Lindburg DG, Durrant BS, Millard SE, Oosterhuis JE. 1993. Fertility assessment of cheetah males with poor quality semen. Zoo Biology 12(1):97-104.

Keywords: Acinonyx jubatus/captive breeding/captivity/cheetah/fertility/inbreeding/male/ reproduction/reproductive success/sperm quality

Abstract: Reports on semen quality of the cheetah *(Acinonyx jubatus)* indicate that high percentages of abnormal morphs and sperm concentrations, 10 times lower than in domestic cats, are found in all populations. These characteristics are believed to result from unusual genetic homozygosity, hypothesized to have been caused by passage of the species through one or more population bottlenecks during its recent history. In a sample of 12 captive living males, we found semen characteristics to be equal or inferior to those previously reported for all males living in other captive facilities. Ten of these males (83.3%) nevertheless produced pregnancies. Seventeen of 19 pregnancies, resulted from mating during a single oestrus. This examination of the reproductive potential of males having comparatively inferior ejaculate quality supports the suggestion that husbandry programs may be more significant than physiological impairment in causing the low birth rates in captive cheetahs. These results also have implications for ascertaining fertility thresholds in mammalian populations undergoing increased levels of inbreeding as a consequence of habit deterioration.

canine vagina. SOUTHWEST VETERINARIAN 27:76–77. 1974.
Purswell, B.J. Differential diagnosis of canine abortion. Pp. 925–929 in CURRENT VETERINARY THERAPY XI. R.W. Kirk and J.D. Bonagura, eds.. Philadelphia, W.B. Saunders Co., 1992.
Scott, F.W.; Wiess, R.C.; Post, J.E.; Gilmartin, J.E.; Hoshino, Y. Kitten mortality complex (neonatal FIP?). FELINE PRACTICE 9:44–47, 1979.
Shille, V.; Lundstrum, K.E.; Stabenfeldt, G.H. Follicular function in the domestic cat as determined as estradiol-17β concentration in plasma: Relation to estsrous behavior and cornification of exfoliated vaginal epithelium. BIOLOGY OF REPRODUCTION 21:953–963, 1979.
Snedecor, G.W.; Cochran, W.G. STATISTICAL METHODS. Ames, Iowa, Iowa State University Press, 1980.
Troy, G.C.; Herron, M.A. Infectious causes of infertility, abortion and stillbirths in cats. Pp. 834–837 in CURRENT THERAPY IN THERIOGENOLOGY II. D.A. Morrow, ed., Philadelphia, W.B. Saunders Co., 1986.
Wildt, D.E. Fertilization in cats. Pp. 299–328 in A COMPARATIVE OVERVIEW OF MAMMALIAN FERTILIZATION. B.S. Dunbar and M. O'Rand, eds.. New York, Plenum Publishing Corp., 1991.
Wildt, D.E.; Brown, J.L.; Bush, M.; Barone, M.A.; Cooper, K.A.; Grisham, J.; Howard, J.G. Reproductive status of cheetahs (*Acinonyx jubatus*) in North American zoos: The benefits of physiological surveys for strategic planning. ZOO BIOLOGY 12:45–80, 1993.
Wildt, D.E.; Bush, M; Howard, J.G.; O'Brien, S.J.; Meltzer, D.; van Dyk, A.; Ebedes, H.; Brand, D.J. Unique seminal quality in the South African cheetah and a comparative evaluation in the domestic cat. BIOLOGY OF REPRODUCTION 29:1019–1025, 1983.
Wildt, D.E.; Monfort, S.L.; Donoghue, A.M.; Johnson, L.A.; Howard, J.G. Embryogenesis in conservation biology—or how to make an endangered species embryo. THERIOGENOLOGY 37:161–184, 1992.
Wildt, D.E.; O'Brien, S.J.; Howard, J.G.; Caro, T.M.; Roelke, M.E.; Brown, J.L.; Bush, M. Similarity in ejaculate-endocrine characteristics in captive versus free-ranging cheetahs of two subspecies. BIOLOGY OF REPRODUCTION 36:351–360, 1987a.
Wildt, D.E.; Phillips, L.G.; Simmons, L.G.; Goodrowe, K.L.; Howard, J.G.; Brown, J.L.; Bush, M. Seminal-endocrine characteristics of the tiger and the potential for artificial breeding. Pp. 255–279 in TIGERS OF THE WORLD: THE BIOLOGY, BIOPOLITICS, MANAGEMENT AND CONSERVATION OF AN ENDANGERED SPECIES. R.L. Tilson and U.S. Seal, eds.. Park Ridge, Noyes Publications, 1987b.
Wildt, D.E.; Schiewe, M.C.; Schmidt, P.M.; Goodrowe, K.L.; Howard, J.G.; Phillips, L.G.; O'Brien, S.J.; Bush, M. Developing animal models systems for embryo technologies in rare and endangered wildlife. THERIOGENOLOGY 25:33–51, 1986.
Wolf, A.M. Infertility in the queen. Pp. 947–954 in CURRENT VETERINARY THERAPY XI. R.W. Kirk and J.D. Bonagura, eds.. Philadelphia, W.B. Saunders Co., 1992.

