REPRODUCTIVE CHARACTERISTICS OF MALE FLORIDA PANTHERS: COMPARATIVE STUDIES FROM FLORIDA, TEXAS, COLORADO, LATIN AMERICA, AND NORTH AMERICAN ZOOS

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Testicular volume, semen traits, and pituitary-gonadal hormones were measured in populations of *Felis concolor* from Florida, Texas, Colorado, Latin America, and North American zoos. More Florida panthers (*F. concolor coryi*) were unilaterally cryptorchid (one testicle not descended into the scrotum) than other populations (43.8 versus 3.9%, respectively). Florida panthers also had lower testicular and semen volumes, poorer sperm progressive motility, and more morphologically abnormal sperm, including a higher incidence of acrosomal defects and abnormal mitochondrial sheaths. Transmission electron microscopy revealed discontinuities in the acrosome, extraneous acrosomal material under the plasma membrane, and remnants of the golgi complex under the acrosome. No differences were detected in mean-circulating follicle-stimulating hormone, luteinizing hormone, or testosterone between Florida panthers and other populations of mountain lions. Seminal traits and concentrations of follicle-stimulating hormone, luteinizing hormone, and testosterone were similar between cryptorchid and noncryptorchid Florida panthers. Animals with *F. concolor coryi* ancestry were categorized on the basis of amount of genetic variation (low = type A; medium = type B; high = captive Piper stock). Compared to counterparts, type A Florida panthers had the lowest testicular volume and sperm-motility ratings and were the only animals exhibiting unilateral cryptorchidism. These results demonstrate the existence of major morphological and physiological differences among populations of *F. concolor*, a finding potentially related to differences in genetic diversity.

Key words: *Felis concolor*, Florida panther, male reproductive traits, cryptorchidism, sperm morphology, testosterone, conservation

The mountain lion (*Felis concolor*), also known as cougar, puma, and panther, has the widest distribution of any terrestrial mammal in the western hemisphere other than man (Hall, 1981). Twenty-seven subspecies or geographic races of mountain lions are recognized, although it is likely that two subspecies (*F. c. cougar* and *F. c. schor-*

geri) are extinct (Anderson, 1983). The Florida panther (*F. c. coryi*), recognized as a distinct subspecies since the late 1800s (C. B. Cory, in litt.), is characterized by a broad, flat frontal region of the skull, shortened rostrum, broad and highly arched nares, a distinct whorl of hair (cowlick) along the back of the thorax, and a 90° kink at the end of the tail (Belden, 1986; Goldman, 1946). Historically, the Florida panther ranged throughout the southeastern United States as far west as eastern Texas and as far north as Tennessee and South Carolina (Goldman, 1946). The current population of 30 to 50 animals, however, is restricted to the Big Cypress Swamp and Everglades ecosystems in southern Florida (Belden, 1986). Human residential and agricultural encroachment leading to rapid and severe habitat loss is the primary cause of the decline of Florida panthers (Belden et al., 1988). These pressures are greatest on private lands, approximately one-half of the documented distribution of Florida panthers (Maehr, 1990). Mountain lions require large, contiguous habitat to maintain the social and reproductive behavior necessary for viable breeding (Seidensticker et al., 1973). Mating, pregnancy, and births occur throughout the year in both free-ranging and captive populations of mountain lions suggesting that there is no synchronized breeding season (Anderson, 1983; Guggisberg, 1975). In a review of the literature by Anderson (1983), no seasonal peaks in reproduction were noted; however, studies providing definitive data are lacking.

The critical status of the Florida panther was recognized as early as 1967 when the United States Fish and Wildlife Service listed the subspecies as endangered. From July 1986 to June 1991, an average of five animals per year died or were killed, with ca. 40% of the mortality attributed to humans (Roelke et al., 1993). Although some reproduction occurs each year, infant mortality is thought to be relatively high with fewer than one-half of all pregnancies resulting in offspring that survive beyond 6 months of age (Roelke et al., 1993). These circumstances and the continuous loss of habitat make it unlikely that the Florida panther population will be self-sustaining. Indeed, a recent Population Viability Analysis predicted that, under existing conditions, the Florida panther will be extinct in 25–40 years (U. S. Seal and R. C. Lacy, in litt.). Other populations of mountain lions in western North America and Latin America, although generally confined to remote regions, currently are not in danger of extirpation (Anderson, 1983; Guggisberg, 1975).

Recent work indicates that the population of free-ranging Florida panthers is composed of two distinct genetic stocks (O'Brien et al., 1990). One (designated type A), living primarily in the Big Cypress National Preserve and surrounding lands, consists of descendants of historic *F. c. coryi.* The other (designated type B), residing primarily in the Everglades National Park, consists of descendents of mountain lions with Latin American heritage, which were introduced by man to the southern Florida ecosystem in the 1950s and 1960s. Between 1956 and 1966, seven mountain lions born in a private collection and designated as Piper stock were released into the Everglades National Park. These mountain lions were descendants of animals obtained from the Big Cypress Swamp in 1940 and a female of South American origin (Roelke et al., 1993). Genetic studies indicate that mountain lions free-ranging in the Everglades National Park (type B), the descendants of Piper stock animals (maintained in captivity), and certain South American mountain lions all have a common mitochondrial DNA haplotype, or a rare allozyme variant or both that does not occur in mountain lions in the Big Cypress (type A) or other North American mountain lions (O'Brien et al., 1990; Roelke et al., 1993).

