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Unique Seminal Quality in the South African Cheetah and a Comparative Evaluation in the Domestic Cat

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ABSTRACT

Analysis of 40 semen samples collected by electroejaculation from 18 cheetahs revealed no major differences in seminal traits among Transvaal, South West (Namibia) or hybrid (Transvaal X South West) males. However, mean spermatozoal concentration (14.5×10^6 spermatozoa/ml of ejaculate) and percent motility (54.0%) were less in cheetahs than in domestic cats (147.0×10^6 spermatozoa/ml of ejaculate, 77.0% motility) subjected to the same electroejaculation regimen. On the average, cheetah ejaculates contained 71.0% morphologically abnormal spermatozoa compared to 29.1% aberrant spermatozoal forms in the domestic cat. These results indicate that seminal characteristics in the cheetah are markedly inferior compared to the domestic cat, particularly with respect to the incidence of pleiomorphic spermatozoa. Because a recent parallel study demonstrates that the cheetah lacks genetic variation, it appears likely that spermatozoal abnormalities are a genetic consequence of genomic homozygosity characteristic of this endangered species.

INTRODUCTION

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 felid genus *Acinonyx*. The physiological data base for this species is extremely limited (Eaton, 1974; Wrogemann, 1975). An abstract by Coubrough et al. (1978) suggests that cheetah spermatozoa exhibit a number of structural defects including coiled and bent flagella; however, no specific details were provided. O'Brien et al. (1983), using allozyme and two-dimensional gel electrophoretic analyses, recently have demonstrated a strikingly reduced amount of biochemical genetic variation in the South African cheetah. This finding in conjunction with the notation of Coubrough et al. (1978) emphasizes the need to examine further the influence of the monomorphic genotype on reproductive function in this species.

In 1971, the National Zoological Gardens of South Africa initiated a comprehensive program for the captive propagation of cheetahs (Brand, 1980). 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

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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 Province and were separated by a distance of 220 km.

Sexual maturity in both the male and female cheetah is thought to occur between 13 and 16 months of age (Wrogemann, 1975). In southern Africa the female is considered seasonally polyestrous, exhibiting overt estrous cycles from December through February (Brand, 1980). During the breeding season at De Wildt, males are maintained approximately 300 m from the female enclosures. A group of males is released daily near the female camps to monitor the onset of sexual receptivity. Estrous females are then permitted to copulate ad libitum for 2 to 3 days with a designated male. Using such methods, a total of 181 offspring have been produced from 1975 through 1982.

The purposes of the present study were to determine ejaculate norms and compare reproductive traits in established populations of male Transvaal, South West (Namibia) or hybrid (Transvaal × South West) cheetahs. Because of the unusual seminal quality observed, a comparative study also was conducted in the domestic male cat.

MATERIALS AND METHODS

Animals and Facilities

Ejaculates were collected in January, 1981 (mid-breeding season) from 11 Transvaal, three South West and eight hybrid cheetahs of Transvaal × South West ancestry. All animals were untamed and averaged (± SEM) 56.0 ± 0.2 kg in weight and 5.3 ± 0.7 years in age. Although the population ranged in age from 2–12 years, the mean ages of the population subgroups were similar ($P>0.05$): Transvaal, 5.9 ± 1.5 years; South West, 6.0 ± 1.9 years; hybrid 3.6 ± 0.4 years. A total of 15 adult males were collected at De Wildt (seven Transvaal, two South West, six hybrid) and seven males were sampled at Lichtenburg (four Transvaal, one South West, two hybrid). At De Wildt, male cheetahs were maintained in groups of three to six in 1-hectare (ha) fenced enclosures. At Lichtenburg and prior to the initiation of the study, males were grouped together with seven female cheetahs and accorded free range of a 400-ha fenced enclosure. All males were separated from females at least 4 weeks before the experiment and, during the 5-day interim of data collection at Lichtenburg, the cheetahs were restricted to a 1-ha fenced camp.

