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Similarity in Ejaculate-Endocrine Characteristics in Captive Versus Free-Ranging Cheetahs of Two Subspecies[1]

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ABSTRACT

Ejaculate-endocrine characteristics were measured in 23 captive cheetahs (Acinonyx jubatus jubatus) in North American zoos and in 8 free-ranging cheetahs (A. j. raineyi) in eastern Africa (Tanzania). A standardized electroejaculation protocol was used, and numbers of motile spermatozoa were similar ($p>0.05$) between groups. Of the spermatozoa collected by electroejaculation, 70.6 ± 3.3% and 75.9 ± 4.4% were morphologically abnormal in the captive "North American" and in the free-ranging, eastern African populations, respectively. Adrenal activity, as measured by an acute, temporal rise and fall in serum cortisol levels during and after electroejaculation, was no different ($p>0.05$) between groups. Although serum luteinizing hormone (LH) levels were less ($p<0.05$) in the free-ranging than in the captive animals, serum testosterone concentrations were similar. The data indicate that the comparatively poor reproductive performance of cheetahs maintained in zoological parks is not attributable to a captivity-induced response afflicting the male. Furthermore, there is no evidence that ejaculate/endocrine characteristics differ between the two subspecies. Because adrenal/gonadal activity and the number of pleiomorphic spermatozoa are similar between the test groups, the results suggest that spermatozoal diversity originates as a result of the extreme genetic monomorphism observed universally in the species.

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INTRODUCTION

Reproductive performance of the cheetah in captivity is notoriously poor, being compromised by a lack of sexual activity and a hyper-

susceptibility to disease (O'Brien et al., 1985, 1986). Because of the sexual inactivity dilemma, we initiated studies of the captive cheetah focusing on the efficacy of hormonal induction of ovulation, laparoscopic characterization of ovarian activity (Wildt et al., 1981; Phillips et al., 1982), and artificial insemination (Wildt et al., 1986c). Failure to achieve any pregnancies after artificial breeding stimulated a number of endocrine studies of male and female cheetahs (Wildt et al., 1984a,b) as well as extensive examination of ejaculate characteristics (Wildt et al., 1983). Of particular interest was the observation of extremely high proportions of morphologically abnormal spermatozoa (71%) in the electroejaculates of captive cheetahs from southern Africa.

The etiology of abnormal spermatozoa, which appears unrelated to sexual abstinence (Wildt et al., 1983) or method of semen collection (Durrant et al., 1985), has at least two possible origins. First, extensive genetic surveys of blood allozyme and cell proteins resolved by electrophoretic analysis revealed an extreme lack of genetic variation in the cheetah (O'Brien et al., 1983). The supplemental finding that unrelated animals accepted allogeneic skin grafts further confirmed that the species was genetically uniform (O'Brien et al., 1985). Together, these observations suggested that the cheetah experienced a severe population contraction in its evolutionary history, having developed a genotype reminiscent of deliberately inbred strains of laboratory or domestic animals. Inbreeding is known to influence male reproductive traits, including spermatozoal integrity in domestic and laboratory animals by producing individuals homozygous for deleterious alleles (Salisbury and Baker, 1966; Krzanowska, 1976; Wyrobek, 1979; Wildt et al., 1982). A second possibility is that increased adrenal function resulting from an inability to adjust to the captive habitat, could adversely affect ejaculate quality, potentially through disruption in gonadotropin or gonadal steroid production (Moberg, 1984; O'Connor et al., 1985; Wildt et al., 1986b).

Little is known about reproduction in the free-ranging cheetah. Taxonomically, the remaining wild cheetahs, probably less than 20,000 (Myers, 1975), have been classified into two major subspecies that exist predominantly in two geographic ranges: 1) *Acinonyx jubatus jubatus*, southern and southwestern Africa (Republic of South Africa, Namibia, Mozambique and Zambia) and 2) *A. j. raineyi*, east-central Africa, Kenya, Tanzania and Uganda (Bourliere, 1963; Guggisberg, 1975; Myers, 1975; Frame, 1984; Caro and Collins, 1986; O'Brien et al., 1986).

Our earlier studies were restricted to captive cheetahs of the *jubatus* subspecies maintained in a single colony in southern Africa. Two questions then evolved. Are ejaculate-endocrine characteristics of the captive southern African population unique to this locale or specific subspecies? To what extent can the finding of aberrant spermatozoal morphology be attributed to the captive habitat? The present study addresses both questions by reporting an extensive reproductive survey of captive cheetahs (*A. j. jubatus*) in North America. Further, to assess the potential influence of environment and taxonomic subspecies, cheetahs (*A. j. raineyi*) free-ranging in native, eastern African habitat were also evaluated.

MATERIALS AND METHODS

Animals

To determine the possible influence of season on results, studbook records (Marker, 1984) were analyzed to establish the distribution of births in zoo-maintained cheetahs in North America. Based on a total of 67 parturitions, births occurred in 10 of 12 mo and in every season of the year. Although 22.4% of all parturitions occurred in October, there was no clear evidence for a distinct breeding season (Fig. 1). Ejaculates and blood were collected from 23 cheetahs maintained in one Canadian and six U.S. zoological parks during 7 different months of the year. All animals were of the southern African subspecies, *A. j. jubatus*, and, with two exceptions, were unproven breeders. Ten had been caught in the wild at indeterminant ages, and the remaining individuals were captive-born, ranging from 1 yr, 9 mo to 12 yr of age. All had resided at each respective zoo for at least 6 mo before sampling.

