

## Reproductive Physiology of the Clouded Leopard: I. Electroejaculates Contain High Proportions of Pleiomorphic Spermatozoa throughout the Year<sup>1</sup>

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### ABSTRACT

Ejaculates were analyzed from clouded leopards (*Neofelis nebulosa*) subjected to a regimented anesthesia/electroejaculation protocol. Group I males ( $n=4$ ), maintained individually in an environment with natural fluctuations in photoperiod, were electroejaculated on the same day at monthly intervals (January–December). Group II clouded leopards ( $n=8$ ), maintained in random zoo populations throughout the U.S., were evaluated on a single occasion.

Phase contrast and electron microscopy indicated a high proportion of structurally abnormal spermatozoa in seminal fluid (Group I range, 14.8–78.9%; Group II range, 32.3–93.0%), the predominant deformity being a tightly coiled flagellum. Semen quality, including spermatozoal concentration and the incidence of abnormal sperm forms, varied ( $p<0.05$ ) among males. Evaluating the numbers of motile spermatozoa/ejaculate (MS/E) among individual males from Group I on a monthly basis suggested a seasonal influence; gradually increasing MS/E values with peaks in June and July were observed in three of four animals. A simultaneous analysis of international breeding records for captive female clouded leopards demonstrated that 46.2% of parturitions occurred in March and April, indicating that most estrual periods occurred from late December through February.

These data suggest that a physiological asymmetry may exist in peak reproductive performance between the male and female clouded leopard, perhaps as a result of differing adaptations to the captive environment. Motile spermatozoa can be recovered throughout the year using electroejaculation and, when used over time, a standardized procedure can determine a hierarchy of seminal quality among males of unknown reproductive potential. The relatively high proportions of structurally abnormal spermatozoa in the ejaculates of the clouded leopard may be related to a low degree of genetic variation within the species and/or hyperadrenal activity in captive populations.

### INTRODUCTION

Investigations of reproductive patterns of nondomesticated species generate a data base necessary for future artificial breeding efforts and provide valuable

information for comparative analysis of rare and previously unstudied species. A variety of wildlife species exhibits unique physiological or genetic traits potentially related to their nondomesticity and intensive, inherent need for survival in natural or captive habitats. The Felidae in particular demonstrate a fascinating array of novel reproductive, endocrine and genetic characteristics. Of particular interest is the observation that the electroejaculates of cheetahs contain high proportions of pleiomorphic spermatozoa (Wildt et al., 1983), a finding which may be related to an extreme genomic monomorphism characteristic of

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the species (O'Brien et al., 1983, 1985) or captivity stress (Wildt et al., 1984).

Using a similar research strategy, information on the reproductive physiology of the male clouded leopard is provided in this and a companion report (Wildt et al., 1986). The clouded leopard, considered the only species (*nebulosa*) in the genus *Neofelis*, is thought still to exist in the forests of Nepal, south-eastern China and the Malayan Peninsula, and on Taiwan, Sumatra and Borneo (Nowak and Paradiso, 1983). In the wild, the species is highly arboreal, hunting from trees by springing on prey from overhanging branches (Nowak and Paradiso, 1983). Information on the reproductive physiology of this species is negligible and consists primarily of unanalyzed breeding records and birth/death data cited in the International Species Studbook (Lewis, 1982). The clouded leopard breeds in captivity, although successful propagation appears closely dependent on prepubertal interaction between individuals designated for mating (Richardson, 1986). Experiences at the National Zoological Park suggest that breeding occurs only when the male and female are introduced at a young age and are permitted to interrelate and mature together. Introducing unfamiliar adults of the opposite sex, even for the purpose of pairing for zoological exhibition, frequently results in the male's killing or traumatizing the female (Richardson, 1986). Consequently, breeding management of adult, singleton male or female clouded leopards potentially could profit from artificial insemination programs.

The present study was conducted to provide a detailed evaluation of electroejaculate characteristics of captive clouded leopards in North America throughout the year. Animals were subjected to the same semen collection procedure to determine if electroejaculation used over time would distinguish males with the greatest reproductive potential. To provide a comparative control and to increase the size of the data base, clouded leopards maintained in other U.S. zoo populations were subjected to the same reproductive evaluation procedures. Particular emphasis was placed on the extent of abnormal spermatozoal morphology, using both phase contrast and transmission electron microscopy.

## MATERIALS AND METHODS

### Animals

Four male clouded leopards (Group I) were maintained at the National Zoological Park's Department

of Conservation (DOC), Front Royal, VA. The DOC is located in the foothills of the Blue Ridge Mountains (latitude 38° 50'N; longitude 78° 15'W). During the year of study, the ranges in daylight and outdoor temperature for each season were approximately as follows: winter (December–February), 9.5–12.0 h, –4.1–7.8°C; spring (March–May), 12.0–15.0 h, 4.9–19.3°C; summer (June–August), 12.0–15.0 h, 15.8–29.8°C; fall (September–November), 9.5–12.0 h, 6.4–20.5°C. All animals were adults ranging from 9 to 12 yr of age and 13.9 to 19.5 kg in body weight. Two of the males (#2 and #4) had been caught in the wild as cubs and two (#1 and #3) had been born in captivity. All had resided at the DOC for at least 3 yr before the beginning of the study.