One recommendation of the Florida Panther Population Viability Analysis was that a captive propagation program be estab-

lished including, if necessary, use of "assisted" reproduction (artificial breeding). Reproductive biotechnologies, such as artificial insemination, in vitro fertilization, and embryo transfer, have been proposed to have considerable potential for propagating rare felids (Wildt, 1990; Wildt et al., 1992*a*, 1992*b*). Nonetheless, a prerequisite to reliable, repeatable use of these strategies is a thorough understanding of the basic reproductive biology of a species. Therefore, the objectives of this study were to: 1) characterize ejaculate traits and pituitary-gonadal hormonal concentrations in Florida panthers; 2) evaluate these data in the context of those obtained from less-threatened wild and captive populations of mountain lions; 3) compare reproductive characteristics of animals with *F. c. coryi* ancestry, but with known differences in genetic variability.

Materials and Methods

This study was conducted from March 1981 to May 1993. Semen and blood samples were collected at various times throughout the year from five populations of mountain lions with most samples collected between October and May. Animals were categorized into one of four groups primarily on the basis of geographic location of capture: Florida (southern Florida, *F. c. coryi*, $n = 16$; type A = 11, type B = 5); Texas (western Texas, *F. c. stanleyana*, $n = 9$); Colorado (southwestern Colorado, *F. c. hippolestes*, $n = 7$); Latin America (Argentina and Uruguay, *F. c. cabrerae*, $n = 10$; Chile, *F. c. patagonica*, $n = 2$, *F. c. puma*, $n = 1$; Guatemala, *F. c. mayensis*, $n = 2$). At the time of sampling, one of the males in the Florida group, 14 in the Latin American group, and all in the Texas group were wild-caught animals that had been living in captivity for 5, 2–13, and ≤ 1 years, respectively. All others were free-ranging at the time of this study. The fifth population consisted of a captive group of pumas ($n = 30$) born in captivity, but of uncertain genetic origin. Three of these males were descendents of Piper-stock animals. Ages of the free-living individuals were unknown, but all males were thought to be adults based upon overall body size. For animals in which birth dates were known, age ranged from 17 to 180 months. Body weight for all males ranged from 39 to 72 kg.

Free-ranging animals were tracked using radiotelemetry and trained dogs. Once chased into a tree, animals were anesthetized with either ketamine HCl (12 mg/kg body weight, intramuscular) or Telazol® (7 mg/kg body weight, intramuscular, A. H. Robbins, Richmond, VA) administered via a syringe dart fired from an air-powered rifle. Animals then either fell from the tree into a net suspended over a crash bag or were lowered from the tree using ropes. Mountain lions maintained in captivity were anesthetized using similar dosages of ketamine HCl or Telazol® administered by dart from an air-powered pistol, pole syringe, or hand syringe. Animals were supplemented with anesthetic as necessary to maintain a surgical plane of anesthesia throughout the electroejaculation procedure.

Testicular width and length were measured with calipers (to the nearest mm), and these values used to calculate total testicular volume (Howard et al., 1986). Semen was collected by a standardized electroejaculation technique using either an AC (P. T. Electronics, Boring, OR) or DC (Lane Manufacturing, Denver, CO) electroejaculator as described previously (Howard et al., 1986, 1990; Wildt et al., 1983). Briefly, 80 stimuli of regimented voltage (2–8 volts) were administered by a rectal probe in three series of 30, 30, and 20 stimuli each with ca. 10 min of rest between series (total $\bar{X} \pm SE$ electroejaculation interval, 33.4 ± 2.1 min).

Blood samples for assessing endocrine status were collected from the cephalic vein preceding onset of electroejaculation, following each electroejaculation series and at 30, 60, and 90 min after the last series. Samples were maintained at ca. 4°C, centrifuged, and recovered sera were stored at −70°C until analyzed for hormonal concentrations.

Semen from all three electroejaculation series was combined and the volume measured. An undiluted aliquot was examined by phase-contrast microscopy for subjective assessment of percent sperm motility (0–100%) and progressive motility (0 for no movement to 5 for rapid linear forward progression—Howard et al., 1986). Sperm concentration was determined using a hemacytometer and erythrocyte–determination kit (Becton-Dickinson, Rutherford, NJ) as described previously (Howard et al., 1986; Wildt et al., 1983). For assessment of sperm morphol-

ogy, an aliquot of raw semen was fixed in 0.3% glutaraldehyde (Howard et al., 1986), and later 200–300 cells were examined using phase-contrast microscopy (1,575×). Sperm were categorized as normal or having one of the following structural defects: abnormal acrosome; other head defects (macro-, micro-, or bicephalic); abnormal mitochondrial sheath (total or partial mitochondrial aplasia); bent midpiece; flagellar defects (tightly coiled or bent flagellum); retained cytoplasmic droplet (proximal or distal); spermatid.