Captive housing conditions varied among zoological parks. However, all males were maintained in facilities with access to outdoor enclosures and natural fluctuations in daylight. The majority of males (79%) lived with at least 1 but as many as 3 adult females. The remaining animals were housed as singletons or in bachelor pairs or trios, usually with visual, aural and olfactory contact with conspecific females. All males had ad libitum access to water and were fed a com-

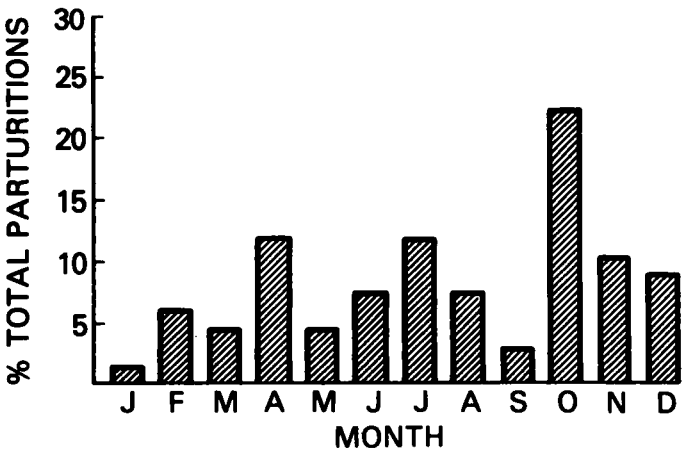

FIG. 1. Distribution of percentage of total parturitions (n=67) in the cheetah based on month of the year.

mercial or noncommercial meat diet consisting of horse, beef, venison and/or chicken with vitamin and mineral supplementation. Animals received from 1.4 to 5.0 kg of meat daily but generally were fasted 1 day/wk.

Free-ranging cheetahs consisted of adult males of the eastern African subspecies, *A. j. raineyi*. A total of 8 males were sampled from the Serengeti ecosystem in Tanzania. Ejaculate and blood samples were obtained in July and August, when males were found in areas of abundant prey. Male cheetahs free-living in the Serengeti National Park can be categorized as territorial or nonterritorial (Caro and Collins, 1986, 1987). The former, which protect areas up to 150 km^2, have yet to be shown to be more reproductively successful than the latter, although territorial cheetahs appear to have a greater survivorship than nomadic males (Caro and Collins, 1987). In the present study, 4 of the 8 animals were known to be territorial and were existing as separate pairs.

A well-defined breeding season does not appear to exist for the free-ranging, eastern African cheetah (Schaller, 1972; Frame, 1984). In a parallel study, abdominal palpations revealed 3 cheetahs to be in the early stages of pregnancy, suggesting that seminal evaluations were being conducted at a time coincident with recent fertile copulations in the study area.

Anesthesia

Captive cheetahs were immobilized for semen/blood collection by using 1 of 2 anesthetic regimens administered by blow dart or hand syringe. On 25 of 29 sampling occasions, males were given ketamine hydrochloride (Ketaset, Bristol Labs., Syracuse, NY, 14.2 mg/kg, i.m.) and maintained in a surgical plane of anesthesia with supplemental ketamine HCl injections (5.5 mg/kg, i.v.). In approximately one-half of these episodes, the animal experienced one or more brief (15–60 s) catatonic seizures while under ketamine HCl anesthesia. At the onset of any third convulsion, diazepam (Valium, Hoffmann-La Roche, Inc., Nutley, NJ, 0.03 mg/kg) was administered i.v. to control the seizures. The remaining 4 captive males were immobilized by using a combination of tiletamine hydrochloride and zolazepam hydrochloride (Telazol, Warner Lambert Co., Ann Arbor, MI, 4.6 mg/kg). In contrast to ketamine HCl, Telazol resulted in a more relaxed anesthesia with less muscular tone and no convulsions. Ancillary sedative therapy was unnecessary, although a surgical plane of anesthesia was maintained with supplemental Telazol doses (2.0 mg/kg, i.m.).

Because of the timidity of the species, free-ranging males were approached by a vehicle used routinely in the Serengeti National Park for cheetah observation (Caro and Collins, 1986, 1987). All animals were dart-injected with Telazol (4.6 mg/kg, i.m.), administered via a blow-pipe extended through the vehicle window. Darted animals usually bolted 30 to 50 m before lying down and becoming recumbent within 10 min. A surgical plane of anesthesia was maintained throughout the blood sampling interval with supplemental Telazol (usually a single injection, 2.0 mg/kg i.m.).