Males were maintained in a building in individual, indoor 3.2- × 5.8-m enclosures containing a nest box, tree limbs for climbing and a 1- × 2-m window which allowed natural fluctuations in photoperiod. Beginning at 0700 h each day, supplemental artificial fluorescent lighting was provided for approximately 8 h. Each enclosure was connected by a trap-door to an outdoor wire-mesh cage containing 21–24 sq m of floor space. Between 1 May and 1 November, males had free access to the outdoor environment; at other times, animals were kept indoors. Throughout the year, indoor enclosure temperature ranged from 21–27°C. All males had ad libitum access to water and were fed a combination of a commercial zoo meat diet (Nebraska Brand Feline Diet, North Platte, NE; 450 g twice weekly) and one chicken, one rabbit and two rats weekly. Each animal had visual access to at least one other male, and all males were in aural proximity to an adult female maintained as a singleton in the same building.

For comparative purposes, a total of eight clouded leopard males (Group II) maintained at seven different zoological parks (Table 1) were subjected to the study protocol. These males, ranging from 3 to 11 yr in age, generally were maintained indoors with exposure to natural photoperiod and fed a diet comparable to that of Group I. Two animals (#7 and #9) were proven breeders and only one (#9) was being maintained with a female.

### Anesthesia and Electroejaculation

Group I males were anesthetized on a single day each month for electroejaculation; Group II males were anesthetized and subjected to semen collection on one occasion only. After induction of a surgical

TABLE 1. Electroejaculate characteristics of male clouded leopards maintained in U.S. zoological parks.<sup>a</sup>

Male no.	Age	Geographic location/month	Motile sperm/ ejaculate ( $\times 10^6$ )	Abnormal spermatozoa (%)		
				1°	2°	Total
5	3	Houston, TX/July	13.5	40.9	44.6	85.5
6	5	Colorado Springs, CO/July	7.6	39.7	36.1	75.8
7	10	Milwaukee, WI/Aug	1.5	33.7	55.8	89.5
8	6	San Antonio, TX/May	28.8	37.8	39.2	77.0
9	11	San Antonio, TX/May	4.3	58.4	34.6	93.0
10	8	Dallas, TX/Jul	90.9	24.1	59.4	83.5
11	3	Cincinnati, OH/Apr	41.9	15.3	49.7	65.0
12	9	Omaha, NE/Mar	51.2	5.7	26.6	32.3

<sup>a</sup>Male #5, 6, 7, 11 and 12 were maintained indoors and exposed to natural fluctuations in photoperiod. Male #8, 9, and 10 were maintained in outdoor enclosures and provided shelters. All were singletons and unproven breeders except male #7, who had sired one litter 2 yr before semen evaluation and #9, who had sired seven litters, the last born 4 yr prior to evaluation.

plane of anesthesia with ketamine hydrochloride (Ketaset, Bristol Labs., Syracuse, NY; 17.0 mg/kg body weight, i.m.), Group I and II clouded leopards were electroejaculated using previously described procedures (Wildt et al., 1983, 1984; Howard et al., 1986). A rectal probe (diameter, 1.6 cm; length, 23 cm) and an electrostimulator (AC, 60-Hz current; P-T Electronics, Boring, OR) were used to deliver a regimented electroejaculation sequence consisting of a total of 80 stimuli given in three series (Wildt et al., 1983, 1984; Howard et al., 1986). Series 1 and 2 consisted of 30 stimuli each, divided into three sets of 10 stimuli at 5, 6 and 7 V (Series 1) and 6, 7 and 8 V (Series 2). A total of 20 stimuli was administered in Series 3, with 10 stimuli each at 7 and 8 V. Electrical stimuli were given in a 3-sec-on and 3-sec-off pattern with a rest period of 3 min between stimulation series. The ejaculate was collected in a warmed (37°C), plastic container.

#### *Semen Evaluation: Light and Transmission Electron Microscopy*

Seminal fluid from each series was combined and a total ejaculate volume recorded. All light microscopic analyses were performed at 37°C using undiluted seminal aliquots and previously described procedures (Wildt et al., 1983; Howard et al., 1986). Percentage of spermatozoal motility and spermatozoal status (speed of forward progressive motility) were evaluated immediately on the basis of observations by two technicians who examined eight separate microscopic fields at 400 $\times$ . Spermatozoal status was a subjective evaluation of the type of forward movement of the sperm cell based on a scale of 0 (no movement) to 5 (steady, rapid forward progression). Sperm concentration/ml of ejaculate was determined using standard

hemocytometer methods. For gross morphological assessments, an aliquot (100  $\mu$ l) of ejaculate was fixed in 1% glutaraldehyde, and 300 spermatozoa/collection were examined using phase contrast microscopy (1000 $\times$ ) (Pursel and Johnson, 1974). Pleiomorphic forms were classified as those related to spermatogenic (primary origin) or excurrent duct system (secondary origin) deformities (Wildt et al., 1983; Howard et al., 1986).