For electron microscopy, semen was fixed with a paraformaldehyde-glutaraldehyde-picric acid fixative (Advanced Biotechnologies, Inc., Columbia, MD) for 1 h and then centrifuged for 10 min at 300 × *g*. The supernatant was discarded, phosphate buffer layered over the sperm pellet, and the sample maintained at ca. 4°C during transport to a commercial laboratory for further processing (Advanced Biotechnologies, Inc.). Briefly, pellets were post-fixed with 1% osmium tetroxide, en bloc stained with 2% aqueous uranyl acetate, dehydrated in a series of ethanol alcohol baths, infiltrated with Spurrs plastic resin, and polymerized overnight. Sections (60–70 nm thick) were mounted on bare grids, post-stained with lead citrate, and examined by transmission electron microscopy.

Serum luteinizing hormone and follicle-stimulating hormone were quantified by specific radioimmunoassays previously validated for serum from nondomestic felids (Brown et al., 1988) using ovine luteinizing hormone (NIH-LH-S18) and ovine follicle-stimulating hormone (NIH-FSH-S8) for the luteinizing-hormone and follicle-stimulating-hormone assay, respectively. Minimum assay sensitivities (defined as 90% of the total binding for 200 μl serum) were 0.1 ng/ml for the luteinizing-hormone assay and 10 ng/ml for the follicle-stimulating-hormone assay. Inter- and intra-assay coefficients of variation were <10% for both assays. Serum testosterone was measured using a radioimmunoassay kit (ICN Biomedical, Carson, CA) previously validated for serum from nondomestic felids (Brown et al., 1988). The assay sensitivity was 0.05 ng/ml. Both inter- and intra-assay coefficients of variation were <10%.

To determine if ejaculate characteristics were influenced by using an AC versus a DC electroejaculator, data were compared using an unpaired Student's *t*-test (Steel and Torrie, 1980). Because no differences were measured, these data were pooled for further analysis. A one-factor analysis of variance (ANOVA) was used to test significance among populations in testicular volume and seminal traits. Hormonal data were analyzed by ANOVA for repeated measures. In both cases, individual means then were compared by the Fisher least-significant-difference procedure for multiple comparisons (Fisher, 1935). The proportion of cryptorchid males among the three populations with *F. c. coryi* ancestry was compared by an extension of the Fisher exact test (Conover, 1980). All other reproductive traits for these three groups were examined by ANOVA, followed by the Fisher least-significant-difference procedure. To reduce the risk of a type I error due to repeated univariate tests, a Bonferroni correction was used to determine the alpha levels for significance (Kleinbaum et al., 1988). All necessary statistical assumptions for ANOVA were met.

Results

Of the Florida panthers examined, 43.8% of 16 were unilateral cryptorchids (one testicle not descended into the scrotum) compared to 3.9% of 51 in the other populations of mountain lions. Mean total testicular volume of Florida panthers was less than that of the other populations (Table 1). Overall, mean quality of semen from Florida panthers was poorer than that of samples collected from some or all of the other populations (depending upon trait; Table 1). Florida panthers had less ejaculate volume, poorer ratings of sperm progressive motility, and fewer morphologically normal sperm than the other populations. Few differences were noted in quality of semen among Texas, Colorado, Latin American, and captive mountain lions. Percentage sperm motility of Colorado and Latin American males was greater than the others, and semen from Latin American mountain lions had more morphologically normal sperm per ejaculate (Table 1).

Florida panthers were unique in having more (ANOVA, $F = 34.8$, $d.f. = 76$, $P = 0.0001$) spermatozoa with abnormal acrosomes (>40%) and more (ANOVA, $F = 6.7$, $d.f. = 76$, $P = 0.0001$) with abnormal mi-

TABLE 1.—*Testicular volume and seminal traits* $\bar{X}$ ± SE) *of free-ranging Florida panthers compared to mountain lions from Texas, Colorado, Latin America, and North American zoos (March 1981–May 1993). Probability values show overall differences among populations.*[a]

Testicular and semen characteristics	Population					
	Florida (n = 16)[b]	Texas (n = 9)	Colorado (n = 7)	Latin America (n = 15)	Captive (n = 30)	P
Total testicular volume (cm^3)	9.6 ± 1.2a	17.8 ± 1.7b	21.5 ± 1.4b	23.3 ± 3.4b	19.0 ± 0.8b	0.0001
Ejaculate volume (ml)	0.7 ± 0.1a	2.0 ± 0.3b	3.9 ± 0.5c	2.1 ± 0.3b	2.9 ± 0.3b,c	0.0001
Sperm motility (%)	38.2 ± 6.7a	49.4 ± 6.2a,c	80.0 ± 2.0b	71.1 ± 3.2b	53.0 ± 3.7c	0.0001
Sperm progressive motility[c]	2.3 ± 0.3a	3.2 ± 0.2b	3.4 ± 0.1b	3.1 ± 0.2b	3.2 ± 0.1b	0.0013
Sperm concentration/ml (× 10^6)	4.8 ± 1.4a	15.4 ± 4.4a,b	10.3 ± 2.8a,b	22.5 ± 9.2b	21.5 ± 3.2b	0.0060
Structurally normal spermatozoa (%)	6.5 ± 0.7a	14.0 ± 3.5b	16.3 ± 2.1b	39.4 ± 2.9c	16.5 ± 1.9b	0.0001

