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Age-Dependent Changes in Sperm Production, Semen Quality, and Testicular Volume in the Black-Footed Ferret (*Mustela nigripes*)[1]

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ABSTRACT

The black-footed ferret (*Mustela nigripes*), which was extirpated from its native North American prairie habitat during the 1980s, is being reintroduced to the wild because of a successful captive-breeding program. To enhance propagation, the reproductive biology of this endangered species is being studied intensively. The typical life span of the black-footed ferret is approximately 7 yr. Female fecundity declines after 3 yr of age, but the influence of age on male reproduction is unknown. In this study, testis volume, seminal traits, sperm morphology, and serum testosterone were compared in 116 males from 1 to 7 yr of age living in captivity. Results demonstrated that testes volume during the peak breeding season was similar ($P > 0.05$) among males 1 to 5 yr of age, reduced ($P < 0.05$) among males 6 yr of age, and further reduced ($P < 0.05$) among males 7 yr of age. Motile sperm/ejaculate was similar in males 1 to 6 yr of age but diminished ($P < 0.05$) in those 7 yr of age. Males at 6 and 7 yr of age produced fewer ($P < 0.05$) structurally normal sperm than younger counterparts; however, serum testosterone concentrations were not reduced ($P > 0.05$) in older males. Histological comparison of testicular/epididymal tissue from 5- and 7-yr-old black-footed ferrets confirmed that the interval between these two ages may represent a transitional period to reproductive senescence. In summary, functional reproductive capacity of male black-footed ferrets exceeds that of females by at least 2 yr. Testes and seminal quality are indistinguishable among males 1 to 5 yr of age, with progressive reproductive aging occurring thereafter.

aging, Leydig cells, male sexual function, sperm, testosterone

INTRODUCTION

The black-footed ferret (*Mustela nigripes*) is one of 65 members in the family Mustelidae, and it is the only ferret native to North America [1, 2]. This endangered species is classified in the subgenus *Putorius*, with the Siberian (steppe) polecat (*M. eversmanni*), European polecat (*M. putorius*), and domestic ferret (*M. putorius furo)* [1–3]. Once widely distributed across the western North American prairies, the black-footed ferret was considered to be extinct during the late 1970s because of habitat loss and the large-scale extermination of the prairie dog (*Cynomys* sp.), upon which the ferret depends for food and shelter. Discovery of a small black-footed ferret population in Wyoming during 1981, however, eventually allowed establishment of a captive-breeding program. Since 1985, the population increased from the last remaining 18 individuals to, at present, approximately 300 black-footed ferrets in captivity. Additionally, a reintroduction program was initiated in Wyoming, Montana, South Dakota, Arizona, and Utah, with an overall goal of establishing 1500 breeding adults in 10 or more widely distributed populations by the year 2010 [4]. Approximately 200 black-footed ferrets currently survive in the wild.

Despite this captive-breeding success, the goal of the recovery and reintroduction program will not be achieved at the current rate of propagation [5]. Therefore, the physiology of the black-footed ferret is being studied to determine causes of reproductive inefficiency and to develop strategies for improving reproductive success. Factors limiting reproduction include a high incidence (~30%) of pseudopregnancy [6], a decline in female productivity after 3 yr of age [6, 7], and a high proportion (>50%) of adult males considered to be of prime-breeding age (1, 2, or 3 yr) that fail to reproduce. Although the typical life span of the black-footed ferret is approximately 7 yr, peak reproductive performance in females occurs at 1, 2, and 3 yr of age (whelping rates, >60%) [6]. Older females (age, 4–7 yr) have reproduced, but at markedly lower whelping rates (<30%). To our knowledge, a systematic investigation of the reproductive life span of the male black-footed ferret has not been conducted. In most species studied, male fertility gradually declines with age beyond sexual maturity [8–11] because of altered endocrine and testicular function [12, 13].

Domestic ferrets, Siberian polecats, and black-footed ferrets are seasonal breeders, with reproductive activity being triggered by a long-day photoperiod [6, 7, 14]. Testes size in black-footed ferrets exposed to natural light increases beginning in December, is maximal from March through May, and then decreases thereafter [6, 7]. Increased testes size correlates with elevated circulating testosterone and spermatogenesis in the domestic [15, 16] and the black-footed [17] ferret. Plasma testosterone in the domestic ferret begins to increase during December, is maximal from April through June, and then returns to baseline during July [15]. Similarly, fecal androgen metabolite excretion in the black-footed ferret is lowest during the summer and highest during the winter and the spring [17].

Although seasonal changes in testicular volume and testosterone concentrations are well established in the black-footed ferret, age-related changes in the reproductive traits have not been evaluated. The objectives of this study were to 1) determine the reproductive life span of the male black-footed ferret; 2) assess the effects of aging on testicular volume, seminal traits, and circulating testosterone; and 3)

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evaluate the histological changes in the testis and epididymis during the transition to reproductive senescence. Because of the endangered status of this species and the geographical disparity among captive-breeding facilities, it was impractical to characterize reproductive traits in individual males over a 7-yr period. Rather, we surveyed a large proportion of the extant population at a single time, ensuring that we evaluated significant numbers of males in all age categories.

MATERIALS AND METHODS

Animals

A total of 116 adult male black-footed ferrets (0.7–1.2 kg body weight) was categorized into seven age groups: 1 yr (n = 25), 2 yr (n = 21), 3 yr (n = 22), 4 yr (n = 11), 5 yr (n = 16), 6 yr (n = 11), and 7 yr (n = 10). All males were healthy at evaluation and were housed individually (under quarantine conditions) at the U.S. Fish & Wildlife Service's National Black-Footed Ferret Conservation Center (Laramie, WY), the National Zoological Park's Conservation & Research Center (Front Royal, VA), or the Cheyenne Mountain Zoological Park (Colorado Springs, CO). Most animals were housed indoors under artificial illumination, which was regulated by automatic timers set to turn on light 15 min before sunrise and turn off light 15 min after sunset. The National Zoological Park's Conservation & Research Center was the only facility in which ferrets (n = 11) were maintained in outdoor enclosures and exposed to a natural photoperiod [7]. Ferrets were fed a commercial mink diet (60%) supplemented with ground rabbit or prairie dog (40%), bioliver, bloodmeal, and occasionally, mouse or hamster carcasses. Fresh water was available ad libitum.