Domestic cat ejaculates were collected in May, 1982, from 16 random source, adult males (3.2 – 5.0 kg body weight) maintained indoors in a colony conducive to year-round production of kittens (Wildt et al., 1978). Like the cheetahs, domestic cats were not used for breeding purposes during the electroejaculation experiment or during at least the 4-week interval preceding the experiment.

Semen Collection

In both species, semen was collected by electroejaculation using similar techniques including anesthesia, voltage and number of electrical stimuli. Individual animals were physically restrained and general anesthesia induced by an i.v. injection of CT 1341 (2.0 mg/kg of body weight, Saffan, Glaxo Labs., Middlesex, England). Semen was collected from cheetahs on one to four occasions/animal and from each domestic cat one time using rectal probe electroejaculation equipment and procedures similar to those described earlier (Platz and Seager, 1978; Platz et al., 1983). To permit comparative analysis of seminal traits, the electroejaculation regimen was standardized so that each animal was allotted 80 electrical stimuli of similar voltage (4 to 7 V) and milliamperage (50–200 MA) given over a 30-min interval. The pattern of applied stimuli was consistent with a previous report (Howard et al., 1981). The ejaculate was collected in a prewarmed vial.

Seman Evaluation

Ejaculate volume was recorded and all microscopic analyses performed at 37°C using undiluted seminal aliquots. Spermatozoal percent motility was evaluated immediately based on observations of four separate microscopic fields at 400×. Spermatozoal concentration (spermatozoal numbers/ml of ejaculate) was calculated using a standard hemocytometer counting procedure, evaluating all 64 squares of both counting chambers of the hemocytometer. Spermatozoal concentration/ejaculate was calculated for the cheetah but not the domestic cat. For the latter species, the very small ejaculate volume (150 μl) and the potential loss of fluid during collection made such a measurement inaccurate.

An aliquot of semen from the first ejaculate containing spermatozoa was fixed in 1% glutaraldehyde according to the protocol of Pursel and Johnson (1974) and 300 spermatozoa/individual were microscopically examined (1000×) for morphological abnormalities. Structural evaluations of spermatozoa were performed in six of the cheetahs twice by fixing an aliquot during a second semen collection occurring 2 to 7 days following the first electroejaculation. Aberrant forms of spermatozoa were classified as primary (a coiled flagellum or a pleiomorphic head defect, which originates during spermatogenesis) or secondary (a bent midpiece or flagellum or a protoplasmic droplet, which originates in the excurrent duct system) deformities.

Data Evaluation

Values reported are means ± standard error of the mean (SEM). Average and SEM values of the subjective estimate trait of percent motility were rounded to the nearest whole percentage. Significant differences were determined by analysis of variance. Individual means were then compared by Student's t test.

RESULTS

Eighteen of 22 cheetahs produced ejaculates containing spermatozoa, the four aspermic males (two Transvaal, two hybrids) all being located at De Wildt. Repeated collections of semen had no discernible influence on standard seminal traits. Fourteen of the males were electroejaculated twice within a 48-h interval. Compared to the first collection, volume of the second ejaculate was greater in seven, less in five and unchanged in two cheetahs. In the second sample, spermatozoal concentration/ml of ejaculate was greater in seven, less in six and the same in one male compared to the spermatozoal numbers from the initial semen sample. Compared to the first sample, spermatozoal concentration/ejaculate in the second sample was greater in nine and less in five cheetahs; however, total sperm numbers varied considerably among individuals and mean values between the first (23.9 ± 5.5 × 10^6 spermatozoa/ejaculate) and second (29.4 ± 5.7 × 10^6 spermatozoa/ejaculate) collection were not different ($P>0.05$). Data from five representative males electroejaculated three times over a 6-day interval are shown in Table 1. Ejaculate volume, spermatozoal concentration, and percent motility fluctuated in a random fashion.