[a] Within rows, means with different letters differ (alpha following Bonferroni correction, P = 0.008).
[b] n = number of animals per group.
[c] Sperm progressive motility was based on a scale of 0 to 5; 5 = most rapid forward progression.

tochondrial sheaths (swollen, partial, or complete aplasia; >5%) than the other populations (Figs. 1 and 2). The mountain lions from Latin America had the most (ANOVA, F = 12.9, $d.f.$ = 76, P = 0.0001) structurally normal sperm (Table 1, Fig. 1). The captive population produced more (ANOVA, F = 7.1, $d.f.$ = 76, P = 0.0001) sperm with flagellar defects (Fig. 1). Transmission electron microscopy of spermatozoa from Florida panthers revealed the presence of what appeared to be extraneous acrosomal material located between the acrosome and the sperm plasma membrane and derangements in acrosomal continuity (Fig. 3). Some cells had vesicular remnants of the golgi complex in the area of the sperm head, either between the chromatin and the acrosome (Fig. 3A) or the acrosome and sperm plasma membrane.

There were no differences among any of the populations with respect to mean (±SE) circulating follicle-stimulating hormone (ANOVA, F = 2.1, $d.f.$ = 64, P = 0.12, overall pre-electroejaculation mean = 34.8 ± 2.8 ng/ml), luteinizing hormone (ANOVA, F = 0.2, $d.f.$ = 64, P = 0.96, overall pre-electroejaculation mean = 0.77 ± 0.09 ng/ml), or testosterone (ANOVA, F = 0.9, $d.f.$ = 64, P = 0.49, overall pre-electroejaculation mean = 0.72 ± 0.13 ng/ml) during the ca. 120 min sampling period. Thus, values for hormones were pooled among populations to evaluate the temporal circulating pattern during the anesthesia interval (Fig. 4). The follicle-stimulating hormone and luteinizing hormone concentrations measured pre-electroejaculation did not change over time. Overall concentration of testosterone, however, decreased (ANOVA, F = 3.2, $d.f$ = 76, P = 0.03) from the pre-electroejaculation mean of 0.72 ± 0.13 ng/ml to 0.32 ± 0.09 ng/ml at 90 min post-semen collection.

For all Florida panthers, testicular volume of unilateral cryptorchids was less (t = 12.6; $d.f.$ = 15, P = 0.0001) than that of noncryptorchids. Seminal traits and serum follicle-stimulating hormone (ANOVA, F =