Semen Collection and Evaluation

For the captive, North American group, a total of 29 electroejaculates was collected from 23 cheetahs. Three males were evaluated on 3 occasions each over the course of 3 consecutive years. Electroejaculates from the 8 free-ranging eastern African cheetahs were collected a single time. Semen was obtained by a standardized electroejaculation protocol for this species (Wildt et al., 1983; 1984b). In brief, an electrostimulator and rectal probe were used to administer 80 incremental stimuli given in an on-off pattern in 3 series consisting of 30, 30, and 20 stimuli, respectively (3–5 min between series).

Each ejaculate was collected in a warmed (37°C), plastic container. After each electroejaculation series, the semen sample from each male was analyzed for volume, spermatozoal concentration, motility, and

status (Wildt et al., 1983; 1984b; 1986b). Spermatozoal status (speed of forward progressive motility) was a subjective estimate of the type of forward movement of the sperm cell based on a scale of 0 (no movement) to 5 (steady, rapid forward progression) (Howard et al., 1986). For the captive cheetahs, semen analyses were performed under standard laboratory conditions. For the free-ranging males, motility and concentration values were evaluated immediately in the field using a phase-contrast microscope powered by a portable, gasoline generator. Morphological assessments of spermatozoa from free-ranging individuals were similar to captive males, except that 900 (rather than 300) individual cells (300/electroejaculation series) were analyzed. Pleiomorphic forms were categorized as those related to spermatogenic (primary origin) or excurrent duct system (secondary origin) deformities (Wildt et al., 1983; Howard et al., 1986).

Serial Bleeding

To determine adrenal-pituitary-gonadal hormone patterns, each male was subjected to repeated blood sampling during anesthesia. The interval between the initial injection of either anesthetic and the first blood sample usually ranged from 8 to 12 min. The mean time intervals between the onset of the first electrical stimulus in each of the three consecutive electroejaculation series were 11, 8, and 7 min, respectively. The first blood sample (10 ml) was obtained from the saphenous vein when the darted animal was tractable, usually 2 to 3 min before the plane of anesthesia was suitable for electroejaculation. Subsequent samples were collected after each stimulus series and 30 and 60 min after the last electrical stimulus (6 samples/male) for a composite bleeding period of ~ 86 min. Blood tubes were allowed to clot for 1 h before centrifugation and serum recovery. Sera from captive cheetahs were stored at −20°C until assayed. Freshly collected serum from the field study was frozen and stored in liquid nitrogen vapor until the samples could be transferred to a conventional −20°C laboratory freezer.

Radioimmunoassays (RIAs)

All serum samples were analyzed for cortisol, luteinizing hormone (LH), and testosterone using specific RIAs. Cortisol was evaluated with a ^{125}I RIA kit (RIANEN™, New England Nuclear, No. Billerica, MA) previously adapted for use in domestic (Carter et al., 1984) as well as wild felids (Wildt et al., 1986a, 1987), including the cheetah (Wildt et al., 1984b). Minimum assay sensitivity was 0.2 ng/tube and the inter- and intraassay coefficients of variation were 9.5% (n=8) and 8.7% (n=6), respectively. A heterologous double-antibody RIA, previously validated for the cheetah (Wildt et al., 1984a,b), was employed to measure serum LH. Minimum assay sensitivity was 0.12 ng/ml, and the inter- and intraassay coefficients of variation were 9.0% (n=5) and 7.5% (n=8), respectively. Testosterone was analyzed using a ^{125}I double-antibody RIA kit (NOSOLVEX™, Radioassay Systems Labs., Inc., Carson, CA). The first and second antibodies were rabbit antitestosterone-19-carboxy-methyl-ester-bovine serum albumin and goat antirabbit gamma globulin, respectively. The lower limit of assay detection was 0.01 ng/tube, and the inter- and intraassay coefficients of variation were 7.9% (n=8) and 8.5% (n=6), respectively. The first antibody in this RIA was more specific than that from a previous assay measuring serum testosterone in cheetahs (Wildt et al., 1984a,b). Antibody cross-reactivity characteristics and the use of the NOSOLVEX system in the domestic cat (Carter et al., 1984; Goodrowe et al., 1985) as well as two species of wild felids (Wildt et al., 1986a, 1987) have been reported.

Statistical Analysis

Values reported are means ± SEM. Data were analyzed by a Statistical Analysis System computer program (SAS, 1982). Significant differences between variables were determined by two-way analysis of variance and individual means compared by Scheffe's Multiple Range Test (Snedecor and Cochran, 1980). A biomedical computer program (BMDP, 1983) was used to evaluate differences in repeated measures of cortisol, LH, and testosterone.

Analyses indicated that neither the origin (wild-caught or captive-bred) nor the simultaneous presence of females influenced seminal characteristics or endocrine profiles in the captive group ($p>0.05$). Likewise, for the free-ranging population, the classification of males as territorial or nonterritorial had no effect ($p>0.05$) on ejaculate/endocrine traits. Therefore, the primary comparison groups were the captive, North American population of the *jubatus* subspecies vs. the eastern African, free-ranging population of *raineyi* . Because two different anesthesia regimens were used in the captive cheetahs

in North America, these subgroups were compared initially to study the effect of immobilizing drugs on endocrine traits. The data then were pooled and contrasted to that from the free-ranging population.

