improve management to enhance the overall health and well-being of captive specimens. These efforts will support the ongoing information used by the CCF and shared with holders of captive cheetahs.

A common problem was facility design. Cheetahs usually were maintained in fair-sized enclosures with no ability to isolate individuals either for handling or for transfer to another site. With the exception of one farm, cheetahs were maintained as singletons or in same-sex groups. Feeding sites, adequate shade, maternity dens and daily fresh water usually were absent. Enclosures ranged in size from 25 to 500 square meters with construction materials including chain link, field fencing and 'chicken' wire. It was not unusual for cheetahs to be held behind a perfectly vertical fence wall (no overhang) no more than 2 to 3 meters high. Substrate primarily was dirt with native grasses, scrub plants and some trees (often numerous thorny plants).

In most cases, it was very difficult to locate animals within the enclosures. In two facilities, animals were not individually identified. Generally, it was fairly simple to dart the first animal in a given enclosure, but then all subsequent captures became challenging. Pen design lacked adequate transfer/shift areas which sometimes resulted in the wrong animal or wrong gender being identified. At one site, poor facility design caused a male to be darted twice (on consecutive days). The lack of isolation sites within the larger enclosures and prolonged darting attempts also caused several cases of animal excitation and hyperthermia, all of which were effectively treated (via the use of cold packs, corticosteroids and intravenous fluids).

A total of 19 cheetahs (16.3) were anesthetized for data collection, and all recovered uneventfully. As during previous studies, Telazol proved to be a safe and reliable anesthetic for the various required manipulative procedures. A single intramuscular injection (180 mg) of Telazol for adult males provided about 1 to 2 hours of surgical anesthesia which allowed electroejaculation followed by endoscopic gastric biopsy (to evaluate the presence of spiral bacteria, see below). Females were effectively anesthetized for gastric endoscopy using 150-180 mg (intramuscular) Telazol. In four animals, Telazol anesthesia was induced on two or three occasions over a 4 to 7 day range with no adverse effects. In our opinion, Telazol continues to be the anesthetic of choice for this 'field' type of study, largely because it provides consistent, rapid, safe results even when multiple anesthesias are required in animals of unknown health status.
The health status of the study animals varied widely, likely due to a general reluctance to provide high quality (and expensive) diets. We believe it was no coincidence that the thinnest, most robust cheetahs producing the best quality semen were most recently captured from the wild and/or were receiving the greatest care and optimal diets. Overall, cheetah health ranged from marginal to excellent, depending on the level of management knowledge of the holder. For example, cheetahs maintained on-site by the CCF were in excellent condition because of optimal diets. In fact, we came to the conclusion that diet probably was the major factor regulating observed differences among animals in general condition and seminal quality (see below). Diets were eclectic and included horse, donkey, zebra, kudu, oryx, domestic cow, warthog and guinea fowl, and the quality of meat ranged from fresh to aged (from road kills). The majority of animals were not provided daily vitamin/mineral supplementation which appeared related to a shabby, matted hair coat. In contrast, cheetahs provided frequent (multiple days/week) supplements of bone meal and/or calcium/minerals (in the form of Calsup®) appeared healthier. Regardless of diet used, we detected minimal dental disease among the study animals.

Blood samples were collected from all animals for clinical hematology and serum chemistries. The majority of the animals had been collected previously, and these subsequent samples allowed further monitoring short- and long-term effects of captivity. Complete blood counts detected normal values for hemoglobin, hematocrit, red and white cell counts, MCV, MCHC and differentials (including neutrophils, lymphocytes, monocytes, eosinophils and basophils). Mean (± SEM) chemistry results (compared to domestic cat values) revealed numerous abnormalities in these cheetahs including decreased alkaline phosphatase (34.6 ± 11.7 U/L; normal range for domestic cats, 100-150 U/L) and elevated levels of SGPT (67.9 ± 10.2 U/L; normal, 5-30 U/L), SGOT (63.9 ± 26.7 U/L; normal, 0-30 U/L), blood urea nitrogen (15.5 ± 1.4 mmol/L; normal, 7-10 mmol/L) and creatinine (180.5 ± 8.4 µmol/L; normal, 80-140 µmol/L). These results suggest abnormalities in liver and kidney function. An inappropriate calcium/phosphorus ratio of 1 to 1 (normal, 1.5-2 to 1) also was detected, which may be associated with inadequate dietary calcium.