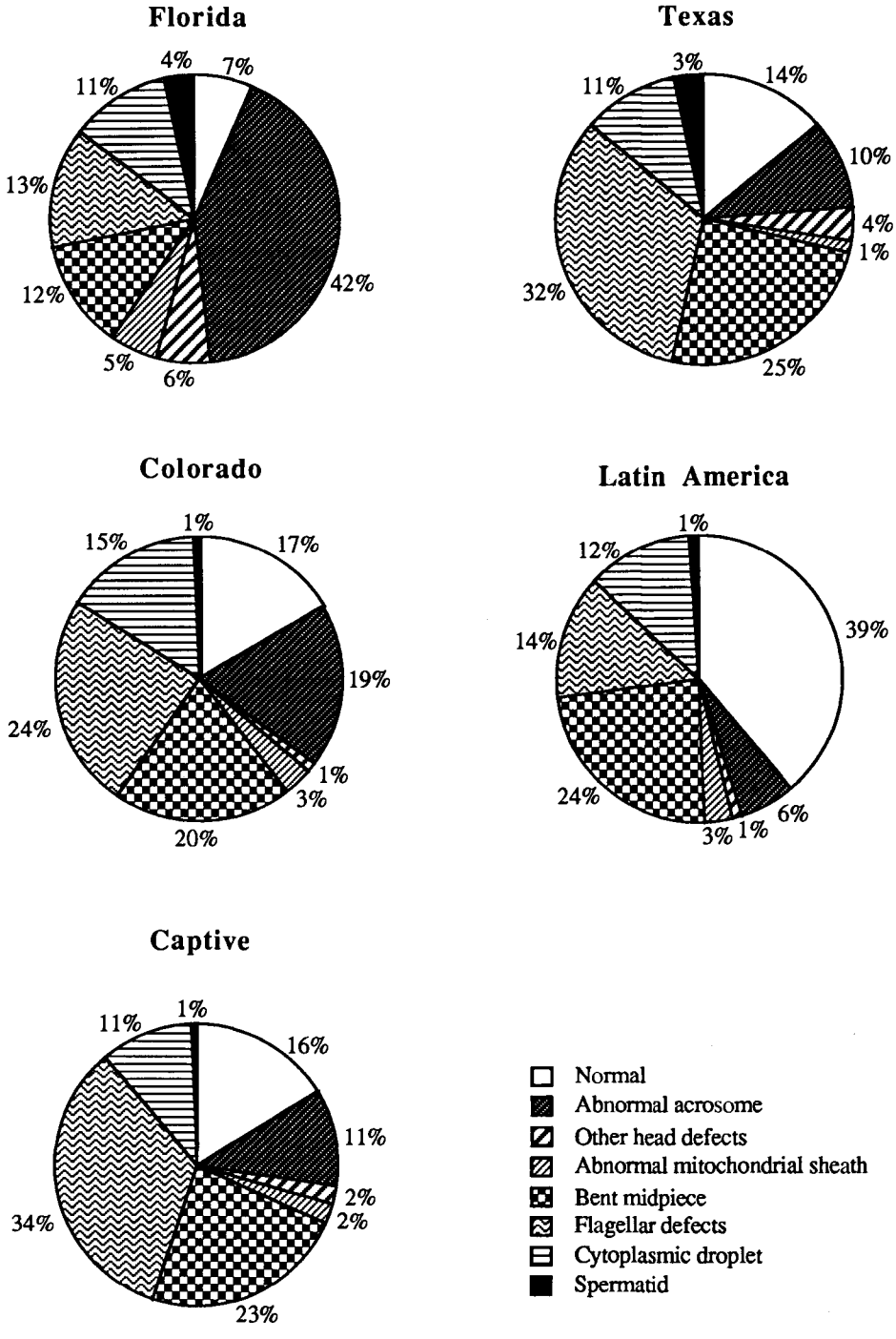
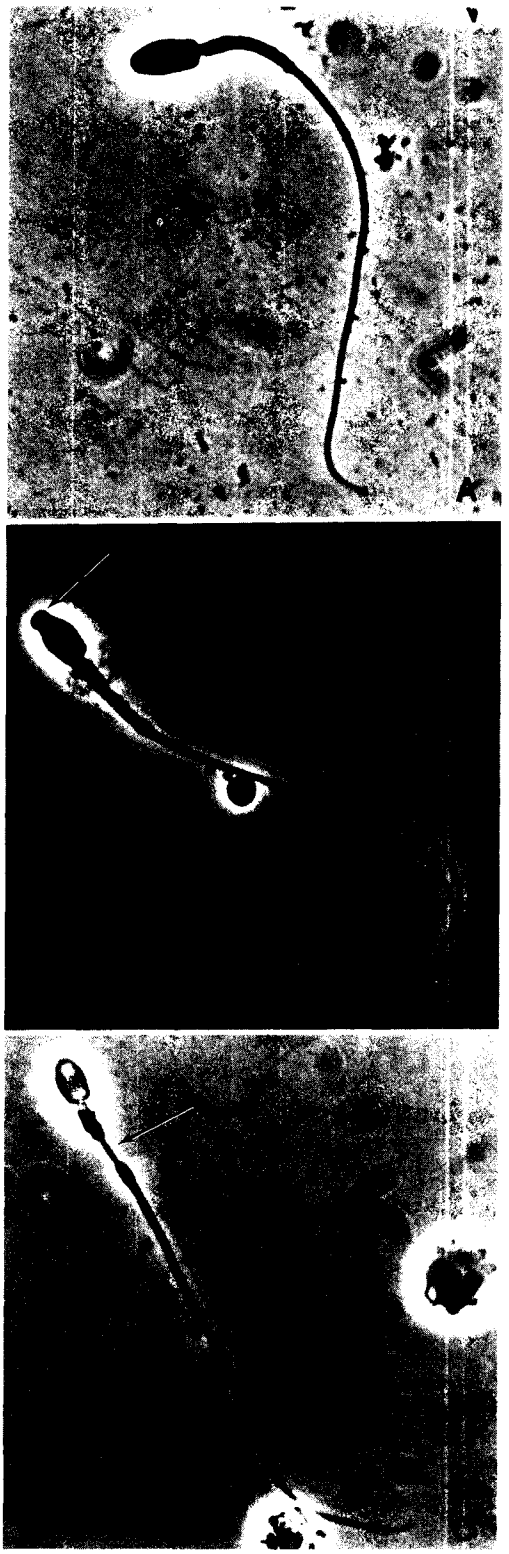


FIG. 1.—Incidence of structural abnormalities in sperm from free-ranging Florida panthers (n = 16) compared to mountain lions from Texas (n = 9), Colorado (n = 7), Latin America (n = 15), and North American zoos (n = 30), March 1981–May 1993.



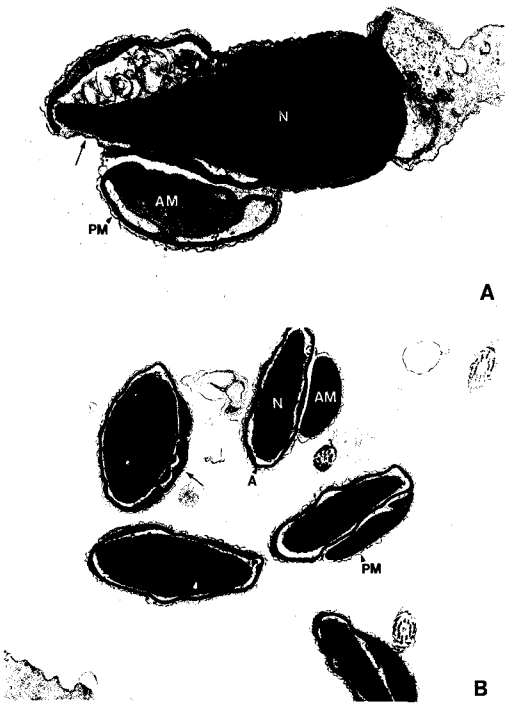


FIG. 3.—Transmission electronmicrographs of spermatozoa from the Florida panther: (A) longitudinal section, 60,000×; and (B) cross section, 36,000×. Discontinuities in the acrosome are evident (arrows). Extraneous acrosomal material (AM) is evident between the sperm plasma membrane (PM) and the acrosome (A). Vesicular remnants of the golgi complex (GC) are located between the acrosome and the nucleus (N).