Zoo Biology 12:97–103 (1993)

Fertility Assessment of Cheetah Males With Poor Quality Semen

D.G. Lindburg, B.S. Durrant, S.E. Millard, and J.E. Oosterhuis

Center for the Reproduction of Endangered Species (D.G.L., B.S.D., S.E.M.), and Veterinary Services Department (J.E.O.), Zoological Society of San Diego, San Diego, California

Reports on semen quality of the cheetah (*Acinonyx jubatus*) indicate that high percentages of abnormal morphs and sperm concentrations, 10 times lower than in domestic cats, are found in all populations. These characteristics are believed to result from unusual genetic homozygosity, hypothesized to have been caused by passage of the species through one or more population bottlenecks during its recent history. In a sample of 12 captive-living males, we found semen characteristics to be equal or inferior to those previously reported for males living in other captive facilities. Ten of these males (83.3%) nevertheless produced pregnancies. Seventeen of 19 pregnancies resulted from matings during a single estrus. This examination of the reproductive potential of males having comparatively inferior ejaculate quality supports the suggestion that husbandry programs may be more significant than physiological impairment in causing the low birth rates of captive cheetahs. These results also have implications for ascertaining fertility thresholds in mammalian populations undergoing increased levels of inbreeding as a consequence of habitat deterioration.

Key words: *Acinonyx jubatus*, reproduction, husbandry, inbreeding, mammal

INTRODUCTION

Inbreeding has been shown to have deleterious effects on reproduction in a wide range of species, including laboratory mice, domestic livestock, and zoo exotics [Johansson and Rendel, 1968; Ralls et al., 1979; Green and Witham, 1991]. From electrophoretic surveys of 128 individuals, the cheetah (*Acinonyx jubatus*) has been shown to exhibit less than 5% heterozygosity at 52 protein loci, a level of genetic uniformity similar to that found in inbred mice [O'Brien et al., 1983, 1985, 1987a]. Acceptance of skin allografts in unrelated pairs of cheetahs has been interpreted as

Received for publication August 3, 1992; accepted October 26, 1992.

The review process for this article was carried out by Dr. Michael J. Raleigh, editor of *American Journal of Primatology*.

Address reprint requests to Dr. Donald G. Lindburg, Zoological Society of San Diego, P.O. Box 551, San Diego, CA 92112.

providing further confirmation of unusually low genetic diversity in the species [O'Brien et al., 1985, 1986]. The cheetah is hypothesized to have passed through one or more population bottlenecks in its recent history, resulting in an extant population that is relatively genetically monomorphic and possibly vulnerable to various forms of environmental adversity [O'Brien et al., 1983, 1985, 1987a]. One indication of this vulnerability could be the failure of the captive-living population to become self-sustaining as the result of a low incidence of births and of high mortality, especially during the first 6 months of life. For example, the effective breeding size (N_e) for the North American zoo population, which numbered 193 individuals in 1986, was 14.5%, and juvenile mortality was 36.7% [Marker and O'Brien, 1989].