Blood samples also were obtained from all cheetahs and processed for plasma, leukocytes and erythrocytes. Plasma will be tested for antibodies to the feline pathogens including feline immunodeficiency virus (FIV), feline infectious peritonitis virus (FIPV) and feline leukemia virus (FeLV). The frozen leukocytes and erythrocytes will provide a source of DNA analysis of the population genetics of the cheetah and the virus genetics of infected individuals including FIPV and the recently discovered 'blue tongue' virus found in erythrocytes. Aliquots of plasma were shipped to a veterinary diagnostic lab in the Republic of South Africa for evaluation of FIP and FeLV. All cats tested negative for FIPV, and one of the 19 cheetahs tested positive for FeLV. This particular male had been housed with a FeLV-positive female that had died recently. Verification of the FIPV and FeLV results and assessment of other feline diseases including FIV will be conducted by either Washington State University or the National Cancer Institute.

**Gastric integrity.** The Cheetah SSP pathology advisor, Dr. Linda Munson, has documented chronic gastritis as occurring among cheetahs maintained in 16 North American zoos. Several institutions have reported cheetahs with clinical and severe symptoms of chronic vomiting. Preliminary studies have revealed the presence of several types of spiral bacteria, the most common being *Helicobacter acinonyx*.

We saw our original plan to intensely study genetics and reproduction also as a unique opportunity to determine the existence and prevalence of gastritis in recently captured versus chronically-maintained cheetahs. Eighteen (15.3) cheetahs were evaluated that had been in captivity 2 days to 12 years. The collection procedure was consistent with earlier methods. A flexible endoscope was used to subjectively evaluate the mucosal surface of the stomach for ulcers and hyperplasia. Ten to 15 pinch biopsies were taken from the cardia, fundus and pyloric regions. Most of these were fixed for histological evaluations; however, some biopsies also were used on-site in an assay to determine the presence of urease, an indirect index of the presence of gastric bacteria.
After interviewing the owners, in no case was there any history of chronic vomiting. The mucosal surface of all cheetahs was grossly normal. Seventeen of the 18 cheetahs (94%) were urease positive (including the male in captivity for only 2 days), indicating a high prevalence of gastric spiral bacteria, regardless of length of time in captivity. None of the cheetahs had discernible mucosal lesions. Spiral bacteria have been detected in recently dead wild cheetahs, and these findings now confirm its presence in recently wild-caught animals.

We now know that (1) Namibian cheetahs (recently wild-caught and long-term in captivity) harbor gastric bacteria and (2) these are observed in the absence of any grossly detected stomach lesions. This finding has practical application, suggesting that management decisions to move cheetahs among institutions (to optimize propagation) should not necessarily be influenced by the presence of gastric bacteria in any given population. These findings also warrant further investigation of alternative etiologies of gastric ulcers and chronic vomiting in cheetahs. We predict that more definitive statements will be possible after histological evaluations are completed.

Genetics. The genetic structure of free-ranging cheetahs, including individuals in Namibia, is genetically reduced relative to other felids due to a historic demographic reduction (population collapse) recently estimated to have occurred at the end of the Pleistocene, approximately 10,000 years ago. Accumulation of rapidly evolving gene families (mini and micro-satelites) allows for estimation of kinship among sampled individuals from the same area. This information is useful for selecting release sites and breeding partners in captive settings, as well as for monitoring the population’s recovery from the historic population bottleneck.

Reproductive physiology, including semen collection/cryopreservation. Sixteen males were anesthetized for electroejaculation. Three males were collected on two or three occasions both to study the kinetics of sperm output over a short interval and to maximize sperm cryobanking from outstanding donors. Cheetahs in North American zoos, including many malformed (64 to 99%) sperm per ejaculate. The mean for various testicular and seminal traits are depicted in Table 1.

There was no clear relationship between the duration in captivity and semen characteristics, largely because most animals had been in captivity for > 30 months. It was important to note that seven of the best quality ejaculates (in terms of total sperm production and percent normal forms) were produced by three males recently captured from the wild (range, 2 days to 3 weeks in captivity). Nonetheless, all of these males produced many sperm pleomorphisms (64%) reconfirming that structurally-abnormal sperm in the cheetah ejaculate is not a consequence of captivity, but an inherent (likely genetically-derived) trait of the entire species.

Twenty-two ejaculates were collected from 16 males. Of these, eight were judged poor or suboptimal in quality and were characterized only. Another five ejaculates were deemed 'marginal', were cryopreserved, but have a high likelihood of not being useful for artificial insemination. Nine ejaculates were estimated to be good-to-excellent quality and were cryopreserved for use in the North American SSP program. These samples now are technically the property of the Cheetah SSP, and their use will be regulated by the Cheetah Working Group under the leadership of the Cheetah SSP Coordinator.