Testes Volume Measurement, Semen Collection, and Evaluation

Each male ferret was examined on a single occasion during the peak breeding season (March through May) [6]. To ensure the breeding season itself did not confound observations, equal proportions of males in each age class were evaluated in each of the months of March, April, and May. Each animal was anesthetized with an intramuscular injection of 38.8 mg/kg ketamine hydrochloride (Ketaset; Bristol Laboratories, Syracuse, NY) combined with 0.06 mg/kg diazepam (Steris Laboratories, Inc., Phoenix, AZ). A surgical plane of anesthesia was reached within 3 min of anesthetic injection and was maintained for approximately 30 min. Length and width of the right and left testis were measured using laboratory calipers, and the volume of each testis was quantified by multiplying the length (cm) by width (cm^2) by 0.524 [16]. Total testicular volume (cm^3) was calculated by adding together the volume of the right and left testis.

Semen was collected by a rectal-probe electroejaculation procedure developed for the domestic ferret [16, 18]. A lubricated rectal probe (diameter, 6 mm) with three longitudinal electrodes (P.T. Electronics, Boring, OR) was placed in the rectum, and the penis was everted manually. Each male was subjected to approximately 30 min of electrical stimulation using an AC 60-Hz sine-wave electroejaculator (P.T. Electronics). Electrical stimuli were delivered in a 3-sec-on, 3-sec-off pattern with a continuous rise in voltage from 0 to the desired peak and then returned to 0. Five series of 30 stimuli (series 1: 2, 3, and 4 V applied 10 times each), 30 stimuli (series 2: 3, 4, and 5 V applied 10 times each), 20 stimuli (series 3: 4 and 5 V applied 10 times each), 20 stimuli (series 4: 4 and 5 V applied 10 times each), and 20 stimuli (series 5: 5 and 6 V applied 10 times each) were given, with a 3- to 4-min rest period between series. The probe was gently manipulated within the rectum during the rest period. Seminal droplets from each series were collected from the glans penis into a warmed (37°C) plastic micropipette.

Seminal volume was recorded, and the sample was placed into a warmed (37°C) microcentrifuge tube containing 100 μl of TEST Egg Yolk Buffer (Irvine Scientific, Santa Ana, CA) modified to contain 4% glycerol. The modified cryodiluent was prepared by combining TEST Yolk Buffer-Freezing Medium containing 12% glycerol (1 part) and TEST Yolk Buffer-Refrigeration Medium containing no glycerol (2 parts) to yield a final, 4% glycerol concentration. After mixing the sperm suspension, a 3-μl sample was placed on a warm slide (37°C) and subjectively evaluated for sperm percentage motility and forward progression (scale of 0–5; best = 5) using methods described in detail elsewhere [16, 19]. Two microliters of raw ejaculate were fixed in 0.3% glutaraldehyde (diluted in PBS) for subsequent evaluation of sperm morphology and acrosomal integrity by phase-contrast microscopy (×1000). Sperm concentration of the suspension was measured using a standard hemocytometer, and actual sperm concentration of the ejaculate was calculated using the known volume of both the cryodiluent and the raw semen in the suspension. The total number of motile sperm/ejaculate was calculated by multiplying the sperm concentration/ejaculate by the sperm percentage motility. Sperm cells were classified as either normal or having one of the following defects: bent midpiece with cytoplasmic droplet, bent midpiece without droplet, coiled flagellum, bent flagellum with droplet, bent flagellum without droplet, residual proximal or distal droplet, or abnormal acrosome [18, 19].

Radioimmunoassay for Testosterone Concentration

A blood sample (2 ml) was collected by jugular venipuncture from each male after the animal had reached a surgical plane of anesthesia and before electroejaculation. The sample was centrifuged for 20 min, and the serum was then collected, stored at −20°C, and later analyzed for testosterone. A double-antibody RIA ^{125}I kit (ICN Biomedicals, Inc., Costa Mesa, CA) was used that relied on a rabbit testosterone-19-carboxymethylether-BSA antibody with the following cross-reactivities: 100% with testosterone, 3.4% with 5α-dihydrotestosterone, 2.2% with 5α-androstane-3β,17β-diol, 2% with 11-oxotestosterone, and less than 1% with 5β-dihydro-testosterone, 5β-androstane-3β,17β-diol, 6β-hydroxytestosterone, androstenedione, androsterone, epiandrosterone, estrone, estradiol-17β, estriol, progesterone, and corticosterone. This assay has been used with other carnivore species [16, 20–22] and was validated for black-footed ferret serum by demonstrating parallelism between binding inhibition curves of serum dilutions and the appropriate standards as well as significant recovery of exogenous testosterone added to black-footed ferret serum. Inhibition curves for serum pools and testosterone standards were parallel. After adding 0.025, 0.050, 0.125, 0.25, 0.50, 1.25, 2.5, and 5.0 ng of testosterone to 25 μl of black-footed ferret serum, 0.001, 0.012, 0.080, 0.124, 0.322, 0.964, 2.014, and 6.254 ng were recovered, respectively, after subtracting endogenous serum testosterone ($y = 0.809\times + 0.122$, $r = 0.97$). Assay sensitivity was 0.01 ng/