RESULTS

The numbers of motile spermatozoa/electroejaculate ranged from 0.1 to 136.8 × 10^6 for captive cheetahs in North American zoos, and from 4.8 to 77.9 × 10^6 for those free-ranging in eastern Africa. The mean number of motile spermatozoa/electroejaculate as well as the individual assessments of spermatozoal count/ml of ejaculate, percent motility, and status rating were similar ($p>0.05$) between groups (Table 1). The total proportion of structurally abnormal spermatozoa for the captive and free-living populations ranged from 31.0 to 97.0% and 56.7 to 91.0%, respectively; these averages also were similar ($p>0.05$, Table 1). Overall, for the captive cheetahs, 33.1 ± 3.6 and 39.0 ± 3.9% of the total spermatozoa were classified as primary and secondary pleiomorphic forms, respectively. The proportions in the free-ranging population were similar ($p>0.05$), with 37.4 ± 1.4 and 36.9 ± 2.9% of all spermatozoa categorized with primary and secondary deformities, respectively. In both groups, secondary abnormalities predominated, usually in the form of a spermatozoon with a bent midpiece and/or residual cytoplasmic droplet. The proportion of various abnormalities was similar ($p>0.05$), except captive cheetah electroejaculates contained more spermatozoa with both bent midpieces and cytoplasmic droplets, whereas free-living males had more spermatozoa with bent midpieces without the residual droplet ($p<0.05$). The major primary defect in both groups was a tightly coiled flagellum, although cells with acrosomal damage and micro- or macrocephaly also were evident. Approximately 3% of all spermatozoa had an aberrant midpiece characterized by a partially or completely missing mitochondrial sheath (Fig. 2a). Although infrequent, bicephalic (Fig. 2b) and biflagellate (Fig. 2c) sperm pleiomorphisms also were evident. Proportions of specific primary deformities were similar except that zoo-maintained cheetahs had more ($p<0.05$) microcephalic spermatozoa than did free-living cheetahs (Table 1).

Within the captive, North American group, endocrine profiles were similar ($p>0.05$) in cheetahs anesthetized with ketamine hydrochloride versus those immobilized with Telazol (Fig. 3). Mean cortisol levels differed ($p<0.05$) only in blood obtained after the second (post-series 2) electroejacula-

TABLE 1. Seminal traits in captive South African (*Acinonyx jubatus jubatus*) and free-ranging eastern African (*Acinonyx jubatus raineyi*) cheetahs.

Item	Captive North America	Free-ranging East Africa
Number of males	20	8
Number of ejaculates	29	8
Motile spermatozoa/ejaculate (X10^6)	26.7 ± 5.8	25.3 ± 9.9
Spermatozoa/ml of ejaculate (X10^6)	25.1 ± 4.4	36.4 ± 12.2
Spermatozoal motility (%)	70.7 ± 3.5	63.1 ± 3.9
Spermatozoal progressive status	3.6 ± 0.1	3.8 ± 0.2
Morphologically abnormal spermatozoa (%)	70.6 ± 3.3	75.9 ± 4.4
Primary (%)		
Tightly coiled flagellum	23.2 ± 3.4	32.3 ± 4.2
Acrosomal defect	3.7 ± 1.0	1.7 ± 0.5
Missing mitochondrial sheath	2.8 ± 0.7	3.4 ± 0.7
Microcephalic	2.3 ± 0.4	0.2 ± 0.1[a]
Macrocephalic	1.1 ± 0.3	1.3 ± 0.5
Bicephalic	0.1 ± 0.1	0.03 ± 0.02
Biflagellate	0.0 ± 0.0	0.04 ± 0.03
Secondary (%)		
Bent midpiece with cytoplasmic droplet	17.7 ± 1.5	6.7 ± 1.8[a]
Cytoplasmic droplet	10.9 ± 1.7	6.6 ± 1.5
Bent tail	4.1 ± 0.6	6.4 ± 1.3
Bent midpiece without cytoplasmic droplet	3.8 ± 1.0	15.7 ± 1.1[a]
Bent neck	0.8 ± 0.3	1.6 ± 0.3

[a]Significantly different ($p<0.05$) from counterpart value.