For electron microscopy, spermatozoa were processed by a modification of an earlier procedure (Jones, 1973). In brief, after centrifugation at 700  $\times$  g for 15 min (21°C) the sperm pellet was resuspended in 4% glutaraldehyde in fixation medium (0.2 M sodium cacodylate with 1.8 g sucrose/100 ml of medium). After 1 h, the spermatozoa were recentrifuged and washed twice more in the fixation medium (5°C). The pellet was resuspended for 1 h in 1% osmium tetroxide before a final wash in fixation medium. The sperm concentrate was supported in agar (2% in 300 mM sucrose) for dehydration and embedding. The agar pellet was formed in a modified 1-ml plastic tuberculin syringe which was centrifuged for 15 min at 1500  $\times$  g before transfer to an ice bath. The hardened pellet was dehydrated in a graded series of ethanol baths and embedded in Epon 812 (TAAB Labs., Great Britain). Thin sections were stained with uranyl acetate and lead citrate before electron photomicrographs were taken.

#### *Demography*

Analyses of ejaculate quality suggested that season affected results in most clouded leopard males. As a consequence, survey data on captive female parturitions were taken from the International Clouded Leopard Studbook (Lewis, 1982) using an approach

similar to that reported recently for Siberian tigers (Seal et al., 1985). Information on the proportion of parturitions for each month of the year and litter size was recorded and analyzed to determine the potential effect of season on female reproductive patterns.

#### *Statistical Analyses*

A Statistical Analysis System program (SAS, 1982) was used to analyze the data by individuals and by seasons for means and standard errors of the means (SEM). Significant differences among the variables were determined by two-way analysis of variance, and individual means were then compared by Scheffe's Multiple Range Test (Snedecor and Cochran, 1980).

## RESULTS

### *Group I*

Seminal fluid was collected by electroejaculation from all Group I clouded leopards during each month of the year. Based on a total of 48 collections, the average total ejaculate volume was  $0.64 \pm 0.03$  ml (individual range, 0.25–1.40) containing  $27.5 \pm 2.3 \times 10^6$  spermatozoa/ml (range,  $3.0\text{--}73.0 \times 10^6$ ) with motility and status ratings of  $71.0 \pm 2.1\%$  (range, 40.0–90.0%) and  $3.9 \pm 0.1$  (range, 3.0–5.0), respectively. A high mean percentage of morphologically abnormal spermatozoa was present in the ejaculate ( $38.9 \pm 1.7\%$ ; range, 14.8–78.9%). The major primary deformity consisted of spermatozoa with tightly coiled flagella, although cells with macro- or microcephalic heads, damaged acrosomes, or missing midpieces were also evident (Table 2, Figs. 1,2). The predominant secondary abnormalities included acute bending of the midpiece or flagellum in the presence or absence of cytoplasmic droplet (Table 2, Figs. 1,2).

The clouded leopard spermatozoon had an electron-dense nucleus (Fig. 2a). The acrosome was moderately dense and enveloped approximately 75% of the nucleus; its anterior region was enlarged, but its width decreased greatly toward the posterior. The nucleus, separated from the acrosome by an opaque cytoplasmic region, rested on a basal plate that abutted the proximal centriole. Cytoplasmic droplets were often observed surrounding the midpiece (Fig. 2b). The midpiece and flagellar regions displayed typical features including axial filaments, outer coarse fibers,

and a mitochondrial sheath. Subcellular abnormalities (including additional acrosomal damage) were not evident, although deformities observed by phase contrast microscopy (bent and coiled flagella) were commonly identified by electron microscopy (Fig. 2c).

When analyzed on a quarterly seasonal basis no differences were observed in ejaculate volume, spermatozoal concentration/ml of ejaculate, percentage of motility, progressive status or percentage of morphological abnormalities over time (Fig. 3). Differences in all ejaculate characteristics were evident between individual males and were particularly pronounced in sperm concentration and abnormality factors (Fig. 3). To investigate individual variations further and to obtain a single index of ejaculate quality, the number of motile spermatozoa/ejaculate (MS/E) was calculated and plotted for each male by month. On this basis, profiles suggested a seasonal influence, since the numbers of motile spermatozoa/ejaculate were observed to rise gradually and peak in June and July in three of four animals (Fig. 4). Additionally, it was evident that electroejaculated males differed in overall MS/E (male #1>4= 2>3).

### *Group II*

The survey results of eight clouded leopards maintained in captivity in other U.S. zoological parks are summarized in Table 1. The numbers of MS/E ranged from 1.5 to  $90.9 \times 10^6$ , values in the range observed in Group I males (Fig. 4). With the exception of male #12, the proportion of pleiomorphic spermatozoa in the ejaculates of the remaining seven animals exceeded 64.0% of all cells evaluated; the number of primary and secondary abnormalities ranged from 5.7- to 58.4% and 26.6 to 59.4%, respectively.

### *Female Seasonality*

Evaluation of 145 clouded leopard parturitions worldwide in the Northern Hemisphere demonstrated that births occurred in 11 different months of the year with the greatest number occurring in March (27.6%) and April (18.6%) (Fig. 5). At the National Zoo's breeding facility, 11 litters were recorded during 6 different months with seven parturitions (63.6%) occurring in February and March. Based on studbook records of all parturitions, the overall mean litter size was  $1.63 \pm 0.06$ , and the sex ratio was 1.08 males for every female produced.