Sperm concentrations 10 times lower than are found in domestic cats and abnormal morphs, often in excess of 70%, are undoubtedly linked to the cheetah's genetic condition, and such findings have raised questions about consequences for male fertility. In their reports on genetic and physiological findings, O'Brien et al. [1983, 1985, 1986, 1987a] and Wildt et al. [1983, 1987] point out that semen parameters of the quality found in the cheetah would connote sterility, if found in cattle or humans. However, although it has been shown that ovum penetration in in vitro studies is compromised in males having these traits [Donoghue et al., 1992], the actual fertilizing ability of teratospermic males, as noted by Wildt et al. [1993], is unknown. In addition, the importance of a possible "inbreeding-infertility" linkage to the survival of wild populations of cheetahs, as well as to other mammalian species, derives from concerns that increasing habitat fragmentation and genetic isolation will ultimately lead to lowered reproductive rates [Wildt et al., 1987; Packer et al., 1991; O'Brien et al., 1987b, 1990].

Here, we report results from tests with captive males, designed to examine the relationship of semen quality to fertility. We hypothesized that infertile males in the sample would either exhibit very little sexual interest in estrous females, i.e., a deficient libido, or would have semen characteristics significantly inferior to those of fertile males. We also hypothesized that overall semen characteristics from those males proven to be fertile would be of significantly higher quality than those for the North American zoo population at large, of which less than 20% have sired litters [Marker and O'Brien, 1989].

MATERIALS AND METHODS

Animals

Twelve male cheetahs maintained at the San Diego Wild Animal Park were evaluated for semen characteristics, responses to estrous females, and success in producing pregnancies. Testing took place over a 10-year period ending in 1991. Males were recruited in the manner typically employed by zoos, i.e., through births, trades, and loans. Eleven had no reproductive history at the time of entry into the test program, thereby precluding the selection of a sample biased in terms of reproductive potential. All individuals were captive-born and were descended from South African stock. However, studbook records indicate that 9 different lineages were represented in the sample.

Semen Evaluation

Semen samples were collected while males were immobilized, for purposes such as quarantine examinations, on arrival in San Diego or at annual physicals.

Anesthesia was induced by application of Ketamine at a rate of 5.5 mg/kg body weight, and Diazapam at 1 mg/10 kg. Standard rectal probe electroejaculation procedures were used. Because the goal was to obtain semen samples, not to test a collection technique, each electroejaculation was customized for maximum response, thus correcting for differences in reproductive anatomy and effects of anesthesia. Samples ranged from 1 to 4 per male subject, for a total of 23 ejaculates from 11 different males. Variation between males in the test group was determined by performing one-way ANOVAs across each of 5 semen parameters: volume, concentration, motility, speed of progression, and percent of abnormal spermatozoa per ejaculate.

Behavior

Behavioral tests consisted of introducing candidate males to the pens of singly housed estrous females as they became available, or introduction of the pair to a neutral pen. Pairings with several different males were often required before mating would occur, despite obvious indications of sexual interest on the part of females. However, once a pair mated, the same male was used for the remaining days of that particular estrus in order to avoid confusion over paternity. Mating tests were terminated after periods ranging from 5 minutes to 4 days, depending on outcome. For example, highly aggressive introductions were abruptly terminated in order to preclude injury, whereas a copulating pair was occasionally allowed to remain together, until interest in mating was no longer evident.

Fertility Measures

Assessments of male fertility in all cases were based on the occurrence of pregnancies in their mates. All females that had produced litters within the previous 2 years were considered fertile for this project. Females with no prior breeding history were counted as fertile, if the test itself resulted in a pregnancy. Females were precluded from testing for up to two years following conception, i.e., during pregnancy and infant rearing, consequently limiting the rate at which the fertility of males could be determined.

RESULTS

Three males of proven fertility produced aspermic ejaculates on 6 occasions (not included in the analysis); none of the ejaculates collected from two nonreproducing males (3 from each) were aspermic. In 22 semen samples, sperm motility ranged from zero ($n = 5$) to 80%. Speed of progression was either 3 or 4 (on a scale from 1 to 5) in all but the 5 nonmotile ejaculates. In 18 samples evaluated morphologically, abnormal spermatozoa ranged from 39 to 100%. Volume ranged from 0.1 to 2.38 ml per ejaculate ($n = 23$), and sperm concentration from 5 to 37.5 ($\times 10^6$/ml, $n = 22$).