4.3, *d.f.* = 15, $P = 0.07$, overall pre-electroejaculation mean = 35.5 ± 2.9 ng/ml), luteinizing hormone (ANOVA, $F = 2.5$, *d.f.* = 15, $P = 0.15$, overall pre-electroejaculation mean = 0.7 ± 0.1 ng/ml) and testosterone (ANOVA, $F = 4.6$, *d.f.* = 15, $P = 0.06$, overall pre-electroejaculation mean = 0.5 ± 0.1 ng/ml) concentrations were not different between unilateral cryptorchid and noncryptorchid animals.

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FIG. 2.—Phase-contrast photomicrographs of spermatozoa from the Florida panther: (A) normal; (B) acrosomal defect (arrow); (C) partial midpiece aplasia (arrow).

A comparison of only the animals with *F. c. coryi* ancestry revealed that none of the type B or captive Piper stock were unilateral cryptorchids, whereas 63.6% of 11 of the type A animals exhibited this trait (Table 2). This difference was not statistically significant, perhaps because of small samples and the conservative nature of the Bonferonni procedure. Type A Florida panthers had smaller testicular volume and poorer ratings of sperm motility than the other two groups (Table 2). Compared to the captive Piper stock, type A males also produced a lower concentration of sperm and fewer structurally normal sperm and sperm with structurally normal acrosomes. No differences were noted among groups in levels of serum follicle-stimulating hormone (ANOVA, $F = 1.6$, $d.f. = 18$, $P = 0.24$, overall pre-electroejaculation mean = 34.3 ± 2.7 ng/ml), luteinizing hormone (ANOVA, $F = 1.1$, $d.f. = 18$, $P = 0.38$, overall pre-electroejaculation mean = 0.69 ± 0.07 ng/ml), or testosterone (ANOVA, $F = 2.1$, $d.f. = 18$, $P = 0.17$, overall pre-electroejaculation mean = 0.5 ± 0.09 ng/ml).

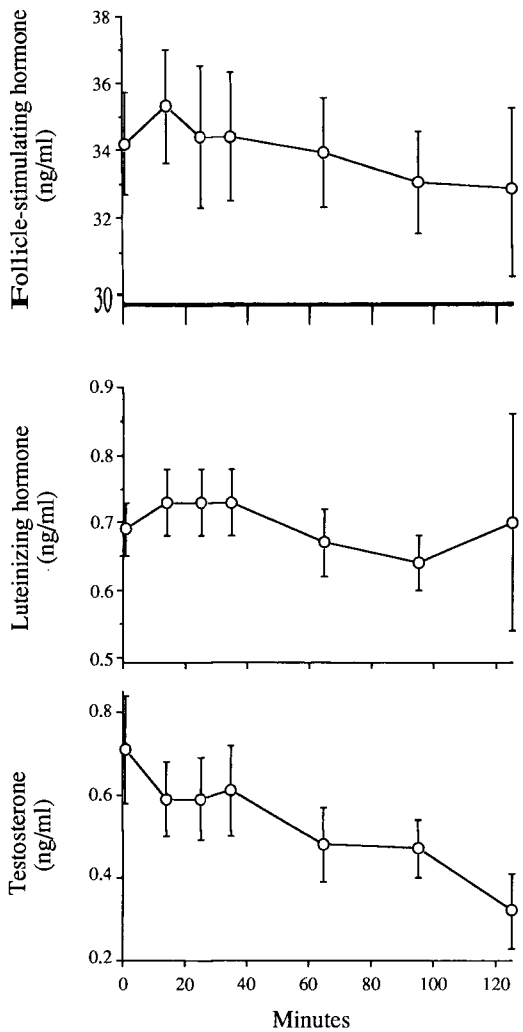

FIG. 4.—Mean (±*SE*) concentrations of circulating follicle-stimulating hormone, luteinizing hormone and testosterone for all populations of mountain lions combined ($n = 65$), during and for 90 min after electroejaculation. Each open circle indicates one sampling time point. Blood samples were collected from the cephalic vein preceding onset of electroejaculation (sample one), following each electroejaculation series (samples two to four), and at 30 (sample five), 60 (sample six), and 90 (sample seven) min after the last series.