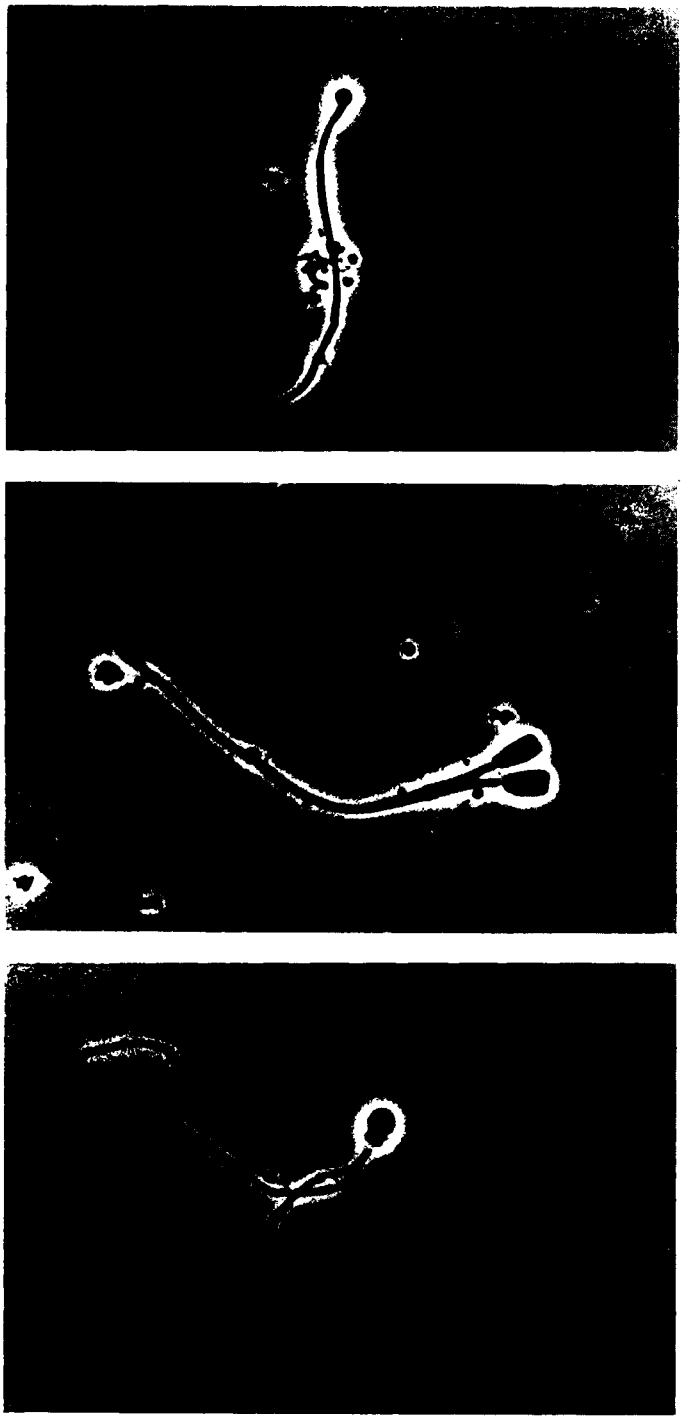

FIG. 2. Spermatozoal forms detected in the cheetah electroejaculate: *a*) microcephalic with missing mitochondrial sheath; *b*) bicephalic; *c*) biflagellate with missing mitochondrial sheath. X 1000.

tion sequence (149.8 ± 8.2 ng/ml in ketamine HCl versus 106.0 ± 23.3 ng/ml in Telazol-injected cheetahs). At all other times, mean concentrations of cortisol, LH, or testosterone were similar, indicating that choice of anesthesia did not influence endocrine results.

Profiles of mean concentrations of cortisol, LH, and testosterone before, during, and after electroejaculation for both cheetah populations are illustrated in Figure 4. Within the captive group, mean cortisol levels were similar ($p>0.05$) at the pre-electroejaculation blood sampling (76.4 ± 6.3 ng/ml) and after the first 30 electrical stimuli (85.1 ± 7.4 ng/ml). A significant rise ($p<0.05$) in serum cortisol was first observed after the second series of stimuli (144.0 ± 8.1 ng/ml) with a further rise and peak of 170.1 ± 8.0 ng/ml detected immediately after the end of electroejaculation. Mean serum cortisol then declined to 101.3 ± 9.1 ng/ml by 60 min after the last stimulation. Serum cortisol in the pre-electroejaculated, free-ranging group averaged 58.5 ± 13.6 ng/ml and also remained at basal levels during the first stimulation series (51.1 ± 9.1 ng/ml). The temporal rise and fall in serum cortisol mimicked that of the captive cheetahs, the