Results of the 5 one-way ANOVAs indicated that the 23 ejaculates in the sample came from males which did not differ significantly in their semen parameters (Table 1). Since the evidence supports a conclusion of sample homogeneity irrespective of individual fertilizing ability, a mean and variance for the entire sample was calculated in order to compare the San Diego males with published results for males living in other North American zoos (Table 2). This comparison revealed that the two populations were similar in percent of morphologically abnormal spermatozoa, but that the San Diego males were actually inferior to the larger population in sperm concentra-

TABLE 1. Results from one-way ANOVAs indicating homogeneity of variance in each of 5 semen parameters for the 11 males sampled

Parameter	DF	F value	P
Volume	10	1.71	0.19
Concentration	9	1.48	0.26
Percent motile	10	1.13	0.42
Progression speed	10	0.25	0.98
Percent abnormal	8	0.84	0.59

TABLE 2. Semen parameters of the San Diego males compared to those of males living in other North American zoos

	San Diego		North America[a]		
Parameter[b]	n	$\bar{x}$ (± SEM)	n	$\bar{x}$ (± SEM)	P
Concentration (× 10^6/ml)	22	11.0 ± 2.2	29	25.1 ± 4.4	<.02
Percent motile	22	42.7 ± 6.7	29	70.7 ± 3.5	<.001
Speed of progression	21	2.4 ± 0.3	29	3.6 ± 0.1	<.001
Percent abnormal	18	66.8 ± 3.7	29	70.6 ± 3.3	NS

[a]Data from Wildt et al., 1987.
[b]Speed of progression is rated from 1 to 5, with 5 being the fastest, and "n" refers to the number of ejaculates examined for each parameter. Two-sample *t*-test, based on a pooled sample variance, was used to assess significance.

tion, percent of motile sperm, and speed of progression. On the basis of semen parameters alone, it could be anticipated that the fertilizing ability of this sample of males would be somewhat compromised.

Only one of the 12 males failed to mate, despite being paired with females on days when they mated with other males. Mean values for ejaculates from this male (n = 3) showed a sperm concentration of 24.5 (× 10^6/ml), 24 percent motility, a progression speed of 2.7, and 80% abnormal morphs. This male did show sexual arousal in the form of penile erections and stutter-barking, and did try to mount estrous females, but was resisted. Both sexes sometimes resisted initial partners during pairings, suggesting that mate preference could be important in this species, but the percent mating overall (91.7%) does not support concerns about a deficient libido in captive-living males [Wildt et al., 1987, 1993; O'Brien et al., 1986]. Failure to manipulate male–female pairings appropriately may be an alternate explanation for the large numbers of "disinterested" males reported by North American zoos [Wildt et al., 1993].

Estrus ranged from 1 to 4 days, and observed copulations during an estrus ranged from 1 to 4. Ten of the 11 males that mated (90.9%) produced pregnancies, with 7 different females. Of the 19 pregnancies produced, 17 occurred in a female's first estrus of availability after lactation or on initial introduction to the study (Fig. 1), and all 10 sires contributed to this result. In the remaining 2 cases, conception occurred in the second estrus. Five pregnancies were known to have resulted from single inseminations by 4 of these males. A male that copulated with 2 females of proven fertility during 7 different estrous periods (male #2 in Fig. 1) was deemed to be the only infertile male among the 11 that mated. Mean values for ejaculates collected from this individual indicated a sperm concentration of 6.7 (× 10^6/ml, n = 3), 50 percent motility (n = 3), a progression speed of 1.5 (n = 2), and, in a single sample, 59% abnormal morphs.