No differences were observed in mean ejaculate volume or spermatozoal concentration and motility among Transvaal, South West and hybrid cheetah groups (Table 2). Combining all data and based on a total of 40 seminal collections containing spermatozoa, an average cheetah ejaculate consisted of 2.1 ± 0.2 ml of fluid. Seminal traits in the cheetah were markedly less ($P<0.05$) than results from domestic cats subjected to the same quantitative and qualitative electroejaculation stimuli. Mean spermatozoal concentration (sperm numbers/ml of ejaculate) for the cheetah was 14.5 ± 1.8 × 10^6 (10 times less than domestic cats), and the percent motility rating of cheetah spermatozoa was 54.0 ± 3.0%, about 70% of that observed in cat ejaculates (Table 3). An average of 71.0 ± 0.9% (range, 44–87%) of the spermatozoa collected in each cheetah ejaculate consisted of abnormal pleiomorphic forms (Fig. 1). The mean percentage of structural deformities in the first ejaculate of males in the Transvaal, South West and hybrid subgroups was 73.2 ± 2.5, 75.7 ± 2.3 and 67.3 ± 3.0%, respectively ($P>0.05$). Overall, of the total defective forms,

TABLE 1. Seminal traits in five representative cheetahs electroejaculated three times over a 6-day interval.

Male	Day[a]	Ejaculate volume (ml)	Spermatozoal		
			Concentration/ml of ejaculate (×10^6)	Concentration/ ejaculate (×10^6)	Motility (%)
1	1	3.3	0.5	1.6	50
	3	1.4	7.0	9.8	35
	7	1.6	11.0	17.6	55
2	1	2.0	11.5	23.0	50
	3	1.9	40.0	76.0	60
	7	0.8	19.0	15.2	40
3	1	1.0	14.5	14.5	65
	3	2.8	17.5	49.0	80
	7	2.1	7.5	15.8	65
4	1	1.8	26.0	46.8	70
	3	3.6	13.0	46.8	55
	7	2.0	13.0	26.0	55
5	1	1.4	28.0	39.2	70
	3	1.2	10.5	12.6	45
	7	1.3	3.5	4.6	60

[a]Day 1-Day of first electroejaculation.

38.6% and 61.4% were in the primary and secondary classification, respectively (Table 3). In the cheetahs evaluated twice, the percent abnormal spermatozoal forms/ejaculate for the group during the second evaluation (73.9 ± 1.9%) was not different ($P>0.05$) from the first (69.4 ± 5.9%). An average of 29.1 ± 3.7% aberrant forms of spermatozoa was noted in the domestic cat samples. Approximately 80% of these were attributable to secondary defects, usually a protoplasmic droplet (Table 3).

DISCUSSION

Seminal traits studied did not vary among cheetahs with Transvaal, South West or hybrid genotypes while ejaculate characteristics in domestic cats were comparable to values reported earlier (Platz and Seager, 1978; Platz et al., 1978). Based on collections from a relatively large population of cheetahs during peak breeding season, spermatozoa concentration, percent motility and normal morphology were less than that observed in domestic cats. It is unlikely that the elevated number of morphologically abnormal spermatozoa in the cheetah was the result of sexual abstinence or degenerative processes associated with elimination of aged spermatozoa. A similar number of defective forms of spermatozoa was observed in cheetahs evaluated twice over a relatively brief interval. Furthermore, both cheetah and domestic cat semen was handled similarly and all precautions were taken to avoid spermatozoal damage from cold shock.

TABLE 2. Seminal trait comparisons among Transvaal, South West and hybrid cheetahs.[a]

	Transvaal	South West	Hybrid
Number of males	9	3	6
Number of ejaculates	19	4	17
Ejaculate volume (ml)	1.6 ± 0.2	3.6 ± 1.1	2.4 ± 0.2
Spermatozoal concentration			
Sperm numbers/ml of ejaculate ($\times 10^6$)	15.6 ± 3.0	13.2 ± 5.0	13.5 ± 2.1
Sperm numbers/ejaculate ($\times 10^6$)	22.1 ± 4.6	39.6 ± 12.9	30.6 ± 4.2
Spermatozoal motility (%)	52.0 ± 5.0	58.0 ± 8.0	57.0 ± 5.0

[a]Values are means ± SEM.