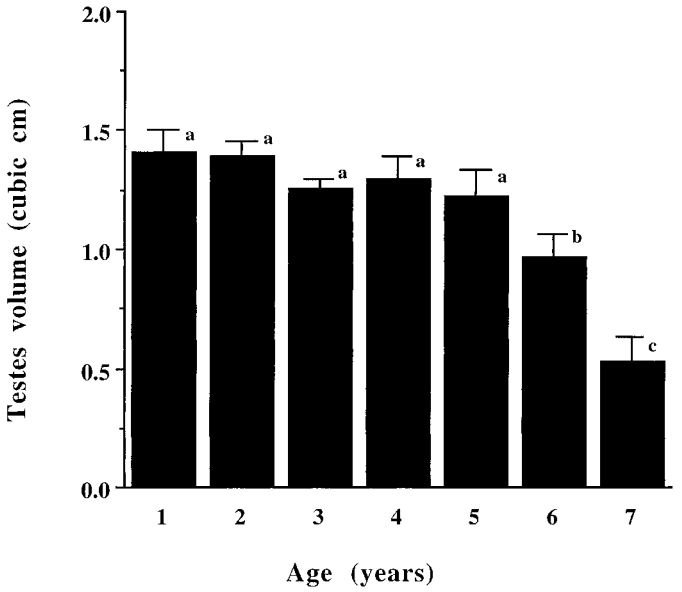

FIG. 1. Age-dependent changes in testicular volume in black-footed ferrets (n = 116) 1–7 yr of age during peak breeding season (March through May). Means (± SEM) with different superscripts differ significantly ($P < 0.05$).

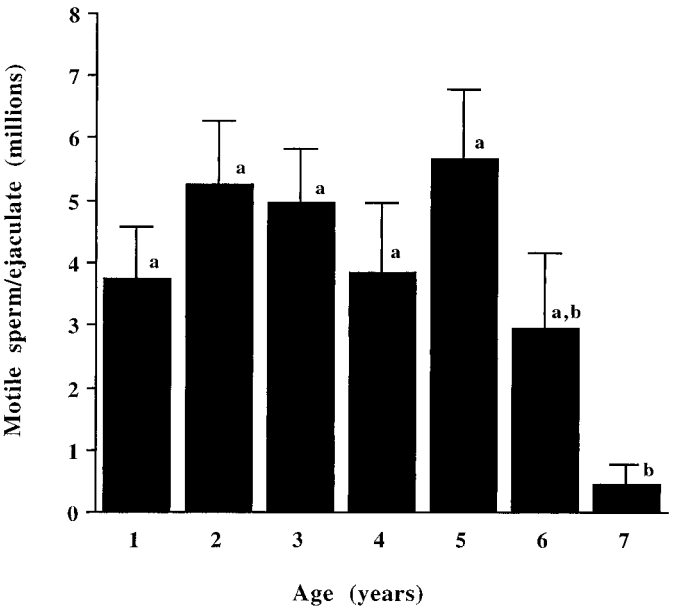

FIG. 2. Age-dependent changes in total number of motile sperm in the ejaculate of black-footed ferrets (n = 116) 1–7 yr of age during peak breeding season (March through May). Means (± SEM) with different superscripts differ significantly ($P < 0.05$).

tube, and the inter-and intraassay coefficients of variation were less than 10%.

Testicular and Epididymal Histology

Testes were excised from four 5-yr-old males and two 7-yr-old males in May during the peak breeding season and processed for histological evaluation. These individuals had completed their breeding assignments (according to genetic protocols) and were being shipped to various zoological institutions for display and education programs. The U.S. Fish & Wildlife Service requires that such exhibit males be castrated. The testis and epididymis from each male were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at a thickness of 5 μm, and stained with hematoxylin-and-eosin. The tissue section of each testis was evaluated for spermatogenesis and spermiogenesis. To compare the size of seminiferous tubules among males, the diameter of 120 tubules (20 per male) were measured (×400) using a stage micrometer. The diameter measurements were taken from the outer basement membrane of the seminiferous tubules. Each epididymal section was examined for stored spermatozoa, and the diameter of 120 epididymal tubules (20 per male) were measured (×400).

Statistical Analysis

Data were analyzed using a general linear models program (SAS Institutes, Cary, NC). Influence of age on reproductive traits (testicular volume, serum testosterone, and semen traits, including sperm morphology) was assessed by ANOVA. Within each reproductive trait and among age groups, differences of the means for selected pairwise comparisons were determined by a Duncan multiple range test. Values are reported as mean ± SEM and considered to be significant at $P < 0.05$.

RESULTS

Testes Volume

Mean testes volume was similar ($P > 0.05$) in 1- to 5-yr-old males, with volume progressively declining ($P < 0.05$) in 6- and 7-yr-old black-footed ferrets (Fig. 1). There also was a broader range in testes volume among males within the 1- to 5-yr age group (0.72–2.65 cm^3, $P < 0.05$) compared with 6-yr-old (0.61–1.52 cm^3) and 7-yr-old (0.12–1.12 cm^3) counterparts. Although 6-yr-old males had smaller testes than younger males, seasonal testicular recrudescence was observed in all individuals among this 6-yr age group. In contrast, four of 10 (40%) 7-yr-old males had no testicular development during the peak breeding season, with testes size being comparable to that during the nonbreeding season.

Ejaculate Traits

Four of 10 (40%) 7-yr-old males failed to produce any seminal fluid during electroejaculation (the same males that experienced no testicular development). In contrast, only two of the 106 (1.9%) 1- to 6-yr-old males failed to produce semen (one 2-yr-old and one 6-yr-old male). Despite this, ejaculate volume was similar ($P > 0.05$) among age groups (data not shown).

