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Abstract: The purpose of the study has been to evaluate and compare reproductive traits in established populations of male Transvaal, South West or hybrid (Transvaal X South West) cheetahs maintained under two captive managements. We have determined that a great proportion of cheetah spermatozoa collected by electroejaculation are morphological abnormal. Furthermore we observed no major differences in reproductive traits based on a comparison of the Transvaal, South West or factor alone. However, a greater proportion of male cheetahs allowed free range with females in a large territorial enclosure produce ejaculates which tend to be of greater quality compared to cheetahs continuously supported in small camps with only occasional female exposure.

REPRODUCTIVE TRAITS IN THE MALE SOUTH AFRICAN CHEETAH

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reproductive-genetic studies in the cheetah are relevant due to this animal's endangered status and unique taxonomic classification as the only species (jubatus) in the feline genus Acinonyx. A question of true subspeciation has evolved, and either two[1], five[2], or eight[3] cheetah subspecies have been recognized. Although all cheetahs in Southern Africa have been classified as Acinonyx jubatus jubatus, a controversy has developed regarding accuracy of existing taxonomic structure[4,1] and the judiciousness of interbreeding cheetahs originating from diverse geographic regions. The purpose of our study has been to evaluate and compare reproductive traits in established populations of male Transvaal, South West or hybrid (Transvaal X South West) cheetahs maintained under two captive management programs. We have determined that a great proportion of cheetah spermatozoa collected by electroejaculation are morphologically abnormal. Furthermore we observe no major differences in reproductive traits based on a comparison of the Transvaal, South West or hybrid factor alone. However, a greater proportion of male cheetahs allowed free range with females in a large territorial enclosure produce ejaculates which tend to be of greater quality compared to cheetahs continuously supported in small camps with only occasional female exposure.

In 1971 the National Zoological Gardens of South Africa initiated a comprehensive program for the captive propagation of cheetahs[5]. The original wild captured breeding stock consisted of males and females from two distinct geographic regions: 1) the northern region of the Transvaal Province of the Republic of South Africa and 2) South West Africa (Namibia). Initial propagative attempts were made at the De Wildt Cheetah Breeding and Research Center. Successful captive breeding at this facility allowed the transfer of adult offspring to the Lichtenburg Nature Preserve and Game Breeding Park

In 1978. These conservation complexes were both located in the Transvaal and were separated by a distance of 220 km.

The female cheetah in southern Africa has been considered seasonally polyestrous, exhibiting overt estrous cycles from December through February[5]. During the breeding season at De Wildt, males were maintained approximately 30 m from the female enclosures. A group of males was released daily near the female camps to monitor the onset of sexual receptivity. Estrous females were then permitted to copulate ad libitum for two to three days with a designated male. Using such methods a total of 175 offspring were produced from 1975 to 1980.

Data from the present study were collected in January, 1981, from 11 Transvaal, three South West and eight hybrid cheetahs of Transvaal X South West ancestry. A total of 15 adult males were supported at De Wildt (seven Transvaal, two South West, six hybrid) and seven males were maintained at Lichtenburg (four Transvaal, one South West, two hybrid). At the former facility, cheetahs were maintained in groups of three to six in one ha fenced enclosures. At Lichtenburg, males were grouped together with seven female cheetahs and accorded free range of a 400 ha fenced enclosure. During the five day interim of data collection at Lichtenburg, the cheetahs were restricted to a one ha fenced camp.

At both locations, individual cheetahs were physically restrained, the saphenous vein isolated and general anesthesia induced by an intravenous injection of CT 1341 (2.0 mg/kg of body weight, Saffan, Glaxo Laboratories). The width and length of each testicle were determined using laboratory calipers, and the measurements converted to volume using a standard conversion formula[6,7].

Semen was collected on one to four occasions/animal using electroejaculation procedures similar to that described for the domestic cat[8]. To permit comparative analysis of ejaculate traits, the electroejaculation regimen was standardized so that each animal was allotted 80 electrical stimuli of similar voltage (four to seven V) and milliamperage (150-200 ma). The pattern of applied stimuli was consistent with a previous report[9]. Semen was immediately evaluated for ejaculate volume, spermatozoa motility (%), progressive motility and spermatozoa concentration (sperm numbers/ml of ejaculate). Speed of progression was a subjective determinant of type of forward movement of spermatozoa using a scale of 1 (lowest rating) to 5 (highest rating). An aliquot of semen from the first ejaculate was fixed in 1% glutaraldehyde and 300 individual spermatozoa assessed under phase contrast microscopy (1000X) for % morphological abnormalities.

Eighteen of 22 cheetahs produced ejaculates containing spermatozoa, the four azoospermic males all being located at De Wildt. Based on a total of 40 seminal collections containing spermatozoa, an average (±SEM) ejaculate consisted of 2.1±0.2 ml of fluid containing 14.5±1.8 X10^6 spermatozoa/ml with a 54.6±3.1% and 3.6±0.1 motility and progressive motility rating, respectively. An average of 71.0±0.9% (range, 41-87%) of the spermatozoa collected/ejaculate consisted of abnormal pleiomorphic forms. Deformities included coiled tails (25.8±2.3%), bent midpieces (23.3±1.1%), bent tails (16.2±1.3%), bent tail tips (2.8±0.6%), protoplasmic droplets (1.3±0.3%) and microcephalic (1.2±0.3%) or macrocephalic defects (0.4±0.2%) (Fig. 1).