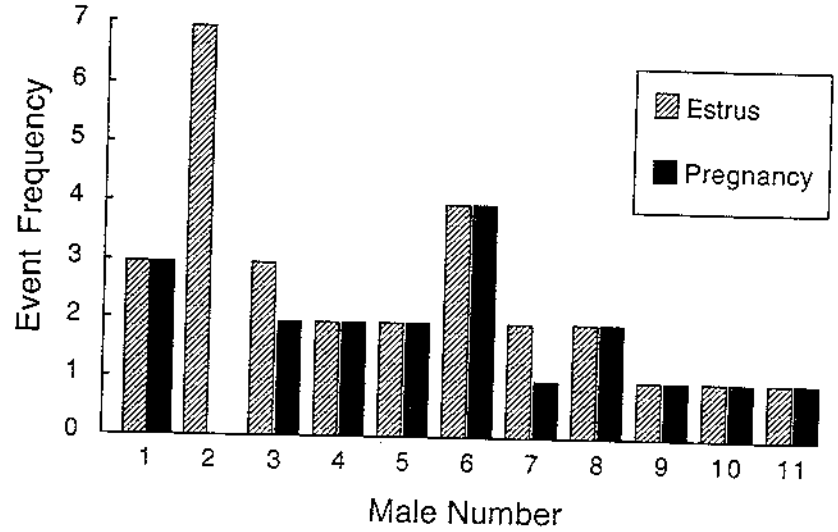

Fig. 1. The distribution of 19 pregnancies in relation to mating episodes (estrus) with each of 11 copulating males, shown in order of the males' entry into the testing program.

DISCUSSION

Spermatozoal deficiencies of the type documented in the San Diego sample might be used to infer a high probability of infertility in male cheetahs. However, the celerity with which fecund females conceived during this study is inconsistent with a presumption of borderline sterility for their mates, inferred from spermatozoal characteristics. Pregnancies were in fact produced by the San Diego males at a far higher rate than for cheetahs in other North American zoos (>80% vs. <20%) [Marker and O'Brien, 1989], suggesting that in cheetahs as in other mammals, specific semen characteristics may be poor predictors of fertility [Amann, 1989; Barth and Oko, 1989].

The results reported here represent the first test of fertility in male cheetahs with known semen parameters. The inferior quality of spermatozoa in all populations tested to date raises legitimate questions about the consequences of inbreeding for fertility in the long run. For the moment, however, it has not been established that the poor conception rates of captive-living cheetahs is attributable to the negative effects of inbreeding on semen characteristics. Alternatively, the results presented here lead to the conclusion that husbandry regimes are more likely to be implicated in the low birth rates of captive-living cheetahs. A finding of poor quality semen in wild populations [Wildt et al., 1987], which show no evidence of reproductive impairment [Laurenson et al., 1992], and the uneven occurrence of breeding among North Amer-

ican zoo collections [Marker and O'Brien, 1989], also suggest a substantial effect of husbandry practices on the reproduction of captive-living cheetahs.

Finally, the lack of diminished fertility in this sample of cheetah males, despite their high rates of teratospermia and other potentially limiting sperm characteristics, is a result that should be of value in appraising the reproductive consequences for wild-living felid populations that are becoming increasingly threatened by habitat fragmentation and genetic impoverishment.

CONCLUSIONS

1. Ten of 12 males evaluated for semen parameters, mating responsiveness, and fertilizing ability sired offspring, despite having semen attributes of inferior quality, compared to other mammals.

2. Seventeen of 19 pregnancies occurred during females' initial estrus, and 5 pregnancies resulted from single inseminations.

3. Semen quality as measured by such traditional measures as concentration, motility, volume, speed of progression, and quantity of abnormal morphs is by itself a poor predictor of fertility in cheetahs, as in other mammals.

ACKNOWLEDGMENTS

We thank P.H. Hawley, Douglas Williams, and Nancy Harvey for assistance with statistical analysis, and Drs. Jim Moore and Tim Caro for commenting on earlier versions of this paper. The logistic support of the animal keeper and veterinary staff at the San Diego Wild Animal Park is gratefully acknowledged. The protocol used for semen collection was reviewed and approved by the Institutional Animal Care and Use Committee of the Zoological Society of San Diego.

REFERENCES

Amann, R.P. Can the fertility of a seminal sample be predicted accurately? JOURNAL OF ANDROLOGY 10:89–98, 1989.

Barth, A.D.; Oko, R.J. ABNORMAL MORPHOLOGY OF BOVINE SPERMATOZOA. Ames, Iowa, Iowa State University Press, 1989.

Donoghue, A.M.; Howard, J.G.; Byers, A.P.; Goodrowe, K.L.; Bush, M.; Blumer, E.; Lukas, J.; Stover, J.; Snodgrass, K.; Wildt, D.E. Correlation of sperm viability with gamete interaction and fertilization in vitro in the cheetah (*Acinonyx jubatus*). BIOLOGY OF REPRODUCTION 46:1047–1056, 1992.