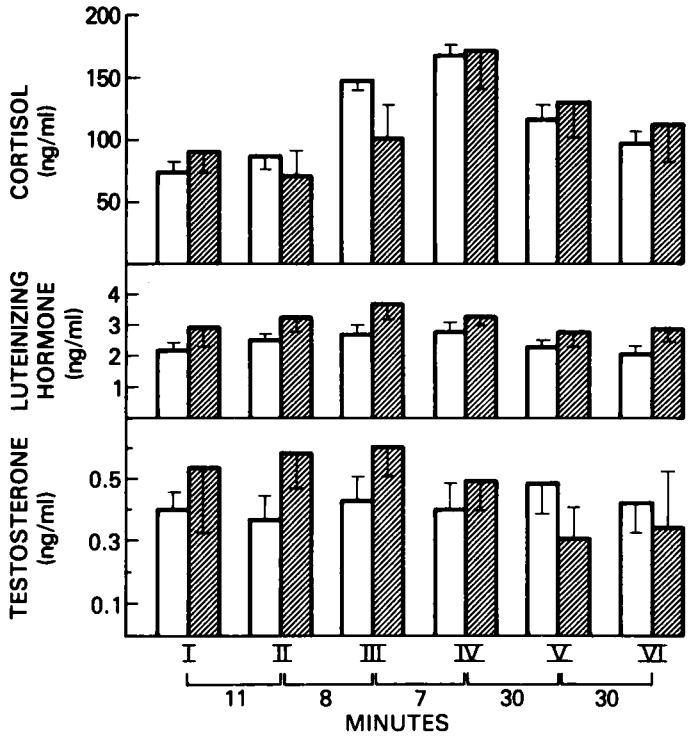

FIG. 3. Mean (± SEM) concentrations of serum cortisol, LH, and testosterone in captive North American cheetahs anesthetized with ketamine hydrochloride (Ketaset, *open bar*, n=25) or tiletamine hydrochloride/zolazepam hydrochloride (Telazol, *batched bar*, n=4). *Roman numerals* indicate time of blood sampling: *I* = post-anesthesia, pre-electroejaculation; *II* = end of electroejaculation Series 1; *III* = end of electroejaculation Series 2; *IV* = end of electroejaculation Series 3; *V* = 30 min after electroejaculation; *VI* = 60 min after electroejaculation.

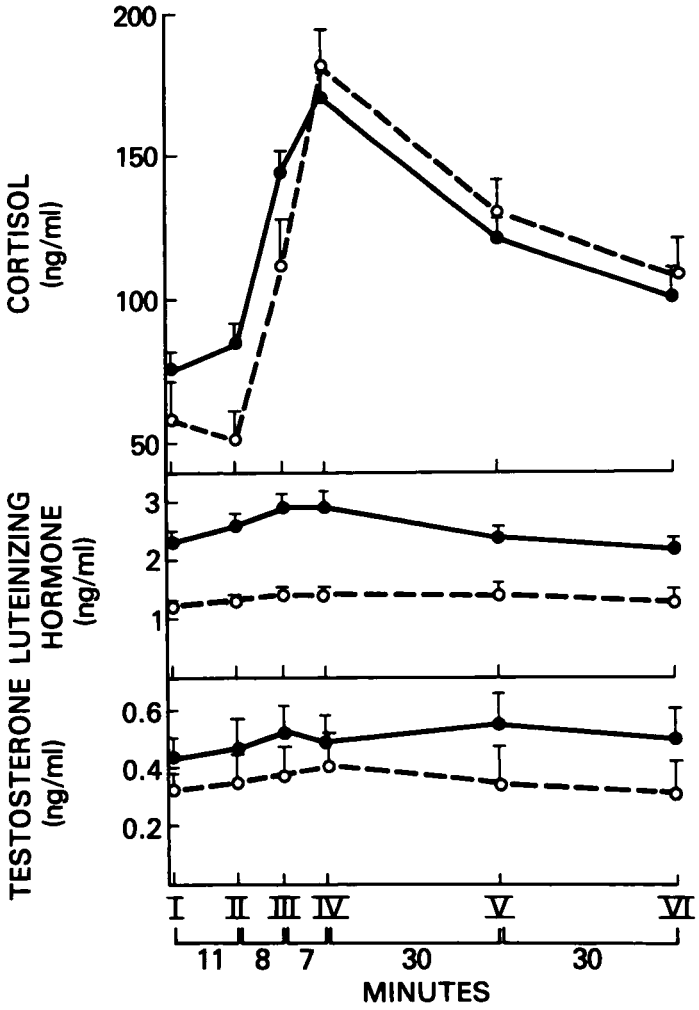

FIG. 4. Mean (± SEM) concentrations of serum cortisol, LH and testosterone in captive North American (n=29, •) and free-ranging eastern African (n=8, ○) male cheetahs. See *Figure 3 legend* for explanation of bleeding times.

first elevation ($p<0.05$) detected 8 min later (112.1 ± 16.7 ng/ml) with peak concentrations (180.3 ± 13.7 ng/ml) coinciding with the end of electroejaculation. By 60 min, circulating cortisol in free-ranging cheetahs averaged 109.6 ± 11.7 ng/ml. With the exception of the second blood sample in which mean serum cortisol concentrations were 60% greater ($p<0.05$) in captive than free-ranging cheetahs, hormonal levels between groups were indistinguishable.

Within captive and free-ranging populations, mean LH concentrations ranged from 2.2 ± 0.1 to 2.9 ± 0.3 and 1.2 ± 0.1 to 1.4 ± 0.2 ng/ml, respectively. Although mean LH patterns did not change over time, concentrations were consistently two-fold greater ($p<0.05$) in the captive than in the free-living animals (Fig. 4). However, the range in mean levels of testosterone for the zoo-maintained (0.44 ± 0.07 to 0.55 ± 0.11 ng/ml) and free-ranging (0.31 ± 0.12 to 0.40 ± 0.13 ng/ml) cheetahs as well as the overall temporal profiles in each group were similar ($p>0.05$, Fig. 4).