Sperm production was influenced by age ($P < 0.05$). Ejaculate quality was consistently highest and comparable among 1- to 5-yr-old males and was reduced in 6- and 7-yr-old males (Figs. 2 and 3). Spermatozoa were not obtained in seven of 10 (70%) 7-yr-old ferrets, compared with only four of 106 (3.8%) 1- to 6-yr-old males (one 2-yr-old, one 3-yr-old, and two 6-yr-old males). Mean total sperm per ejaculate was similar ($P > 0.05$) in 1- to 4-yr-old males (mean range, $5.4 \pm 1.0 \times 10^6$ to $7.4 \pm 1.2 \times 10^6$). Ejaculates from 5-yr-old males contained more ($P < 0.05$) sperm ($11.7 \pm 2.1 \times 10^6$) than those from 1-yr-old ($5.4 \pm 1.0 \times 10^6$) and 6-yr-old ($5.2 \pm 1.9 \times 10^6$) ferrets. Ejaculates from 7-yr-old males produced the fewest sperm ($1.7 \pm 1.6 \times 10^6$) compared with all other age groups ($P < 0.05$). Three of the 7-yr-old males producing aspermic ejaculates had normal testicular development.

The highest mean percentage sperm motility scores were observed in 1- to 5-yr-old males (mean range, 49.7% ±

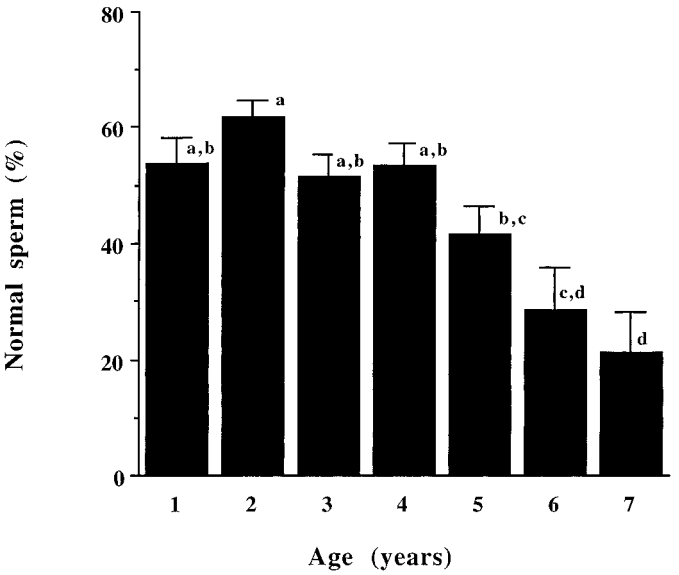

FIG. 3. Age-dependent changes in the incidence of structurally normal sperm in the ejaculate of black-footed ferrets (n = 116 males) 1–7 yr of age during peak breeding season (March through May). Means (± SEM) with different superscripts differ significantly ($P < 0.05$).

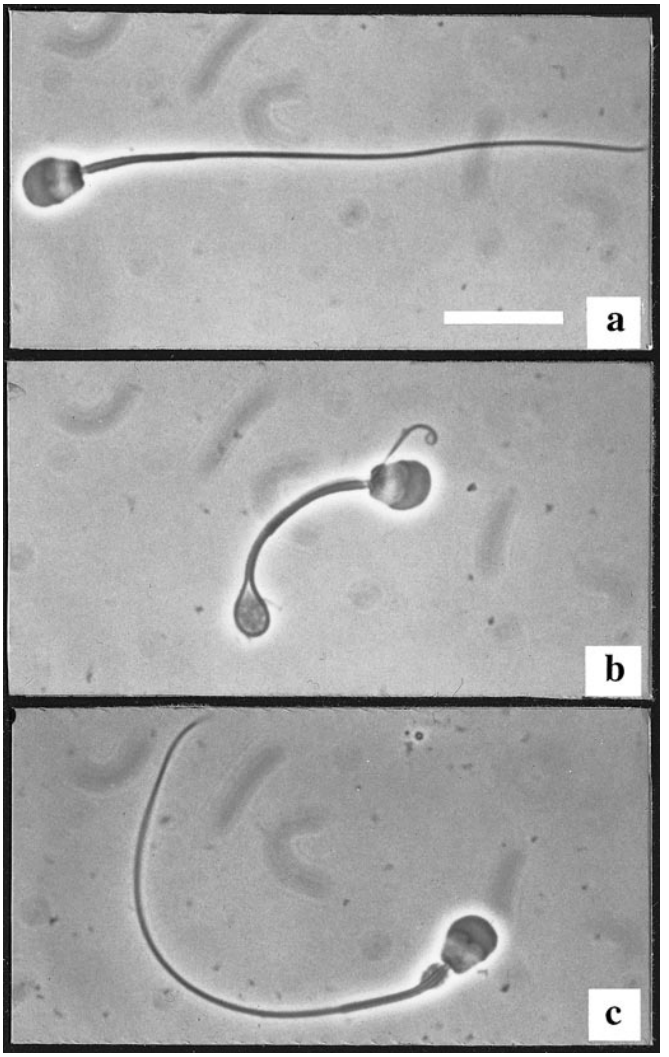

FIG. 4. Age-related changes in sperm morphology in black-footed ferrets categorized as a normal spermatozoon (**a**) or abnormal cell with a bent flagellum with cytoplasmic droplet (**b**) or proximal cytoplasmic droplet (**c**). Bar = 20 μm.

3.7% to 67.0% ± 2.3%; $P > 0.05$). The motility score for ejaculates from 6-yr-old males (40.5% ± 6.8%) differed ($P < 0.05$) only from the 2-yr age group (67.0% ± 2.3%). Mean percentage sperm motility for the 7-yr age group (20.0% ± 2.0%) was less ($P < 0.05$) than that for all other ages. In contrast to the proportion of sperm showing some motion (i.e., percentage motility), sperm forward progression (i.e., speed and type of forward movement) did not differ ($P > 0.05$) among all age groups (mean range, 2.3 ± 0.3 to 3.3 ± 0.1).

The total number of motile sperm/ejaculate also was influenced ($P < 0.05$) by age (Fig. 2). Ferrets 1 to 6 yr of age produced ejaculates containing comparable ($P > 0.05$) numbers of motile sperm/ejaculate (mean range, 3.0 to 5.7 × 10^6). In contrast, 7-yr-old males produced the fewest motile sperm/ejaculate (mean, 0.5 × 10^6), a value that differed ($P < 0.05$) from those of their 1- to 5-yr-old counterparts.