DISCUSSION

There are major physiological and morphological differences among populations of *Felis concolor*, especially in the incidence of unilateral cryptorchidism and seminal traits. This supports previous assertions that wildlife populations have unique physiological mechanisms that must be considered when conducting basic comparative studies or examining reproductive characteristics (Wildt et al., 1992*a*). We believe these differences are related, in part, to genetic background. The Florida panther, which exhibited the poorest overall semen quality, coincidentally is facing the severest loss of habitat and inbreeding pressures. A recent survey of molecular genetics (Roelke et al., 1993) revealed that 7.5% of the 41 loci examined in the Florida panther were polymorphic compared to 10% in Texas mountain lions, 17% in Arizona mountain lions and >26% in mountain lions maintained in zoos. The average heterozygosity for the Florida panther was 0.028 compared to 0.042, 0.069 and 0.066 for Texas, Arizona, and captive mountain lions, respectively (Roelke et al., 1993). If the Florida panther is experiencing

TABLE 2.—*Reproductive characteristics* ($\bar{X} \pm SE$) *of animals with* Felis concolor coryi *ancestry (March 1981–May 1993). Probability values show overall differences among groups.*[a]

Testicular and semen characteristics	Type A ($n = 11$)[b]	Type B ($n = 5$)	Captive Piper Stock ($n = 3$)	P
Cryptorchid (%)	63.6	0.0	0.0	0.0280
Testicular volume (cm^3)	8.1 ± 1.2a	13.8 ± 1.9b	21.6 ± 0.5c	0.0001
Ejaculate volume (ml)	0.8 ± 0.2a	0.6 ± 0.2a	1.9 ± 0.4a	0.0001
Sperm motility (%)	25.6 ± 7.1a	61.0 ± 5.6b	80.0 ± 0.0c	0.0004
Sperm progressive motility[c]	1.7 ± 0.3a	3.3 ± 0.1b	3.7 ± 0.2b	0.0001
Sperm concentration/ml ($\times 10^6$)	4.1 ± 1.5a	6.1 ± 2.9a	32.5 ± 7.1b	0.0050
Structurally-normal spermatozoa (%)	5.7 ± 0.8a	7.8 ± 1.2a,b	22.8 ± 15.3b	0.0025
Abnormal acrosomes (%)	40.8 ± 2.7a	43.4 ± 3.1a	13.8 ± 2.8b	0.0001

[a] Within rows, means with different letters differ (alpha following Bonferroni, $P = 0.006$).
[b] n = number of animals per group.
[c] Sperm progressive motility was based on a scale of 0 to 5, 5 = most rapid forward progression.

inbreeding depression (as data on polymorphism and heterozygosity suggest), then it is not surprising that the subspecies expresses poor-quality semen. Inbreeding depression has been related to decreased semen quality and lowered fertility and neonatal survival in a variety of domesticated and wild species (Lasley, 1978; O'Brien et al., 1985; Ralls and Ballou, 1982; Wildt et al., 1982). The hypothesis of lost genetic variability in the population of Florida panthers also appears supported by an extraordinarily high incidence of cryptorchidism, a trait that is highly heritable in other species (Rothschild et al., 1988; Thomas and Howard, 1975). Unilateral cryptorchidism also has been reported in captive-bred maned wolves (*Chrysocyon brachyurus*—Burton and Ramsay, 1986), a population originating from four founders (Rodden, 1985).

Additional evidence for genetics regulating male reproductive characteristics was derived from findings indicating physiological and anatomical differences within animals of *F. c. coryi* ancestry. Studies of molecular genetics have revealed polymorphism and heterozygosity values to be 5.0% and 0.018, respectively, in type A Florida panthers compared to 7.5% and 0.020 for type B, and 12.5% and 0.045 for the captive Piper stock (Roelke et al., 1993). Type A animals, which expressed less than one-half the genetic variability of the captive Piper stock, exhibited lower testicular volume and poorer semen quality and were the only group demonstrating the cryptorchid condition.

Almost a decade ago, we reported an extreme incidence of structurally abnormal sperm in the African cheetah (Wildt et al., 1983), a species later reported to be genetically monomorphic (O'Brien et al., 1985). We also have detected fewer polymorphic loci, lower rates of heterozygosity, and relatively high numbers of pleiomorphic sperm in lions (*Panthera leo*) descended from isolated populations in the Serengeti ecosystem or in a geographic range (India) no longer supportive of large-sized predators (Wildt et al., 1987). The Florida panther also produces many pleiomorphic sperm that are unique in the proportion (>40%) that are afflicted with an acrosomal defect. In an earlier study in which we assessed sperm structure in 28 species of felids, we never observed >5% of the sperm with deformities in this structural region (Howard et al., 1984). Electron microscopy revealed that the acrosome, which plays a key role in fertilization (Yanagimachi, 1981), is severely deranged. Observations of vesicular remnants of the golgi complex and extraneous acrosomal material between the acrosome and sperm plasma membrane were similar to the "miniacrosome defect" condition described in humans (Baccetti et al.,

1991). The acrosome, which is formed following the second meiotic division, is derived from the golgi apparatus (Kaye, 1984). In humans, acrosomal defects have been attributed to degeneration during spermiogenesis or absorption of the acrosome by the supporting Sertoli cells (Baccetti et al., 1991).