TABLE 3. Seminal traits in the South African cheetah compared to the domestic cat[a].

	Cheetah	Domestic cat
Number of males	18	16
Number of ejaculates	40	16
Spermatozoal concentration (sperm numbers/ml of ejaculate) ($\times 10^6$)	14.5 ± 1.8	147.0 ± 39.5[b]
Spermatozoal motility (%)	54.0 ± 3.0	77.0 ± 3.0[b]
Morphological abnormalities of spermatozoa (%)	71.0 ± 0.9[b]	29.1 ± 3.7
Primary		
Coiled flagellum	25.8 ± 2.3[b]	5.5 ± 0.8
Microcephalic defect	1.2 ± 0.3[b]	0.2 ± 0.1
Macrocephalic defect	0.4 ± 0.2	0.1 ± 0.04
Secondary		
Bent midpiece	23.3 ± 1.1[b]	6.4 ± 0.8
Bent flagellum	16.2 ± 1.3[b]	5.1 ± 0.7
Bent flagellum tip	2.8 ± 0.6[b]	0.02 ± 0.01
Protoplasmic droplet	1.3 ± 0.3	11.8 ± 1.7[b]

[a]Values are means ± SEM.

[b]Significantly greater ($P<0.05$) than counterpart value.

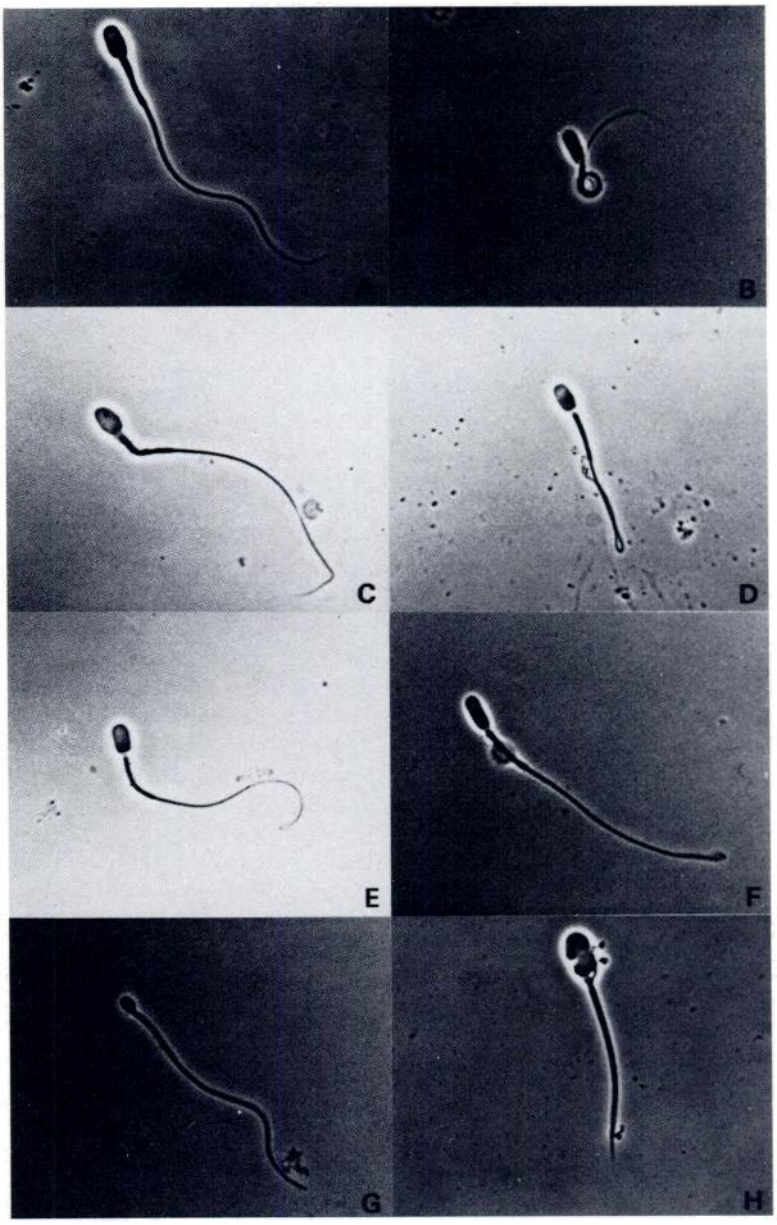

FIG. 1. Spermatozoal forms detected in the ejaculate of electroejaculated cheetahs: *A*) normal; *B*) coiled flagellum; *C*) bent midpiece; *D*) bent flagellum; *E*) bent flagellum tip; *F*) protoplasmic droplet; *G*) microcephalic defect; *H*) macrocephalic defect.

The results confirm and extend the preliminary data of Coubrough et al. (1978) who noted similar spermatozoal defects in cheetahs classified as either fertile or infertile. Other abnormalities of gametic substructure including acrosomal integrity could not be accurately evaluated using the microscopic methods of the present study, The acrosomal ridge of the cheetah spermatozoon is extremely narrow and fails to protrude beyond the apex of the head region. However, scanning electron microscopy provides preliminary evidence that acrosomal defects also exist in this species (Coubrough et al., 1978).