Regardless of the number of ejaculates cryobanked, there was a general concern about the incidence of acrosomal damage in electroejaculates (range, 8 to 50%). Historically, we know that these sperm do not withstand cryopreservation well, and this characteristic will be assessed in laboratory tests in the near future. Additionally, it was interesting that one male was aspermic (no sperm produced). This was a highly unusual finding (only one of 60 adult male cheetahs in the original North American cheetah survey was aspermic), and we suspect that this specific case was related to poor dietary mineral/vitamin supplementation.

One of the best single ejaculates was produced by a male only 28 months of age. Ejaculate characteristics in this young male were comparable to those of the best (and much older) males measured in the North American SSP-sanctioned survey conducted in 1992. However, it was interesting that this male was re-examined 4 days later, and only a few motile sperm were recovered. We interpret this new information to mean that males as young as 30 months of age can
produce ejaculates comparable in quality to ‘prime’ age counterparts. However, there may be an age-related inability to sustain spermatogenesis at a high level until a more advanced age, and it remains unknown if these young males express equivalent libido to older cheetahs.

Adult (> 4 year old) cheetahs electroejaculated on three occasions over a 4 to 7 day interval, in general, were able to sustain semen volume and sperm concentration. However, the average number of malformed sperm increased by 7 to 15% by the second or third collection (Table 2). This increased number of pleiomorphisms resulted from an increase (9 to 23%) in abnormal acrosomes (Table 2).

Blood sera (two samples per animal) were collected from each male, will be assessed for serum testosterone and compared to previous endocrine data collected from captive and free-living cheetahs.

**Training and education.** Our contributions to conservation can be increased exponentially by transferring our skills and talents to managers and explorers of biodiversity in other countries. One important new component of the Mobile Laboratory Team in ensuring that there is a training component to all field studies abroad. This was particularly appropriate in the case of this project because a primary mission of the CCF is education. Training/education was provided in three ways.

1. The CCF organized a 2 day Wildlife Seminar in Biotechnology for Namibian veterinarians. The seminar, held in Otjiwarongo, was attended by approximately 40 participants including four students of the Otjiwarongo High School who were preparing a science project on cheetah conservation for participation in CONSERVO, a national environmental competition. A briefing book (see enclosure) was provided to all participants that included: a) acknowledging contributors to the project; b) schedule of events; and c) various scientific papers relevant to seminar topics. Jack Grisham, North American SSP Cheetah Coordinator discussed the status of captive breeding in North America with an emphasis on the importance of SSP-like programs. David Wildt addressed the relevance of global/regional planning, the mission of the CBBG and the general reproductive research efforts of NOAHS Center including the development of genome resource banks. Stephen O’Brien discussed the application of molecular genetics to conservation biology issues, relating the importance of gene diversity to the health and welfare of African species. Mitchell Bush explained the new and emerging role of the veterinarian in conservation biology, while providing specific clinical cases relevant to free-living species viability. Specific disease/management challenges of cheetahs in North American zoos were addressed by Lynn Kramer. A final presentation was provided by JoGayle Howard who discussed specific aspects of using assisted reproduction for helping manage captive and free-living species. All presentations were followed by a question and answer session. The following day, a ‘practical’ demonstration was provided by team members at the Otjiwarongo Veterinary Clinic to the participants. Demonstrations including use of anesthetics, dart preparation and delivery, anesthesia monitoring, electroejaculation, semen evaluation/processing/cryopreservation and endoscopic gastric biopsy.

2. During 2 days of collecting data at the CCF base (near Otjiwarongo), high school students (from the Conservo Club, Otjiwarongo Secondary School and the Science Club of the German High School) were invited to attend and watch all aspects of anesthesia, semen collection/processing and gastric biopsying. Approximately 40 students and their science teachers enthusiastically participated asking many questions and showing obvious interest in cheetah conservation and the science associated with our work.

3. The team also provide a morning series of lectures (same as described in #1 above) to undergraduates at the University of Namibia and Technikon. The audience was comprised of faculty and students from the zoology and wildlife departments.

**Recommendations**

The data summarized in this report has motivated us to make the following general recommendations:
1. There is a need to continue a longitudinal dialogue with wildlife researchers in Namibia (especially in the Etosha National Park), demonstrating the tremendous potential of cooperation. Continued dialogue with the Namibian government and local veterinarians also is warranted to emphasize in situ cheetah conservation and cooperative research. It is important that these new contacts receive the Cheetah Newsletter, the NOAHS Center Newsletter, CCF Newsletter, all relevant AZA and CBSG reports and recent scientific publications.