TABLE 2. Structural morphology of clouded leopard spermatozoa.<sup>a,b,c</sup>

	Male				Total
	#1	#2	#3	#4	
Morphologically normal	65.6 ± 3.5 <sup>d,e</sup>	68.5 ± 3.6 <sup>d</sup>	50.3 ± 3.1 <sup>f</sup>	59.7 ± 2.8 <sup>e</sup>	60.9 ± 2.0
<i>Primary defects</i>					
Coiled flagellum	5.5 ± 1.2 <sup>d</sup>	5.8 ± 1.9 <sup>d</sup>	13.8 ± 1.7 <sup>e</sup>	14.7 ± 2.6 <sup>e</sup>	10.3 ± 1.2
Macro/microcephalic	0.5 ± 0.4 <sup>d</sup>	1.6 ± 0.6 <sup>d,e</sup>	2.9 ± 0.9 <sup>e</sup>	1.6 ± 0.6 <sup>d,e</sup>	1.7 ± 0.3
Acrosomal defect	1.8 ± 1.3 <sup>d</sup>	2.2 ± 1.1 <sup>d</sup>	1.3 ± 0.6 <sup>d</sup>	0.8 ± 0.4 <sup>d</sup>	1.5 ± 0.4
No midpiece	0.1 ± 0.1 <sup>d</sup>	1.4 ± 0.8 <sup>d</sup>	0.9 ± 0.5 <sup>d</sup>	0.1 ± 0.1 <sup>d</sup>	0.6 ± 0.2
<i>Secondary defects</i>					
Bent midpiece with cytoplasmic droplet	5.3 ± 2.5 <sup>d</sup>	2.5 ± 0.7 <sup>d</sup>	5.1 ± 2.4 <sup>d</sup>	4.1 ± 1.6 <sup>d</sup>	4.2 ± 0.9
Bent midpiece without cytoplasmic droplet	5.7 ± 1.2 <sup>d</sup>	2.8 ± 1.0 <sup>e</sup>	7.2 ± 0.9 <sup>d</sup>	5.9 ± 1.0 <sup>d</sup>	5.4 ± 0.6
Bent flagellum	8.8 ± 1.3 <sup>d</sup>	8.0 ± 1.7 <sup>d</sup>	10.2 ± 1.3 <sup>d</sup>	8.9 ± 1.1 <sup>d</sup>	9.0 ± 0.7
Cytoplasmic droplet, proximal	3.6 ± 0.7 <sup>d</sup>	4.8 ± 1.1 <sup>d</sup>	3.4 ± 0.6 <sup>d</sup>	1.9 ± 0.4 <sup>e</sup>	3.4 ± 0.4
Cytoplasmic droplet, distal	1.1 ± 0.5 <sup>d</sup>	0.5 ± 0.4 <sup>d</sup>	0.4 ± 0.2 <sup>d</sup>	0.5 ± 0.3 <sup>d</sup>	0.6 ± 0.2
Bent neck	2.0 ± 0.6 <sup>d</sup>	2.0 ± 0.5 <sup>d</sup>	4.2 ± 0.7 <sup>e</sup>	1.8 ± 0.5 <sup>d</sup>	2.5 ± 0.3

<sup>a</sup>Values are mean percentages ± SEM.

<sup>b</sup>Based on 12 electroejaculates/animal taken throughout the year.

<sup>c</sup>Values within rows with different superscripts are significantly different ( $p < 0.05$ ).

## DISCUSSION

Under a standardized electrostimulation protocol, clouded leopards studied over time as well as in random zoo-maintained populations produced electroejaculate norms different from those reported for domestic cats and cheetahs (Wildt et al., 1983; Carter et al., 1984). Although the proportions were not as extreme as those reported for the cheetah, clouded leopards produced high proportions of pleiomorphic spermatozoa/ejaculate ranging from 14.8 to 78.9% in males monitored monthly (Group I) and from 32.3 to 93.0% in males surveyed on a single occasion (Group II).

When the data from Group I males were averaged and evaluated on a quarterly basis, seasonality showed no discernible effect on ejaculate traits. Plotting results from individual males, however, demonstrated that three of four animals produced gradually increasing numbers of MS/E that peaked in the summer months of June–July; the MS/E of the remaining male with the poorest overall ejaculate quality did not vary over time. Analysis of birth records indicated that most clouded leopard parturitions occurred from early March through April. Because the gestation interval is estimated at 86–93 days (Nowak and Paradiso, 1983), the incidence of estrus appears to be

greatest from late December through early February, which strongly suggests that increasing photoperiod influences female reproductive patterns. Therefore, a potential asymmetry may exist between male and female peak reproductive performance, perhaps as a result of chronic exposure to a captive environment far removed from the natural tropical habitat. Females, because of the more extreme fluctuations in North American daylength and temperature, may have become more sensitive to photoperiodicity, evolving seasonal estrual patterns similar to those reported in free-ranging domestic cats (Paape et al., 1975; Jemmett and Evans, 1977) and more recently in captive Siberian tigers (Seal et al., 1985). In contrast, the male's reproductive performance in terms of ejaculate quality may excel under environmental conditions more similar to the native habitat, thus explaining the improved seminal traits observed after the males experienced the longer, warmer days of late spring and early summer.