There were no differences ($P>0.05$) in ejaculate volume, spermatozoa count/ml of ejaculate, testes volume, spermatozoa motility or % morphological sperm abnormalities among the Transvaal, South West or hybrid males (Fig. 2).

ced of progression of spermatozoa greater (P<0.05) in the South West
cetahs than the Transvaal or hybrid groups. This observation may have been
function of the few number (three) of South West males available.

More dissimilarities were detected when the results were evaluated
the basis of demographic management systems (Fig. 3). Although ejaculate
lume was not different between De Wildt and Lichtenburg males, spermatozoa
unt/ml of ejaculate, speed of progression and % spermatozoa motility
nsignificantly favored the Lichtenburg group. Combined testes volume was
eater (P<0.05) in the De Wildt cheetahs and may have been influenced by
e differences, since De Wildt cheetahs were, on the average, 3.5 years
der (6.5±0.9 years) than their Lichtenburg counterparts (3.0±0.0 years).
erall the Lichtenburg males averaged 8.4% fewer (P>0.05) abnormal spermatozoa
an the De Wildt group. As detailed in Figure 4, Lichtenburg cheetahs
oduced more normal spermatozoa and tended to have fewer spermatozoa with
oiled tails, bent midpieces, proximal droplets and micro- or macrocephalic
eformities.

We conclude from these results that, in general, reproductive traits
tudied do not vary among cheetahs with genetic origins from the Transvaal or
outh West regions of southern Africa. Additionally, hybrids resulting from
rossbreeding Transvaal and South West cheetahs demonstrate similarity in
eproductive characteristics to their "purebred" parents. Based on the
e Wildt versus Lichtenburg comparison, available territorial size and constant
emale exposure may (and may continue to) influence male reproduction in that
greater proportion of Lichtenburg males produce ejaculates which tend to have
reater sperm concentrations and fewer abnormal spermatozoa.

Overall, ejaculate quality in the cheetah appeared inferior compared to previous information obtained in electroejaculated domestic cats. Using similar semen collection procedures, Platz et al.[10] reported an average spermatozoa count and % motility rating of 1.52 $X10^6$ spermatozoa/ml of ejaculate and 70.4%, respectively, for domestic cats. More recent unpublished data from our laboratory indicate that the great proportion of morphological sperm abnormalities observed in the cheetah are not apparent in the domestic cat. Six adult cats exposed to the same quantitative and qualitative electroejaculation stimuli as the cheetah produced ejaculates with 30% or fewer aberrant sperm forms. Additionally, it cannot be reasoned that the elevated number of morphologically abnormal spermatozoa in the cheetah is the result of sexual abstinence or degenerative processes associated with elimination of aged spermatozoa. Morphological evaluations of spermatozoa were performed in six of the cheetahs during the second semen collection occurring two to seven days following the first electroejaculation. The total % of abnormal spermatozoal forms/ejaculate during the second evaluation (74.0+2.3) was not significantly different from the first (71.0+0.9).

The proportion of abnormal spermatozoa in the ejaculate has been considered to be related to fertility in man[11] as well as most conventional domesticated species including the bull[12], rat[13], boar[14] and dog[15]. Aberrant sperm forms are generally classified as primary (coiled tail, pleiomorphic head defects which originate during spermatogenesis) or secondary (bent midpiece or tail, protoplasmic droplet which originate in the excurrent duct system) deformities. In the bull[12] and dog[15], primary abnormalities exceeding 20% of the spermatozoal population may indicate fertility dysfunction. Another species, the gorilla, is considered to produce a proportionately large (29+2.5%) of pleiomorphic spermatozoa in the ejaculate[16,17]. However, the significance of this finding

regard to fecundity is unknown. The captive cheetah appears unique in such a consistently great proportion of both primary and secondary abnormal spermatozoa are observed across a wide range of individuals and a relatively successful breeding population.

A concurrent evaluation of the genetic variance in this same cheetah population reveals monomorphic blood chemistries indicative of genetic homogeneity[18]. Whether the ontogeny of the latter finding is the result of protracted inbreeding within the species remains unknown. However, it is well established that reproductive traits including ejaculate quality may be adversely affected in highly inbred, homogeneous populations[19-21]. Furthermore, recent examinations of records of captive zoo stock reveals alarming degree of inbreeding which appears responsible for numerous deleterious effects including increased juvenile mortality[22]. It could be speculated that the limited genetic variance and the pleiomorphic spermatozoa of the cheetah may be related. If confirmed, spermatozoal morphology may be useful as a taxonomic guide in assessing relationships among felid species, similar to that demonstrated for man and the great apes[16]. It remains to be determined whether the comparatively poor ejaculate quality of the cheetah is a genetic consequence or a unique species norm.

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