In general, endocrine profiles of individual cheetahs mimicked average patterns (Fig. 5). Cortisol level in the first blood sample was <90.0 ng/ml on 22 of 29 occasions in the captive group and in 7 of 8 of the free-ranging males. The range in maximally detected cortisol varied from 95.1 to 267.8 ng/ml and 131.7 to 227.5 ng/ml in the captive and free-ranging groups, respectively. Twenty-three of 29 captive cheetahs and 8 of 8 free-ranging males produced peak cortisol concentrations at the bleeding immediately after the third electroejaculation series. Within individual

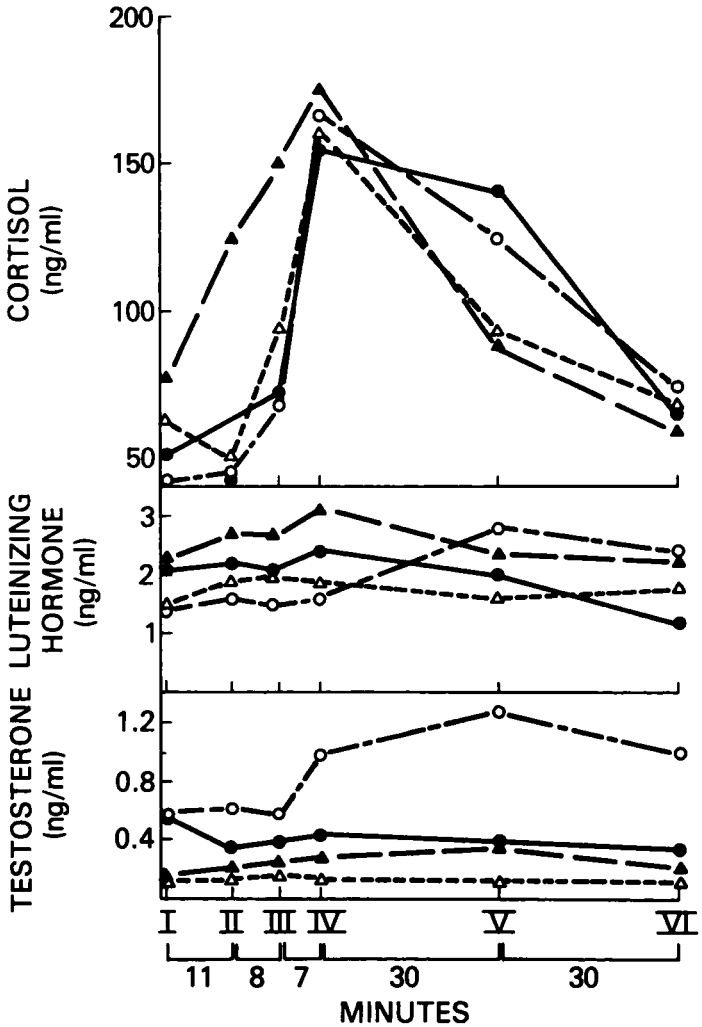

FIG. 5. Serum cortisol, LH, and testosterone concentrations in 2 male cheetahs maintained in captivity in North American (•,▲) or free-ranging in eastern Africa (○,△). See *Figure 3 legend* for explanation of bleeding times.

cheetahs, LH fluctuated by only 4.6 and 1.4 ng/ml in the captive and free-ranging populations, respectively, over the ~ 86-min interval. Concentrations of LH rarely (on only 7 of 29 occasions) exceeded 4.0 ng/ml in the captive cheetahs; LH levels greater than 2.0 ng/ml were observed in only 1 free-ranging male. Testosterone patterns within individual males in both groups usually were stable, although 1 captive and 1 free-ranging cheetah's testosterone concentrations varied by 2.3 and 0.7 ng/ml, respectively. Serum testosterone exceeded 1.0 ng/ml in at least 1 blood sample on only 2 of 29 and 1 of 8 bleeding occasions in the captive and free-ranging groups, respectively.

DISCUSSION

These data represent the first information on reproductive-endocrine characteristics of the free-ranging cheetah and provide a retrospective opportunity for analyzing the influence of captivity on physiological function. This approach appears appropriate for the cheetah, a species that, when evaluated with conventional methods of fertility assessment, demonstrates unique ejaculate characteristics, particularly in spermatozoal morphology. In the present study, the number of motile spermatozoa/electroejaculate was similar between captive and free-living cheetahs of 2 previously classified subspecies. Further, spermatozoal number and motility ratings in each group were similar to values measured earlier in a large captive population of *A. j. jubatus* in southern Africa (Wildt et al., 1983). The similarity in the proportion of pleiomorphic spermatozoa/ejaculate in captive cheetahs (*A. j. jubatus*) in North America (70.6%) and southern Africa (71.0%) and free-ranging cheetahs (*A. j. raineyi*) in eastern Africa (75.9%) indicated that spermatozoal diversity was unrelated to habitat status or taxonomic classification. Overall, the basis of these electroejaculate traits, cheetahs of the 2 subspecies, maintained in captivity in North America or southern Africa, or free-living in eastern Africa, were indistinguishable from each other.

Considerable numbers of spermatozoa were afflicted with primary abnormalities associated with spermatogenic dysfunction. Our recent evaluations of felid spermatozoa have involved more sophisticated microscopy, which has improved detection of subtle defects in acrosomal and midpiece integrity. Consequently, a partial or complete derangement of the acrosomal cap or mitochondrial sheath around the midpiece has been found to contribute significantly to the overall pleiomorphism rate. Recent reports in other wild felids, including the clouded leopard (Wildt et al., 1986b) and tiger (Wildt et al., 1987), have demonstrated spermatozoal micro- and macrocephaly as well as the tightly coiled flagellum and absent mitochondrial sheath defects. At present, the cheetah appears unique, primarily because of the relatively high proportion of aberrant forms among all collected spermatozoa.