Definitive conclusions on seasonality patterns are difficult to establish in many wildlife species maintained under captive conditions. In the clouded leopard, accurately monitoring estrual behavior is complicated by the nocturnal nature of the species, and weaning or loss of a suckling litter can induce a fertile

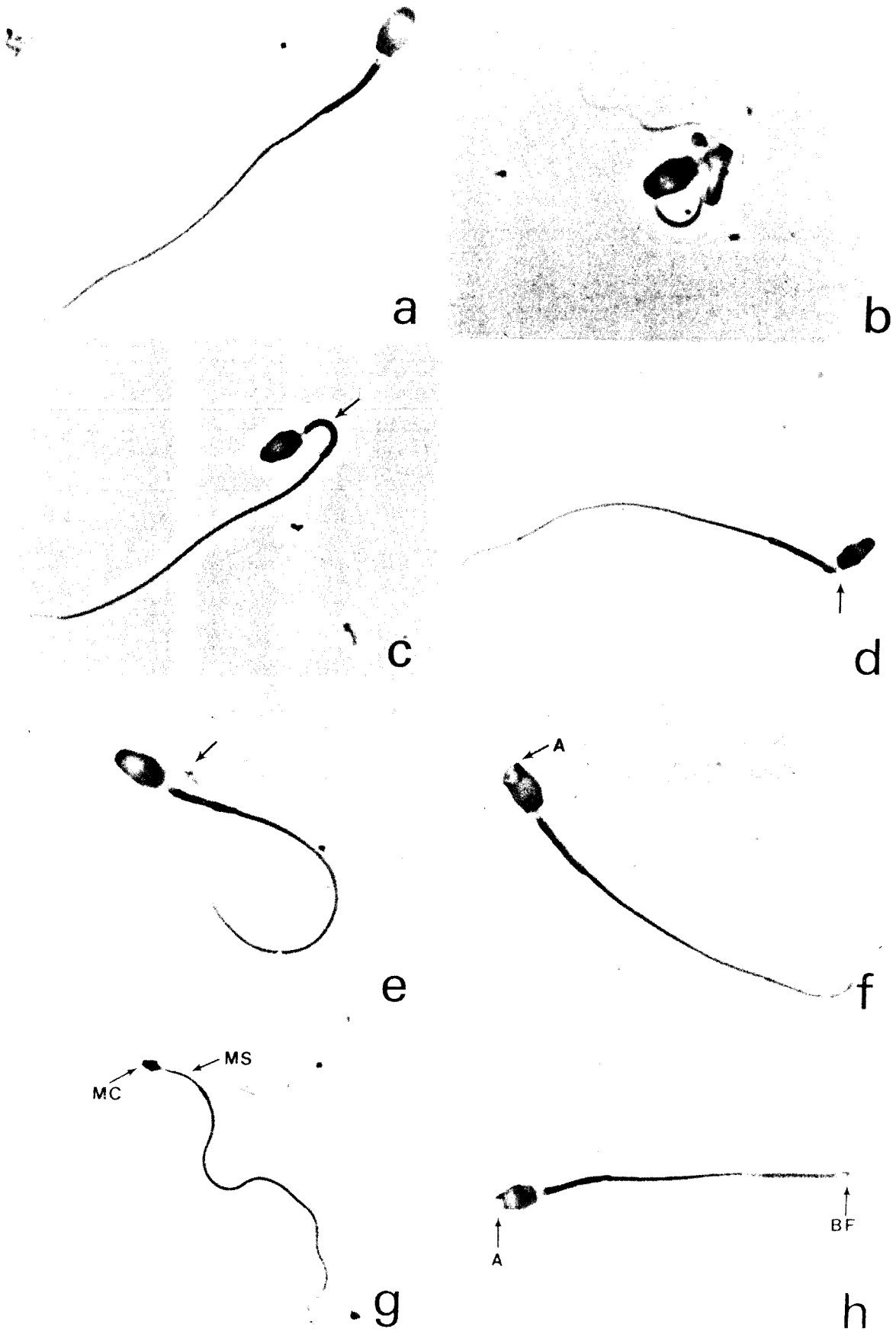


FIG. 1. Spermatozoal forms detected in the clouded leopard electroejaculate: a) normal; b) coiled flagellum; c) bent midpiece with cytoplasmic droplet; d) bent neck; e) proximal cytoplasmic droplet; f) acrosomal (A) defect and distal cytoplasmic droplet; g) microcephalic (MC) defect and missing mitochondrial sheath (MS); h) bent flagellum (BF) and acrosomal defect (A).  $\times 1000$ .

estrus (Richardson, 1986), a factor that could influence the birth distribution. Although effects attributable to season may have existed in Group I males, the sample size was small, and variations were observed only in the three individuals with the greatest seminal quality. The survey data of Group II failed to assist in evaluating seasonality effects because of the marked diversity in animal age and geographic location and the further complication of only a single electroejaculation episode. Accuracy of the reproductive evaluation is improved when analyses employ a standardized electrostimulation protocol on multiple occasions (Howard et al., 1986). With this approach it is possible to determine if differences over time or between animals are related to environmental factors or physiological variations in reproductive potential. Although these data were inconclusive in clearly establishing male seasonality, the results demonstrate that clouded leopards do produce recoverable, motile spermatozoa through the year. When the standardized electrostimulation protocol is used over time, definitive patterns in ejaculate quality emerge that eventually permit establishing a range of expected species norms. Most importantly, a consistently used electroejaculation technique employed over time provides information helpful in establishing a hierarchy of reproductive potential within a specific group of males.