The semen of the domestic cat consisted of few primary or secondary defects in spermatozoa (Table 3). In contrast, the captive cheetah appeared unique in that such a consistently great proportion of both primary and secondary spermatozoal abnormalities were observed across a wide range of individuals and in a relatively successful breeding population. The latter finding does not appear to be population or geographically specific. Comparable high percentages of aberrant spermatozoal morphology were observed in ejaculates of eight other South African cheetahs sampled from the Blijdorp Zoo, Rotterdam, Holland, the Henry Doorly Zoo, Omaha, Nebraska and Wildlife Safari, Winston, Oregon (D.E.W., M. B. and J.G.H., unpublished observations).

There is lack of agreement in the literature concerning the importance of spermatozoal morphology in fertility (Salisbury and Baker, 1966). However, in general, the vast majority of the abnormalities detected in spermatozoa is found in mammals exhibiting pronounced infertility (Salisbury et al., 1977). In man (Chandley et al., 1975), as well as the bull (Chenoweth and Ball, 1980), ram (Rhodes, 1980), boar (Gibson and Johnson, 1980) and dog (Larson, 1980), the proportion of abnormal spermatozoa in the ejaculate has been related to fertility. Primary spermatozoal defects generally are considered more detrimental than secondary deformities (Chenoweth and Ball, 1980). When they exceed 20% of the spermatozoal population, fertility dysfunction may be indicated in the bull (Chenoweth and Ball, 1980) and dog (Larson, 1980). Until this report, human (MacLeod, 1964) and gorilla (Seuanez et al., 1977) spermatozoa were considered to show far greater variation in structure than male gametes from other species. Even in fertile men, 20 to 35% of spermatozoa have a structural defect (Afzelius, 1981). The gorilla is considered to produce a preponderance (29 to 92.5%) of pleiomorphic spermatozoa in the ejaculate (Seuanez et al., 1977; Platz et al., 1980; Afzelius, 1981); however, the significance of this finding to fecundity is unknown.