The incidence of structurally normal sperm also declined ($P < 0.05$) with increasing age (Fig. 3), primarily because of an increased incidence of flagellar defects and residual cytoplasmic droplets (Fig. 4). Males 1–4 yr of age produced ejaculates containing more than 50% normal sperm (mean range, 51.4% ± 3.9% to 61.7% ± 2.8%; $P > 0.05$). More ($P < 0.05$) malformed sperm were measured in 5-yr-old males (mean, 58.5% ± 4.8%), with decreasing proportions of structurally normal cells continuing through 7 yr of age (mean, 21.0% ± 7.0%; Fig. 3). The highest percentage of abnormal acrosomes was measured in 6-yr-old males (Table 1), but this value differed ($P < 0.05$) only from that of the 2-yr age group. No differences were observed among all age groups in sperm with a midpiece defect, coiled flagellum, bent flagellum without droplet, or distal cytoplasmic droplet ($P > 0.05$). Seven-yr-old males produced more ($P < 0.05$) sperm with a bent flagellum with droplet than those

TABLE 1. Influence of age on proportions of morphologically abnormal sperm in black-footed ferrets.[a]

	Age (yr)						
Sperm defect	1	2	3	4	5	6	7
Abnormal acrosome (%)	8.9 ± 1.0^{b,c}	6.8 ± 1.0^{c}	10.7 ± 1.5^{b,c}	9.6 ± 1.7^{b,c}	12.2 ± 2.7^{b,c}	17.3 ± 6.7^{b}	10.5 ± 3.5^{b,c}
Coiled flagellum (%)	4.2 ± 1.9	2.0 ± 1.3	1.9 ± 0.5	3.3 ± 1.6	4.0 ± 1.8	8.0 ± 3.5	1.0 ± 1.0
Bent midpiece with droplet (%)	11.9 ± 2.9	12.6 ± 2.7	14.4 ± 2.2	13.5 ± 3.0	15.8 ± 2.5	15.9 ± 4.1	12.5 ± 6.5
Bent midpiece without droplet (%)	1.9 ± 0.3	2.9 ± 1.0	2.8 ± 1.0	2.3 ± 0.8	4.7 ± 1.2	1.8 ± 0.8	2.0 ± 2.0
Bent flagellum with droplet (%)	1.0 ± 0.4^{b}	0.7 ± 0.4^{b}	1.4 ± 0.5^{b}	1.3 ± 1.0^{b}	1.1 ± 0.6^{b}	0.9 ± 0.4^{b}	4.0 ± 2.0^{c}
Bent flagellum without droplet (%)	4.7 ± 1.3	4.8 ± 1.6	3.1 ± 1.0	5.0 ± 2.7	4.9 ± 2.0	3.9 ± 2.4	1.0 ± 1.0
Proximal droplet (%)	7.1 ± 2.8^{b}	2.0 ± 0.3^{b}	4.4 ± 1.0^{b}	6.0 ± 2.4^{b}	7.2 ± 2.0^{b}	19.1 ± 6.1^{c}	45.5 ± 9.5^{d}
Distal droplet (%)	6.6 ± 1.3	6.5 ± 1.3	9.9 ± 2.1	5.6 ± 2.1	8.6 ± 3.8	4.7 ± 1.7	2.5 ± 0.5

[a]Values are means ± SEM, n = 116 males. Within a row, means with different superscript letters differ significantly ($P < 0.05$).

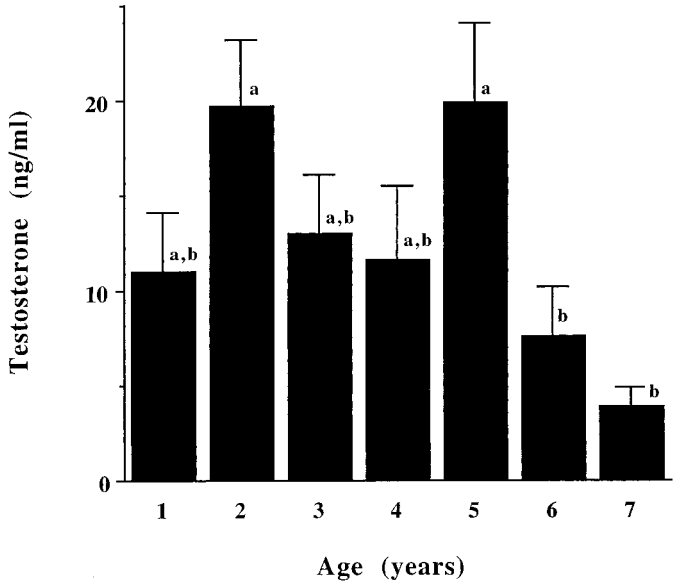

FIG. 5. Serum testosterone in anesthetized male black-footed ferrets 1–7 yr of age during peak breeding season (n = 116). Means (± SEM) with different superscripts differ significantly ($P < 0.05$).

in the other age groups. Likewise, a higher incidence ($P < 0.05$) of sperm with proximal droplets was observed in 7-yr-old compared with 6-yr-old ferrets, and the latter group also differed ($P < 0.05$) in this regard from all other age groups.

Serum Testosterone

Mean serum testosterone was statistically similar ($P > 0.05$) in males 1–5 yr of age (Fig. 5). The lowest mean testosterone concentration was observed in 6- and 7-yr-old males, but these values only differed ($P < 0.05$) from those of the 2- and 5-yr age groups. This resulted, in part, from variability among males within groups (i.e., range in 1-yr-old males, 0.2–36.1 ng/ml; in 2-yr-old males, 1.3–41.0 ng/ml; in 3-yr-old males, 0.4–42.0 ng/ml; in 4-yr-old males, 1.2–33.8 ng/ml; in 5-yr-old males, 1.3–47.6 ng/ml; in 6-yr-old males, 0.7–30.0 ng/ml; and in 7-yr-old males, 0.5–7.5 ng/ml).