Electron microscopy revealed morphological traits common to other species (Nicander et al., 1962; Hadek, 1963; Pedersen and Hammen, 1982) and no obvious subcellular abnormalities. Overall, of the pleiomorphic spermatozoa observed, 36% (Group I) and 40% (Group II) were of primary origin, suggesting severe spermatogenic dysfunction. Although a portion of these defective spermatozoa could be presumed to be the result of aged or degenerating spermatozoa within the vas deferens or epididymis, cheetahs evaluated three times within a single week continued to produce high proportions of abnormal spermatozoa/ejaculate (Wildt et al., 1983). More recently, the electroejaculation technique has been eliminated as a cause of aberrant sperm production. Ejaculates collected from a domesticated cheetah using an

artificial vagina (Durrant et al., 1985) contained numbers of pleiomorphic spermatozoa comparable to numbers found in electroejaculated males. Additionally, hemicastration and extraction of fluid from the vas deferens of a free-ranging wild-caught bobcat (*Felis rufus*) and puma (*Felis concolor*) revealed a predominance of structurally defective spermatozoa (total abnormalities, 73.0% and 94.0%, respectively; Howard et al., in preparation).

Ejaculate characteristics of the cheetah may in part result from a striking lack of genetic variation within the species (O'Brien et al., 1983). Biochemical analyses of over 200 structural loci indicate that the cheetah has one to two logarithms less genetic variation than eight other species of felids (O'Brien et al., 1983, 1985). In addition, the cheetah has demonstrated remarkable genetic uniformity at the major histocompatibility complex, a very high infant mortality rate, and a hypersusceptibility to a coronavirus associated with feline infectious peritonitis (O'Brien et al., 1985). Genomic monomorphism could influence reproductive traits including spermatozoal integrity of a wild species, particularly since inbreeding adversely affects seminal quality in homogeneous, inbred populations of domestic mammals, including the mouse (Krzanowska, 1976; Wyrobek, 1979), bull (Salisbury and Baker, 1966), and dog (Wildt et al., 1982; Hall et al., 1985). A recent genetic analysis of 20 captive clouded leopards (Newman et al., 1985) indicated that only one of 50 biochemical loci examined (2.0%) were polymorphic, the lowest rate in felids with the exception of the cheetah. The relatively high degree of genetic uniformity in the clouded leopard may be related in part to a propensity for assortive mating among adult animals observed in zoological collections. If free-ranging adult males aggressively attack unfamiliar adult females in the natural habitat, it is understandable that pair bonding may occur at a relatively young age, a circumstance which may result in an increased incidence of incestuous matings.

Increased adrenal function due to a normally wild and aggressive nature or to captivity stress could also adversely affect the quality of ejaculate, potentially through disruptions in gonadotropin or gonadal steroid production (Moberg, 1984). Various factors including social, thermal, pharmacologic, and nutritive stressors adversely influence ejaculate quality of certain domestic species (Austin et al., 1961; Rhynes and Ewing, 1973; Wettemann et al., 1976; Rama-

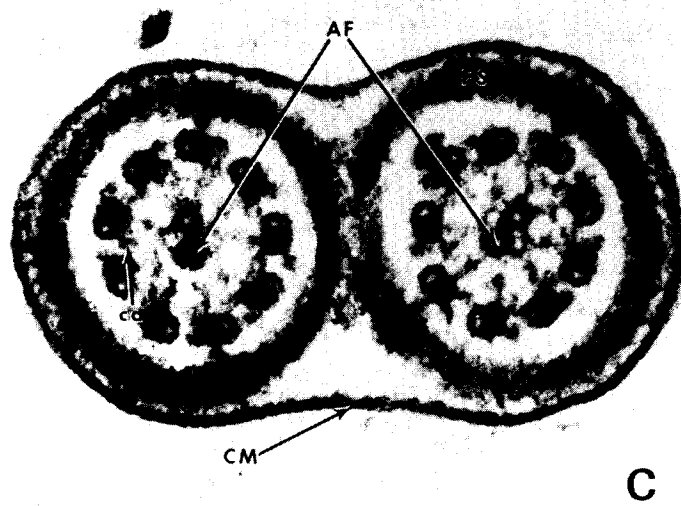
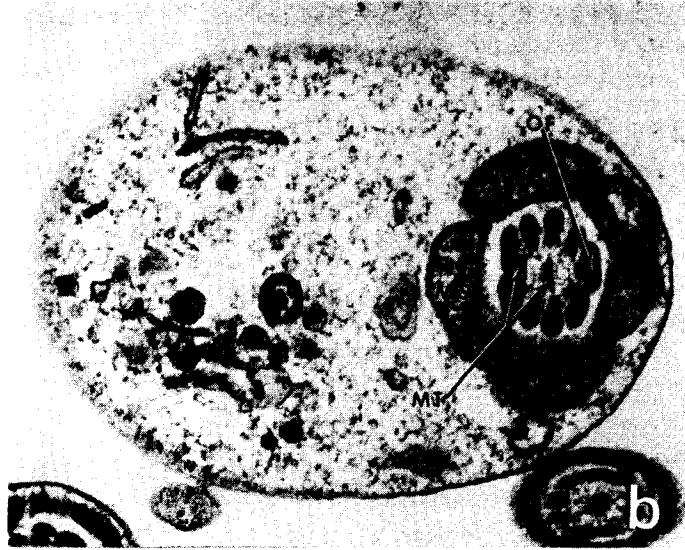
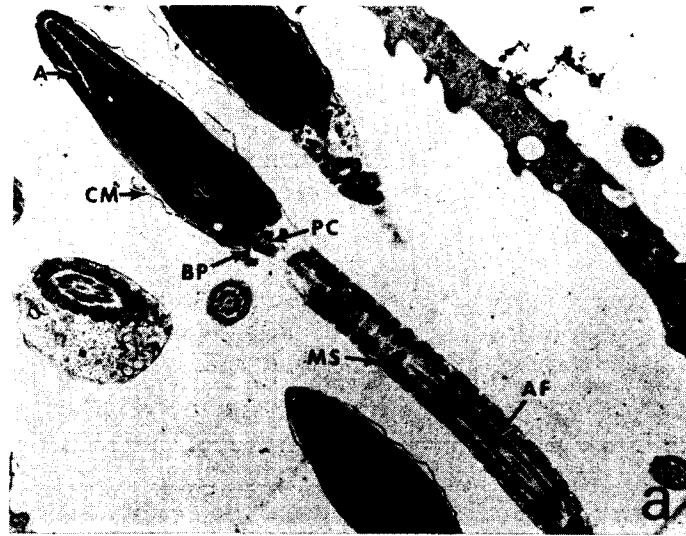