The etiology of abnormal spermatozoal characteristics in the cheetah is unknown. The possibility exists that the chronic stress associated with captivity has adversely affected testicular function. However, in general, captive cheetahs are neither aggressive nor hyperactive and usually exhibit outwardly serene behavior. In a concurrent study, markedly low levels of genetic variation have been detected in the De Wildt cheetah population (O'Brien et al., 1983). A comprehensive biochemical genetic analysis of approximately 200 structural loci of 55 cheetahs indicates that less than 1% of the loci are polymorphic, a value 10 times less than the extent of variation detected in man (Harris and Hopkinson, 1972), feral mice (Rice et al., 1980) or domestic cats (O'Brien, 1980). The level of variation in the cheetah approaches that observed in inbred mouse strains after 10 or 20 generations of sib mating (Green, 1982).

Numerous studies have established that spermatozoal development and morphology are under rigorous genetic control (Beatty, 1970; Krzanowska, 1976; Wyrobek, 1979). Using variation between inbred mouse strains as a monitor, Wyrobek (1979) has suggested that the contribution from biological (nongenetic) factors to spermatozoal morphology is generally trivial. Furthermore, it is well established that seminal quality can be adversely affected in highly inbred homogenous populations of mammals (Salisbury and Baker, 1966; Rice et al., 1967; Johansson and Rendel, 1968; Wildt et al., 1982). For example, approximately 66% of spermatozoa from the BALB/c inbred mouse strain are abnormally shaped compared to <5% abnormal sperm in noninbred mice (Wyrobek, 1979). The frequency of abnormals in inbred mice returns to normalcy (circa 2% abnormals) in hybrid progeny of inbred parents, suggesting complementation of a variety of chromosomal genes which contribute to the integrity of mammalian spermatozoa. Possibly more salient to the cheetah data described here is the recent examination of records of various species of captive zoo stock which reveals a high degree of inbreeding correlated with numerous deleterious effects, including increased juvenile mortality (Ralls et al., 1979). Whether the poor ejaculate

quality of the cheetah is a genetic consequence or a unique species norm cannot be determined by the present study; however, it is indeed possible that both are the case.

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REFERENCES

Afzelius, B. A. (1981). Abnormal human spermatozoa including comparative data from apes. Am. J. Primatol. 1:175–182.

Brand, D. J. (1980). Captive propagation at the National Zoological Gardens of South Africa. Int. Zoo. Yearb. 20:107–112.

Beatty, R. A. (1970). The genetics of the mammalian gamete. Biol. Rev. 45:73–119.

Chandley, A. C., Edmond, P., Christie, S., Gowans, L., Fletcher, J., Frackiewicz, A. and Newton, M. (1975). Cytogenetics and infertility in man. I. Karyotype and seminal analysis. Ann. Hum. Genet. 39:231–254.

Chenoweth, P. J. and Ball, L. (1980). Breeding soundness evaluation in bulls. In. Current Therapy in Theriogenology (D. Morrow, ed.). W. B. Saunders, Philadelphia, pp. 330–339.

Coubrough, R. I., Bertschinger, H. J. and Solely, J. T. (1978). Scanning electron microscopic studies on cheetah spermatozoa. Proc. Elect. Micro. Soc. So. Afr. 8:57–58.

Eaton, R. L. (1974). The Cheetah, Van Nostrand Reinhold, New York.

Gibson, C. D. and Johnson, R. C. (1980). Evaluation of the boar for breeding soundness. In: Current Therapy in Theriogenology (D. Morrow, ed.). W. B. Saunders, Philadelphia, pp. 1034–1037.

Green, E. (1982). Genetics and Probability in Animal Breeding Experiments, Oxford University Press, Oxford.

Harris, H. and Hopkinson, D. A. (1972). Average heterozygosity per locus in man: an estimate based upon incidence of enzyme polymorphisms. Ann. Hum. Genet. Lond. 36:9–20.

Howard, J. G., Pursel, V. G., Wildt, D. E. and Bush, M. (1981). Comparison of various extenders for freeze-preservation of semen from selected captive wild ungulates. J. Am. Vet. Med. Assoc. 179:1157–1161.