Histology

Testis sections from all 5-yr-old male ferrets contained histological evidence of active seminiferous tubules undergoing spermatogenesis and spermiogenesis (Fig. 6a). Spermatogonia, primary spermatocytes, round spermatids, elongated spermatids, and spermatozoa were identified in all sections, and epididymides contained stored spermatozoa (Fig. 7a). In contrast, testis sections from 7-yr-old males revealed collapsed seminiferous tubules lined by Sertoli cells (Fig. 6b). Spermatogenesis was incomplete, and the seminiferous tubules and efferent ductules were devoid of spermatozoa. The mean diameter of the seminiferous tubules in 7-yr-old males (150.1 ± 3.7 μm) was reduced ($P < 0.05$) compared with that in their 5-yr-old counterparts (189.6 ± 2.8 μm). Likewise, the epididymides of older males appeared to be shrunken and contained no spermatozoa (Fig. 7b). Epididymal tubules were smaller ($P < 0.05$) in 7-yr-old ferrets (100.8 ± 3.0 μm) compared with those in younger males (162.6 ± 2.6 μm).

DISCUSSION

To our knowledge, this is the first study to clearly characterize the age-related decline of testes function in the endangered black-footed ferret. The period from 5 to 7 yr is an interval of transition, with reproductive senescence occurring in a significant proportion of the population by 7 yr of age. In addition to poor testes development, 7-yr-old males produced oligospermic or azoospermic ejaculates with lower sperm motility and fewer structurally normal sperm than in their younger counterparts. All 7-yr-old ferrets evaluated in this study demonstrated normal testicular development during earlier breeding seasons. Most (n = 7) had sired offspring, one as recently as the previous breeding season (at 6 yr of age). This age-related decline of black-footed ferret testes function agrees with similar age-dependent changes reported in the mouse [23], rat [24, 25], bull [10, 26], and human [27].

The deleterious effects of aging on seminal traits appeared to be gradual in black-footed ferrets. The 7-yr-old males experienced marked reductions in total testicular volume, motile sperm/ejaculate, and incidence of normal sperm compared with the younger age groups. The 6-yr-old males appeared to be the group in transition, often with intermediate values between those of their 5- and 7-yr-old counterparts, especially valid regarding testis volume, number of motile sperm/ejaculate, and percentage of morphologically normal sperm. Our overall results revealed that the optimal reproductive life span in the male black-footed ferret coincided with individuals in the 1- to 5-yr age group. This concurs with similar findings in male domestic ferrets documenting a 1- to 5-yr breeding life [28].

Diminished reproductive capacity was further supported by testicular/epididymal histology in 5-yr-old versus 7-yr-old males. The former retained maximal spermatogenic capability; the latter did not. Similar histological changes have been reported for nonmustelid species [23, 25, 29–33] and in regressed testes of male domestic ferrets [15, 34] and stoats (*M. erminea*) [35] during the nonbreeding season. In the regressed state, the seminiferous tubules of the domestic ferret and stoat (also a mustelid) lack spermatozoa but usually contain occasional spermatogonia, primitive Sertoli cells, inactive interstitial (i.e., Leydig) cells, and greatly reduced diameters in the tubule lumens. The epididymides also do not contain sperm during the nonbreeding season.

For many species, the proportion of structurally normal sperm in the ejaculate varies with the season. Numbers of pleiomorphic sperm are higher at the onset and the end of seasonal spermatogenesis, with the most normally shaped sperm being measured during the peak breeding season [36–38]. Black-footed ferrets normally ejaculate approximately 50% pleiomorphic sperm, which is a trait perhaps related to the small number of founders (n = 7) comprising the original species recovery plan [39]. Despite rigorous genetic management, the black-footed ferret produces more malformed sperm than the domestic ferret or Siberian polecat [19], including high proportions of flagellar defects [40]. Additionally, another study (currently in progress) has determined that the incidence of abnormally shaped sperm is approximately 65% in black-footed ferrets at the beginning (February) and end (June) of the breeding season, compared with 40–50% during the peak breeding season (unpublished data). Present data revealed that age also affected the numbers of morphologically normal spermatozoa. The most defects were observed in aged males and