FIG. 2a. Longitudinal electron micrograph section of head, neck and anterior midpiece of normal spermatozoon. Beginning at the exterior, the head is composed of acrosome (A), cell membrane (CM) and a cytoplasmic region between the acrosome and nucleus (N). The ruptured CM was presumed to be an artifact of the fixation process. Neck region contains proximal centriole (PC) and basal plate (BP). Midpiece consists of the axial filament (AF) surrounded by the mitochondrial sheath (MS). X5500.

b. Cross-section of midpiece enveloped by a proximal cytoplasmic droplet. Midpiece consists of a central 9 by 2 arrangement of microtubules (MT), surrounded by nine outer coarse fibers (OF) and the mitochondrial sheath (MS). X33,000.

c. Cross-section of bent flagellum illustrating adjacent axial filaments (AF) and fibrous sheaths (FS) within the cell membrane (CM). Axial filament displays the 9 by 2 microtubules arrangement with connecting arms (ca). X90,000.

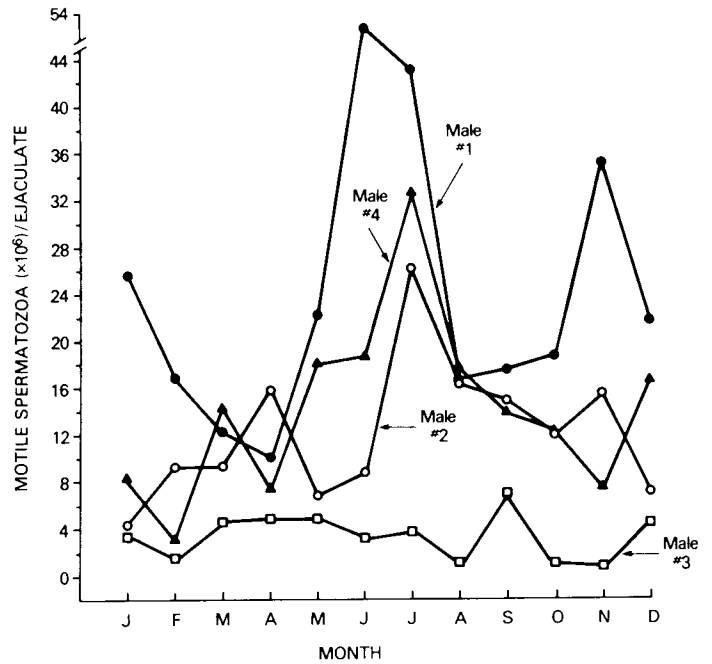
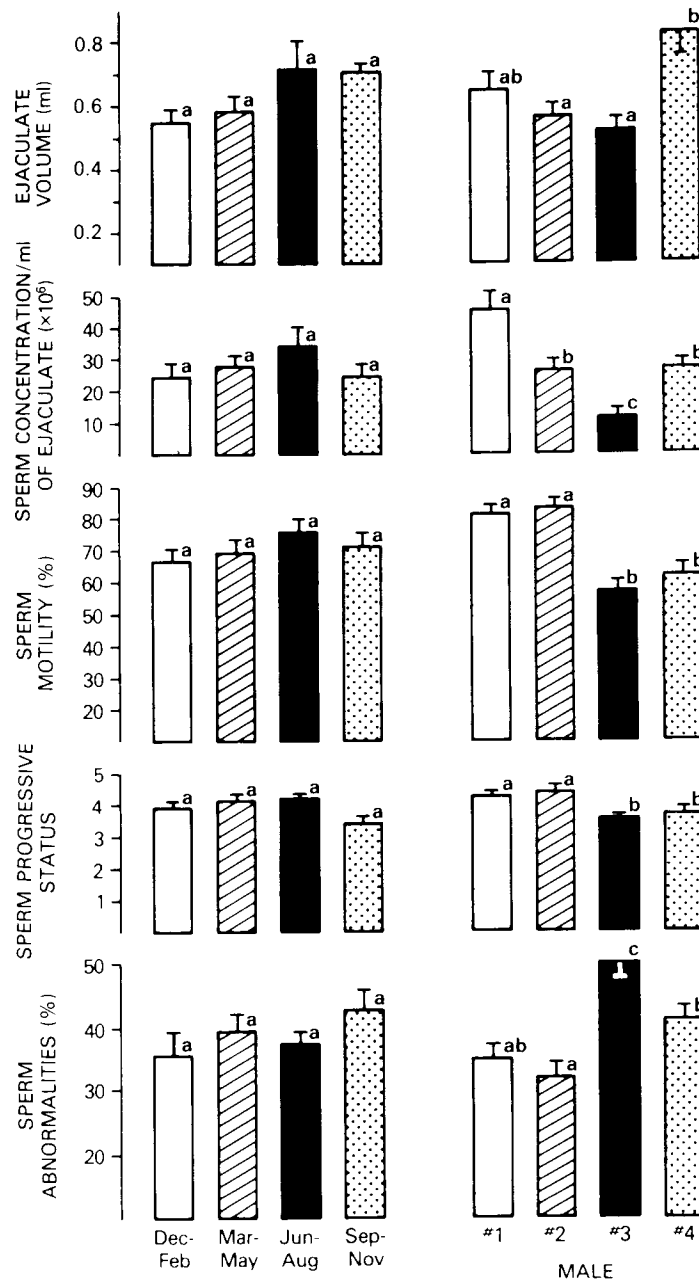