Johansson, I. and Rendel, J. (1968). Genetics and Animal Breeding, Oliver & Boyd, Edinburgh, pp. 257, 373–377, 378–382.

Krzanowska, H. (1976). Inheritance of sperm head abnormality types in mice –the role of the Y chromosome. Genet. Res. 28.189–198.

Larson, R. E. (1980). Infertility in the male dog. In: Current Therapy in Theriogenology (D. Morrow, ed.). W. B. Saunders, Philadelphia, pp. 646–654.

MacLeod, J. (1964). Human seminal cytology as a sensitive indicator of the germinal epithelium. Int. J Fertil. 9:281–295.

O'Brien, S. J. (1980). The extent and character of biochemical genetic variation in the domestic cat. J. Hered. 71:2–8.

O'Brien, S. J., Wildt, D. E., Goldman, D., Merril, C. R. and Bush, M. (1983). The cheetah is depauperate in genetic variation. Science 221:459–461.

Platz, C. C. and Seager, S.W.J. (1978). Semen collection by electroejaculation in the domestic cat. J. Am. Vet. Med. Assoc. 173:1353–1355.

Platz, C. C., Wildt, D. E. and Seager, S.W.J. (1978). Pregnancy in the domestic cat after artificial insemination with previously frozen spermatozoa. J. Reprod. Fertil. 52:279–282.

Platz, C. C., Wildt, D. E., Howard, J. G. and Bush, M. (1983). Electroejaculation and semen analysis and freezing in the giant panda (*Ailuropoda melanoleuca*). J. Reprod. Fertil. 67:9–12.

Platz, C. C., Wildt, D. E., Bridges, C. H., Seager, S.W.J. and Whitlock, B. S. (1980). Electroejaculation and semen analysis in a male lowland gorilla, *Gorilla gorilla*. Primates 21:130–132.

Pursel, V. G. and Johnson, L A. (1974). Glutaraldehyde fixation of boar spermatozoa for acrosomal evaluation. Theriogenology 1:63–68.

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

Rhodes, A. P. (1980). Semen collection and evaluation (in the ram). In: Current Therapy in Theriogenology (D. Morrow, ed.). W. B. Saunders, Philadelphia, pp. 944–947.

Rice, M. C., Gardner, M. B. and O'Brien, S. J. (1980). Genetic diversity in leukemia prone feral house mice infected with murine leukemia virus. Biochem. Genet. 18:915–928.

Rice, V. A., Andrews, F. N., Warwick, E. J. and Legates, J. E. (1967). Breeding and Improvement of Farm Animals, McGraw-Hill Book Co., New York, pp. 188–190.

Salisbury, G. W. and Baker, F. N. (1966). Nuclear morphology of spermatozoa from inbred and linecross Hereford bulls. J. Anim. Sci. 25:476–479.

Salisbury, G. W., Hart, R. G. and Lodge, J. R. (1977). The spermatozoan genome and fertility. Am. J. Obstet. Gynecol. 128:342–350.

Seuanez, H. N., Carothers, A. D., Martin, D. E. and Short, R. V. (1977). Morphological abnormalities in spermatozoa of man and great apes. Nature 270:345–347.

Wildt, D. E., Guthrie, S. C. and Seager, S.W.J. (1978). Ovarian and behavioral cyclicity of the laboratory maintained cat. Horm. Behav. 10:251–257.

Wildt, D. E., Baas, E. J., Chakraborty, P. K., Wolfe, T. L. and Stewart, A. P. (1982). Influence of inbreeding on reproductive performance, ejaculate quality and testicular volume in the dog. Theriogenology 17:445–452.

Wrogemann, N. (1975). Cheetah Under the Sun. McGraw-Hill Book Co., New York.

Wyrobek, A. J. (1979). Changes in mammalian sperm morphology after x-ray and chemical exposures. Genetics 92:105–119.