Green, M.C.; Witham, B.A., eds. HANDBOOK ON GENETICALLY STANDARDIZED JAX MICE. Bar Harbor, Maine, The Jackson Laboratory, 4th ed., 1991.

Johansson, I.; Rendel, J. GENETICS AND ANIMAL BREEDING. San Francisco, W. H. Freeman, 1968.

Laurenson, M.K.; Caro, T.; Borner, M. Female cheetah reproduction. NATIONAL GEOGRAPHIC RESEARCH AND EXPLORATION 8:64–75, 1992.

Marker, L.; O'Brien, S.J. Captive breeding of the cheetah (*Acinonyx jubatus*) in North American zoos (1871–1986). ZOO BIOLOGY 8:13–16, 1989.

O'Brien, S.J.; Wildt, D.E.; Goldman, D.; Merril, C.R.; Bush, M. The cheetah is depauperate in genetic variation. SCIENCE 221:459–462, 1983.

O'Brien, S.J.; Roelke, M.E.; Marker, L.; Newman, A.; Winkler, D.; Colly, L.; Evermann, J.F.; Bush, M.; Wildt, D.E. Genetic basis for species vulnerability in the cheetah. SCIENCE 227:1428–1434, 1985.

O'Brien, S.J.; Wildt, D.E.; Bush, M. The cheetah in genetic peril. SCIENTIFIC AMERICAN 254: 84–92, 1986.

O'Brien, S.J.; Wildt, D.E.; Bush, M.; Caro, T.M.; FitzGibbon, C.; Aggundey, I.; Leakey, R.E. East African cheetahs: Evidence for two population bottlenecks? PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE USA 84:508–511, 1987a.

O'Brien, S.J.; Martenson, J.; Packer, C.; Herbst, L.; de Vos, V.; Joslin, P.; Ott-Joslin, J.; Wildt, D.E.; Bush, M. Biochemical genetic variation in geographic isolates of African and Asiatic lions. NATIONAL GEOGRAPHIC RESEARCH 3:114–124, 1987b.

O'Brien, S.J.; Roelke, M.; Yuhki, N.; Richards, K.W.; Johnson, W.E.; Franklin, W.L.; Anderson, A.E.; Bass, O.L., Jr.; Belden, R.C.; Martenson, J.S. Genetic introgression within the Florida panther (*Felis concolor coryi*). NATIONAL GEOGRAPHIC RESEARCH 6:485–494, 1990.

Packer, C.; Pusey, A.E.; Rowley, H.; Gilbert, D.A.; Martenson, J.; O'Brien, S.J. Case study of a population bottleneck: Lions of the Ngorongoro Crater. CONSERVATION BIOLOGY 5:219–230, 1991.

Ralls, K.; Brugger, K.; Ballou, J. Inbreeding and juvenile mortality in small populations of ungulates. SCIENCE 206:1101–1103, 1979.

Wildt, D.E.; Bush, M.; Howard, J.G.; O'Brien, S.J.; Meltzer, D.; Van Dyk, A.; Ebedes, H.; Brand, D.J. Unique seminal quality in the South African cheetah and a comparative evaluation in the domestic cat. BIOLOGY OF REPRODUCTION 29:1019–1025, 1983.

Wildt, D.E.; O'Brien, S.J.; Howard, J.G.; Caro, T.M.; Roelke, M.E.; Brown, J.L.; Bush, M. Similarity in ejaculate–endocrine characteristics in captive versus free-ranging cheetahs of two subspecies. BIOLOGY OF REPRODUCTION 36:351–360, 1987.

Wildt, D.E.; Bush, M.; Goodrowe, K.L.; Packer, C.; Pusey, A.E.; Brown, J.L.; Joslin, P.; O'Brien, S.J. Reproductive and genetic consequences of founding isolated lion populations. NATURE 329:328–331, 1987.

Wildt, D.E.; Brown, J.L.; Bush, M.; Barone, M.A.; Cooper, K.A.; Grisham, J; Howard, J.G. Reproductive status of cheetahs (*Acinonyx jubatus*) in North American zoos: The benefits of physiological surveys for strategic planning. ZOO BIOLOGY 12:45–80, 1993.