On the basis of endocrine function, adrenal activity, as measured by serum cortisol levels, was no different between the captive and free-ranging cheetahs and was comparable to the previous report of captive cheetahs in southern Africa (Wildt et al., 1984b). On the basis of the temporal cortisol profiles, there was no evidence that captive and free-living cheetahs differed in basal adrenal activity or the ability to respond to a manipulatory stress. In both groups, the finding that cortisol concentrations generally were less than 90.0 ng/ml before electroejaculation onset, peaked coincident with the end of the electroejaculatory sequence, and declined over time exactly mimicked earlier observations of captive cheetahs in southern Africa (Wildt et al., 1984b).

Serum testosterone concentrations during the sampling period were similar between captive, North American (0.44 to 0.55 ng/ml) and free-ranging, eastern African (0.31 to 0.40 ng/ml) cheetahs, but slightly less than the mean range of 0.51 to 0.79 ng/ml for the captive colony in southern Africa (Wildt et al., 1984b). This probably can be attributed to different specificities of the testosterone antibodies used in the 2 RIAs. The cross-reactivity of the testosterone antibody with dihydrotestosterone was 56% in the earlier study and only 10.3% in this study. Regardless, serum testosterone concentrations in all 3 groups of cheetahs were only about half the level recently reported in the domestic cat (Carter et al., 1984; Goodrowe et al., 1985) clouded leopard (Wildt et al., 1986a), and tiger (Wildt et al., 1987) using the same RIA system.

Although the range in mean LH concentration did not differ appreciably between captive, male cheetahs in North America (2.2 to 2.9 ng/ml) and southern Africa (3.3 to 5.2 ng/ml) (Wildt et al., 1984b), free-ranging animals generally produced LH profiles ranging from only 1.2 to 1.4 ng/ml. These concentrations, which were reminiscent of levels observed in serially bled, anesthetized females from southern Africa (Wildt et al., 1984a), did not appear related to

electroejaculation-induced adrenal activity since prestimulation LH levels already were low. As LH activity was comparable in captive North American cheetahs anesthetized with ketamine HCl and those immobilized with Telazol, it is unlikely that the reduced LH pattern in the free-ranging group is related to anesthesia. Because LH in captive cheetahs probably is released in an episodic fashion (Wildt et al., 1984b), it is likely that the infrequent sampling protocol in the present study was insufficient to identify sporadic LH pulses. A suggestion that the eastern African cheetah differs from its southern African counterpart on the basis of pituitary LH secretion could only be warranted after further studies involving a more frequent and prolonged blood sampling interval. Nonetheless, the potential differences in pituitary function may be physiologically inconsequential since testosterone concentrations and ejaculate traits were comparable between captive, North American and free-ranging, eastern African populations.

A sufficient data base now is available to assess more accurately the potential variables associated with the poor reproductive performance of captive cheetahs. Because of the similarity in electroejaculate characteristics and adrenal/gonadal function in captive and free-ranging males, there is no evidence that zoo-maintained captivity appreciably influences reproductive or endocrine characteristics. On the basis of behavioral observations and successful captive breeding, zoologists traditionally have presumed that many wild-caught species readily adapt physiologically to the captive environment. However, this premise is confounded when a highly specialized species such as the cheetah fails, routinely, to reproduce in captivity. The issue is complicated further by an extensive array of environmental variables as well as genotype, the latter often severely compromised in small, zoo-maintained animal populations (Ralls et al., 1979). Therefore, the present data are significant because they suggest that in the case of the cheetah, zoo habitat is not necessarily directly detrimental to physiological function or responsible for the difficulty of breeding in captivity. On the contrary, because of the captive cheetah's history of poor reproductive performance and susceptibility to disease (O'Brien et al., 1985), it is rather remarkable that zoological parks have succeeded at all in sporadically propagating the species.

One theory concerning the difficulty of captive management and reproduction of the cheetah is a profound lack of genetic variation in the overall species. Initially an electrophoretic survey of allelic isozyme variation in 55 captive cheetahs in southern Africa (*A. j. jubatus*) revealed an absence of polymorphism for each of 52 loci (O'Brien et al., 1983). More recently, similar analyses have been performed in 43 additional captive North American cheetahs (originating in southern Africa) and in 30 captive and free-ranging animals from eastern Africa (*A. j. raineyi*) (O'Brien et al., 1987). One polymorphic locus was discovered in 2 sibling cheetahs in 1 North American zoo. The same locus as well as a second were found to be polymorphic in the eastern African population. The recalculated frequency of polymorphism for *A. j. jubatus* now is 2% and for *A. j. raineyi*, 4%. Compared to other felid species (Newman et al., 1985), the cheetah clearly has the least detectable genetic variation. All evidence to date suggests that the genetic uniformity of the species probably is the primary contributor to the diverse morphological characteristics of the cheetah spermatozoon.

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