2. In general, farmers maintaining cheetahs in captivity need to improve their management standards. The CCF already is attempting to make these changes, but old attitudes 'die hard'. The development of a 'basic fact' sheet could be distributed to farmers maintaining cheetahs. We hope that our findings of suboptimal reproductive characteristics in some individuals will provide motivation to improve diet. There also is the need to design all new facilities to ensure that vertical fences are at least 3 meters high with 0.6 meters of overhang and that all enclosures have the capability of allowing animal restraint and transfer. Adequate shelter and a clean supply of water are essential, and a separate 'feeding' yard is preferable. If propagation is an objective, then maternity areas need to be constructed.

3. There is a need to establish or continue to explore the following research issues systematically in cheetahs: a) the measurable impact of supplementary calcium and vitamins on ejaculate characteristics; b) an empirical assessment of the specific nutrient deficiencies of various diets fed cheetahs maintained on Namibian farms; c) determining why cheetahs develop gastric ulcers and clinical symptoms in captivity (i.e., is it stress-induced from crowding or small caging?); d) developing methods allowing the culture of gastric biopsies under field conditions, serologic testing and the effective treatment of gastric ulcers; e) continuing disease screening for evidence of feline pathogens; f) assess the incidence of anthrax in free-ranging cheetahs and the potential development of an effective vaccine; and g) long term monitoring of African populations for FIV exposure and the probable introduction of FIV into Namibia.

4. Given the gradually increasing success rate of natural and assisted (via artificial insemination) reproduction in the North American Cheetah SSP, major captive breeding of cheetahs within Africa appears illogical, especially if the purpose is to meet market demands of other regions including the U.S. We predict that in the future, our need for new genes can be met using germ plasm recovered from free-ranging, briefly captured males (and perhaps females) using the field approach developed in this project. Rather than concentrating on further development of captive breeding within Africa, more emphasis should be on supporting cheetahs living in native habitat. For Namibia, this includes developing novel approaches for reducing conflict between the cheetah and the farmer, the mission of the CCF. For this reason, and because progress is being made, it appears prudent that we and our North American colleagues find ways of (a) supporting the excellent work of the CCF and (b) assisting in developing regional plans to protect the cheetah in situ. Given the high profile of the species world-wide, the substantial biological database and its continued precarious status, this species appears to be an ideal candidate for a population habitat viability assessment (PHVA), facilitated by the CBSG, led by appropriate Namibian authorities and with the cooperation of many experts from throughout continental Africa.
Table 1. Comparison of semen and testicular traits in Namibian and North American cheetahs.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Namibian cheetahs (n = 16 males)</th>
<th>North American cheetahs (n = 60 males)</th>
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<tbody>
<tr>
<td>Number of ejaculates</td>
<td>22</td>
<td>60</td>
</tr>
<tr>
<td>Testicular volume (mm³)</td>
<td>18.1 ± 0.2</td>
<td>13.9 ± 0.4</td>
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<tr>
<td>Ejaculate volume (ml)</td>
<td>1.7 ± 0.3</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Sperm concentration (x10⁶/ml)</td>
<td>17.6 ± 5.2</td>
<td>29.3 ± 5.6</td>
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<tr>
<td>Total sperm/ejaculate (x10⁶)</td>
<td>48.5 ± 14.6</td>
<td>67.5 ± 5.4</td>
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<tr>
<td>Sperm motility (%)</td>
<td>85.0 ± 1.5</td>
<td>67.1 ± 2.0</td>
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<tr>
<td>Sperm forward progression (scale, 0-5)</td>
<td>3.5 ± 0.2</td>
<td>3.6 ± 0.1</td>
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<td>Normal spermatozoa (%)</td>
<td>27.5 ± 0.2</td>
<td>21.3 ± 2.0</td>
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Table 2. Influence of repeated ejaculations on sperm morphology and incidence of abnormal acrosomes in Namibian cheetahs.

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<thead>
<tr>
<th>Male 1 (C7M)</th>
<th>Normal sperm (%)</th>
<th>Abnormal acrosome (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>36</td>
<td>15</td>
</tr>
<tr>
<td>Day 4</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>Day 8</td>
<td>15</td>
<td>24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Male 2 (C8M)</th>
<th>Normal sperm (%)</th>
<th>Abnormal acrosome (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td>Day 4</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Day 8</td>
<td>8</td>
<td>37</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Male 3 (C13M)</th>
<th>Normal sperm (%)</th>
<th>Abnormal acrosome (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>Day 5</td>
<td>2</td>
<td>50</td>
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