FIG. 4. Numbers of motile spermatozoa/ejaculate for individual clouded leopards electroejaculated once monthly throughout the year.

ley, 1981; Widdowson, 1981; Collu et al., 1984); effects include increasing numbers of abnormal spermatozoa (Skinner, 1981; Meyerhoeffer et al., 1985; O'Connor et al., 1985). A stress hypothesis may be implausible in the case of the cheetah, a species neither aggressive nor producing comparatively high circulating concentrations of adrenal glucocorticoids (Wildt et al., 1984). In contrast, clouded leopards produce markedly elevated cortisol concentrations (Wildt et al., 1986), certainly much greater than levels reported in many common domesticated or laboratory species (Welsh and Johnson, 1981; Fujieda et al., 1982; Vernikos et al., 1982; Dantzer and Mormede, 1983).

The direct correlation of either loss of genetic diversity or hyperadrenal function as causative factors in the unique ejaculate characteristics of certain wild felids remains to be determined. Of greater interest is the significance of the high proportions of abnormal sperm forms to subsequent fertility, a point that

FIG. 3. Mean (± SEM) values for ejaculate characteristics of clouded leopards on the basis of season or individual animals. Within comparison groups, bars with different superscripts are significantly different (p<0.05).

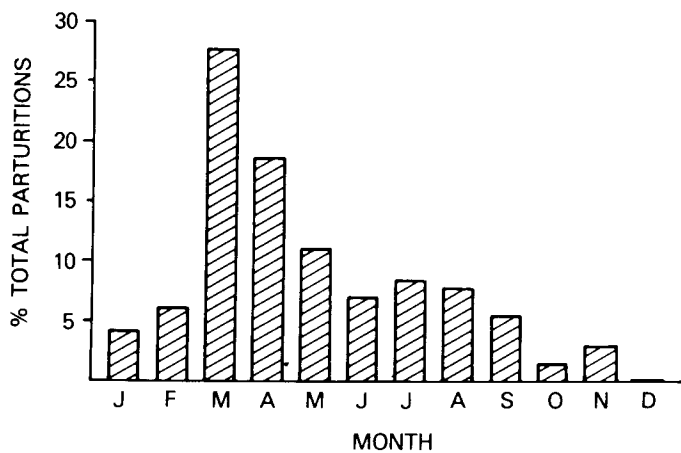


FIG. 5. Distribution of percentage of total parturitions in the clouded leopard based on month of the year (n=145).

continues to stimulate controversy when ejaculate quality in domestic species and even man is discussed (Wildt et al., 1983). As demonstrated by the parturition survey, the captive clouded leopard does not excel in reproductive performance. The mean birth rate of 1.6 cubs/litter was considerably less than that reported in domestic cats (mean, 3.3 kittens/litter; Schmidt et al., 1983) or tigers (mean, 2.4 cubs/litter; Seal et al., 1985). Whether this finding is the result of male subfertility or environmental stress on the dam remains to be determined. However, there is a need to analyze ejaculates from wild cats unaffected by the rigors of captivity stress and polymorphic in genomic background. As a result, studies are in progress to evaluate spermatozoal morphology in selected felids, with known histories of breeding success, free ranging in wild habitats.

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