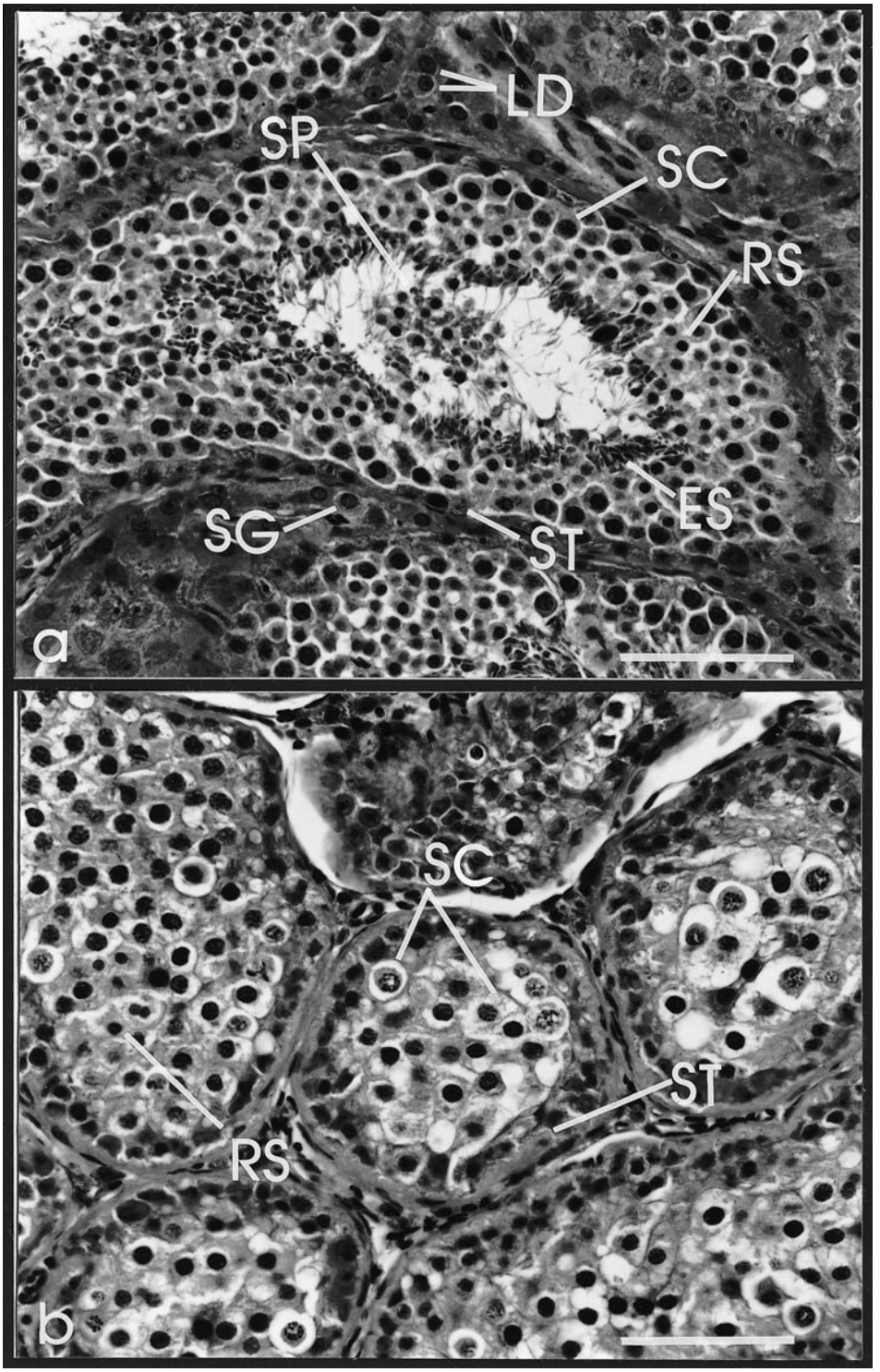

FIG. 6. Testis section of a 5-yr-old (**a**) and a 7-yr-old (**b**) black-footed ferret during peak breeding season (May). Seminiferous tubules in the 5-yr-old male contain Sertoli cells (ST) and active germinal cells in various stages of spermatogenesis and spermiogenesis, including spermatogonia (SG), spermatocytes (SC), round spermatids (RS), elongated spermatids (ES), and spermatozoa (SP). Leydig cells (LD) are present in the interstitial spaces between tubules. Age-associated, collapsed tubules in the 7-yr-old male are shrunken with no lumen and contain Sertoli cells (ST), spermatocytes (SC), and round spermatids (RS) with no evidence of spermatozoa. Bar = 80 μm.

were characterized by more sperm with bent flagella and residual cytoplasmic droplets. Increased proportions of abnormally shaped sperm, including sperm with bent flagella, have been associated with aging in other species, including humans [41] and hamsters [42, 43]. Sujarit and Pholpramool [44] provided evidence that epididymal sperm transport is increased in rats after androgen withdrawal. Therefore, it is logical to speculate that the high proportion of cytoplasmic droplets in the ejaculates of aged, 7-yr-old black-footed ferrets results from deficient maturation and accelerated epididymal sperm passage, perhaps related to low androgen support.

Differences in circulating testosterone concentrations were detected between 6- and 7-yr-old ferrets and their 2-