Increased numbers of pleiomorphic sperm impair fertility in a variety of nonfelid species (Singleton and Shelby, 1972; Soderberg, 1986). The success of artificial insemination (Marshburn et al., 1991) and in vitro fertilization in humans (Jeulin et al., 1986) is inversely correlated with the percentage of sperm pleiomorphisms in the inseminant. The influence of teratospermia (structurally abnormal sperm) on the fertilization process is becoming more clear. Structurally abnormal human sperm are less likely to undergo the acrosome reaction either spontaneously or following treatment with human follicular fluid (Fukuda et al., 1989). Felid sperm with structural defects are unlikely to participate in fertilization (Howard et al., 1991). Although structurally abnormal cells occasionally bind to the oocyte or perhaps even penetrate into the outer layer of the zona pellucida, they do not enter the inner layer of the zona or the perivitelline space (Howard et al., 1991). Even morphologically normal sperm from teratospermic (<40% normal sperm) domestic cats are less able to bind and penetrate conspecific oocytes of cats in vitro compared to sperm from normospermic (>60% normal sperm) males (Howard et al., 1991). From preliminary in vitro fertilization studies using sperm from Florida panthers, we have determined that ca. 30% of oocytes of mountain lions fertilize in vitro, but all fail to form cleaved embryos (Miller et al., 1990). In contrast, rates of in vitro fertilization cleavage in the tiger (*Panthera tigris*), a species routinely producing ca. 80% structurally normal sperm, usually approach 60% (Donoghue et al., 1990). Despite these observations, we still lack definitive data on the effect of teratospermia on fertility of the Florida panther. Certainly, at least some of the free-ranging males sire young. The one adult male in captivity, however, is infertile on the basis of numerous copulations with western mountain lions in estrus (Miller et al., 1990).

If the Florida panther is indeed reproductively compromised, then a plausible cause is the sperm acrosomal anomaly. Acrosomal defects in sperm adversely affect fertility of humans (Jeulin et al., 1986), cattle (Andersson et al., 1990), and pigs (Bane and Nicander, 1966). The round-headed sperm defect of humans includes the absence of an acrosome and an inability of the cell to bind or fuse to an oocyte (von Bernhardi et al., 1990). These sperm are capable of undergoing decondensation and pronuclear formation following microsurgical injection into the oocyte (Lanzendorf et al., 1988), indicating that they are biologically functional if the outer oocyte barrier (zona pellucida) can be bypassed.

Testosterone is important for normal spermatogenesis and epididymal sperm maturation (Mann and Lutwak-Mann, 1981), and a high incidence of structurally abnormal sperm appears related, in part, to low concentrations of circulating testosterone. Male domestic cats consistently producing ca. 30% normal spermatozoa have lower testosterone (ca. 0.4 ng/ml) than normospermic (ca. 1.2 ng/ml) males (ca. 70% structurally normal sperm—Howard et al., 1990). The adult tiger, considered a normospermic species (ca. 80% normal sperm forms), produces 0.88–6.75 ng/ml testosterone compared to ca. 0.47–1.17 ng/ml for the teratospermic cheetah (ca. 30% normal sperm forms—Wildt et al., 1988). The Asiatic lion produces fewer normal sperm (ca. 50%) and lower circulating testosterone (ca. 0.5 ng/ml) than the more genetically diverse lions of the Serengeti National Park (ca. 75%, ca. 1.3 ng/ml, respectively—Wildt et al., 1987). Populations of mountain lions examined in the present study all had low circulating testosterone (0.72 ng/ml) and also high numbers of structurally abnormal

sperm (>60%). Because all circulating hormones (including gonadotropins) were similar among the five populations of mountain lions, it appears that differences in semen quality observed were not being mediated via endocrine mechanisms, at least based upon the monitoring strategy used (window blood sampling). If the more uniform genotype of the Florida panther is contributing to poor seminal characteristics, the effect likely is mediated at the level of the testis.

Unilateral cryptorchidism reduces semen quality in dogs (Kawakami et al., 1984) and humans (Scott, 1961). This was not the case in the Florida panther, perhaps because semen quality already was poor. Three of the six Florida panthers known to have sired young in the wild were unilateral cryptorchids, making it unclear what effect cryptorchidism has on overall fertility of the population. Cryptorchidism does, however, appear to serve as a marker of genetic relatedness within the population. Currently, 70% of free-ranging adult males are cryptorchid, compared to an incidence of ca. 20% 9 years ago (Roelke et al., 1993).

The Florida panther exhibits poorer male reproductive characteristics when compared to other populations of mountain lions in North or Latin America. Although the etiology of cryptorchidism and teratospermia appears to be genetic in origin, the direct effect of these traits upon reproductive efficiency and the long-term survival of the Florida panther remains to be determined. We are not prepared to predict unequivocally the demise of this subspecies on the basis of data presented in the present study. Because of the importance of reproductive health in sustaining robust population viability, however, it is difficult to believe that this subspecies can avoid extirpation without more vigorous intervention. The most logical response would be to begin selecting against some of the physiological traits most counterproductive to reproductive efficiency, especially cryptorchidism and the acrosomal defect, in both captive and free-ranging populations. The introduction of genetic material from other more vigorous populations of mountain lions may be one solution (O'Brien and Mayr, 1991). Regardless of the outcome for this endangered subspecies, studies to assess the effect of lost genetic diversity on reproductive characteristics will provide valuable lessons for continuing to address the consequences of rapidly declining populations.

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