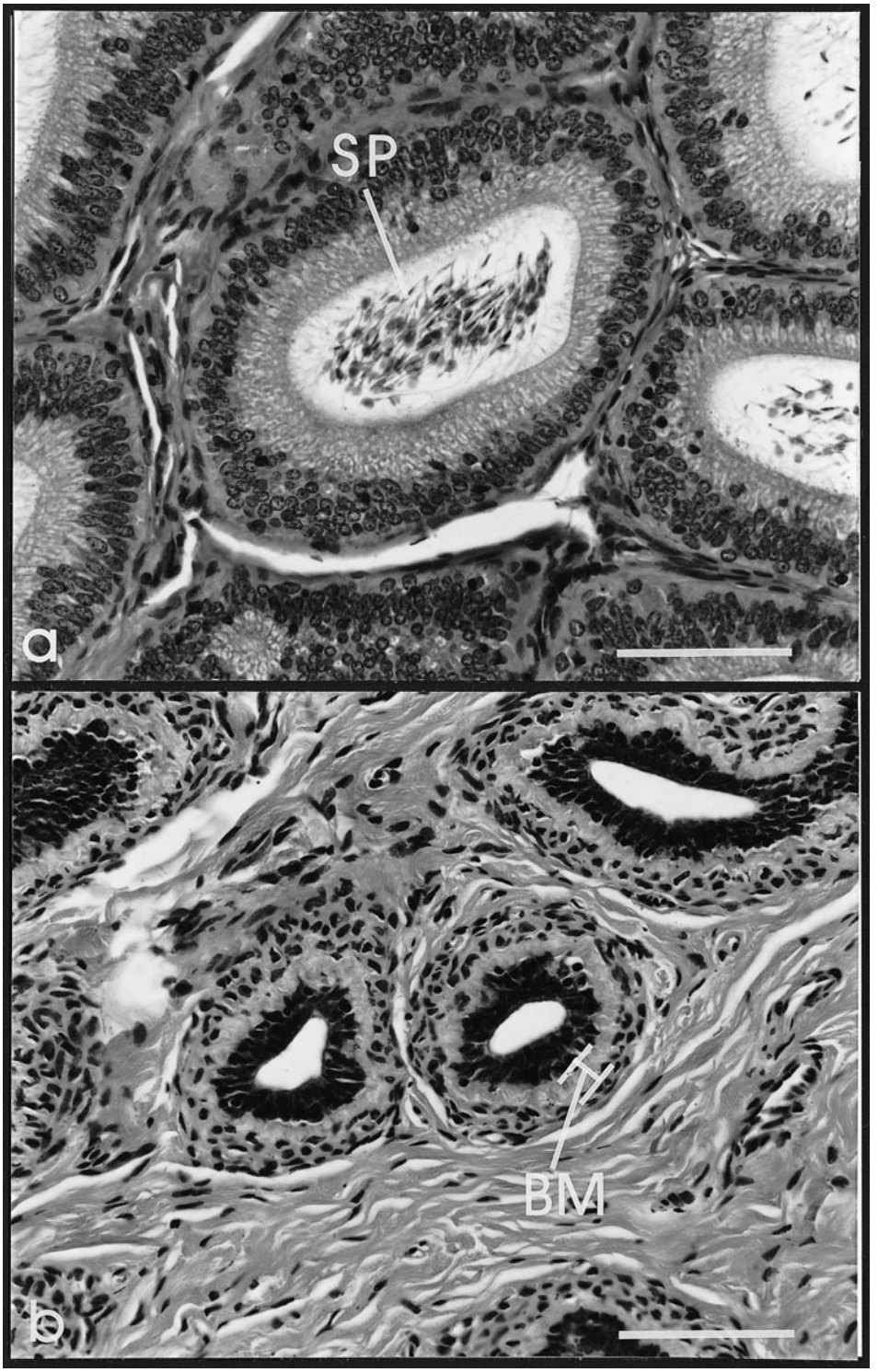

FIG. 7. Epididymal section of a 5-yr-old (**a**) and a 7-yr-old (**b**) black-footed ferret during the breeding season (May). Caput epididymal tubules in the 5-yr-old are packed with spermatozoa (SP). Epididymal tubules in the 7-yr-old male are shrunken with thickened basement membranes (BM) and devoid of spermatozoa. Bar = 80 μm.

and 5-yr-old counterparts. However, no clear pattern of declining testosterone with advancing age was seen, despite it seeming logical that older ferrets with smaller testes would be less androgenically active. Interestingly, many studies on the relationship of age and blood testosterone concentrations have reached contradictory conclusions [12, 13]. Initial studies failed to demonstrate an age-dependent decline in circulating testosterone [12, 45–49]. However, subsequent studies in humans [50, 51], mice [8, 23], and rats [11, 52, 53] demonstrated that testosterone decreases with age. One factor is that males of the same age are not necessarily at the same reproductive state (i.e., one individual may be aging more or less rapidly). When data are averaged over a number of individuals, it becomes difficult to see clear changes unless all individuals have become senescent. Some of the difficulty in interpreting data also

may arise from the episodic pulsatility of pituitary and gonadal hormones. Release of LH and subsequent secretion of testosterone are episodic in domestic ferrets [54], mice [55], rats [56], and humans [57]. As with testosterone, there also appear to be contradictory data among studies as well as species-specific differences in LH profiles because of aging. In humans, LH concentrations tend to rise as a function of age [57], whereas such concentrations decrease with age in male rats [58] and mice [8, 23]. However, more recent studies have revealed no changes in absolute circulating LH in old rats [59–61], although pulse frequency and amplitude decrease with age in both rats [61, 62] and mice [55]. Thus, a more frequent blood sampling protocol in ferrets may reveal an episodic pattern of hormone release and more marked age-related differences in testosterone patterns. Nonetheless, to more accurately understand age-dependent kinetics on testosterone profiles, a longitudinal assessment of hormonal activity is required. Because the black-footed ferret is a wild and intractable species, such a study could only be conducted using noninvasive monitoring of fecal hormone metabolites, which is a technique already validated by Brown [17] for this species.

Our current findings have important implications for managing the contemporary black-footed ferret population. Although females older than 3 yr have compromised reproductive capacities [6], males are reproductively sound through their fifth breeding season. A high proportion of these males will breed naturally, although many 5-yr-old males demonstrate a decreased interest in sexual activity (unpublished observations). A decrease in libido before age-related changes in seminal traits is common among aging males [12, 13]. Substantial data indicate that sperm from 5-yr-old males can be used to produce young by artificial insemination. In our laboratory, six of eight (75%) black-footed ferrets inseminated in utero with sperm from 5-yr-old males became pregnant and whelped 16 kits (unpublished observations), which is similar to the overall success rate of more than 70% using laparoscopic artificial insemination and sperm from younger age groups [63]. Six-yr-old males appear to be in transition from retaining normal reproductive activity to becoming senescent. The effect of aging on individual male reproductive function in this age group is variable. By 7 yr of age, male black-footed ferrets demonstrate profound reproductive failure. Nonetheless, occasional motile sperm can be recovered from both 6- and 7-yr-old males.

In summary, black-footed ferrets (males and females) typically have a life span of approximately 7 yr when maintained in captivity [6]. This study indicated that contrary to female black-footed ferrets, males retain their reproductive capacity for most of their life span. However, space in captive breeding facilities is limited, and the need for maximal productivity is essential to produce sufficient kits to supply the reintroduction program. Therefore, males older than 5 yr do not benefit the captive breeding program, because they no longer are reliably fit reproductively. Because the proportion of males older than 5 yr ranges from 12% to 25% of the total male population each year, these older males occupy significant resources in the breeding program. Given that these males already have contributed genetically to the population or have adequate numbers of sperm cryopreserved for future artificial insemination, these individuals should not occupy valuable breeding space. One logical option is translocation to appropriate zoological institutions for use in community outreach and education programs, thereby helping to illustrate one of the most successful species-recovery programs in modern conservation.

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