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Seasonal variation in serum testosterone, testicular volume, and semen characteristics in the coyote (*Canis latrans*)

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Abstract

The coyote is a seasonally breeding mammal, with most copulations occurring between December and April (depending on location). The objective of this study was to characterize seasonal changes in serum testosterone concentrations, testicular volume, and ejaculate quantity and quality in captive male coyotes. There were seasonal differences in testicular volume, with the greatest volume (20.2 ± 5.4 cm^2, mean ± S.E.M.) in February, corresponding with peak breeding season. Circulating serum testosterone concentrations peaked (3.31 ± 0.9 ng/mL) during January and were positively correlated ($P \leq 0.001$, $r = 0.413$) with testicular volume. Ejaculate volume (1.67 ± 0.4 mL) and sperm concentration ($549.2 \times 10^6 \pm 297.7$ spermatozoa/mL) both peaked during January and February, consistent with the height of the breeding season. Ejaculate volume and sperm concentrations were positively correlated with testicular size ($r = 0.679$, $P \leq 0.001$ and $r = 0.499$, $P \leq 0.001$, respectively) and with serum testosterone concentrations ($r = 0.368$, $P \leq 0.01$ and $r = 0.208$, $P \leq 0.05$). Progressively motile, viable, and morphologically normal spermatozoa fluctuated seasonally, peaked (90.4 ± 4.5, 84.8 ± 4.1, and 87.9 ± 2.9%) during the breeding season, and then subsequently declined (period of aspermatogenesis). All three of these end points were positively correlated with testicular size ($r = 0.589$, $P \leq 0.001$; $r = 0.586$, $P \leq 0.001$; and $r = 0.469$; $P \leq 0.001$) and serum testosterone ($r = 0.167$, $P \leq 0.05$; $r = 0.190$, $P \leq 0.05$; and $r = 0.221$, $P \leq 0.01$). In conclusion, there were intricate relationships among testosterone concentrations, testicular volume, and the production of both functionally intact and morphologically normal spermatozoa.

Keywords: Coyote; Seasonality; Semen; Electroejaculation; Testosterone

1. Introduction

The coyote (*Canis latrans*) is a social canid, living throughout North and Central America [1,2]. Over the past 200 y, extirpation of competitors, introduction of livestock, and growth of agricultural lands have allowed coyotes to steadily increase their range outward from the western United States [2–5]. Due to widespread predation on domestic livestock and their fur-bearing status, coyotes are one of the most widely studied canids.

As with most wild canids, coyotes are classified as seasonally breeding, with the majority of copulations occurring between December and April, depending on location [6,7]. Numerous studies have described the reproductive biology and physiology of male coyotes [6–12], with electroejaculation successfully utilized on multiple occasions to obtain semen for analysis [13–15]. Although seasonal changes in spermatogenesis and its association with both serum testosterone concentrations [11] and the annual cycle of testicular

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recrudescence and involution [14] have been previously reported, to our knowledge, a comprehensive seasonal profile of seminal characteristics has not been described in coyotes. This information will increase understanding of the reproductive cycle of male coyotes and could augment research on chemosterilants for controlling coyote populations [16–19].

The general objective of the present study was to elucidate new knowledge regarding the reproductive biology of male coyotes. The specific objectives were to characterize seasonal changes in: (1) serum testosterone concentrations, (2) testicular volume, and (3) ejaculate quantity and quality in captive male coyotes.

2. Materials and methods

2.1. Animals

Semen was collected from 10 sexually mature male coyotes (2–6 y old, body weight 10–14 kg body weight), from the captive breeding colony at the United States Department of Agriculture, National Wildlife Research Center, Predation Ecology and Behavior Field Station in Millville, UT, USA. They were born in captivity and hand-reared by staff to reduce stress from routine handling. The coyotes were housed in individual kennels (4.3 m^2), identified by ear tags and subcutaneous microchips, and fed a daily ration of meat slurry, with water provided ad libitum. The NWRC, Institutional Animal Care and Use Committee approved all procedures in this study (Protocol QA-862).

2.2. Anesthesia and electroejaculation procedure

Once monthly for 12 mo, semen collection (electroejaculation) was attempted in every coyote, using methods described by Minter and Deliberto [15] and Wildt et al. [20]. On the day of semen collection, coyotes were fasted and transported to an indoor collection site. They were anesthetized with 100 mg ketamine (Ketaved, Vedco Inc., St. Joseph, MD, USA) and 30 mg xylazine (Tranquived, Vedco Inc.) given im by hand syringe. While the animals were anesthetized, the length and width of each testis was measured using digital calipers. Testicular volume was estimated based on the formula for a cylinder with spherical ends $((\pi \text{width}^2 \times (\text{length} - \text{width})/4) + (\pi \text{width}^3/6))$ [21]. The volumes for the right and left testes were combined to obtain total testicular volume for each male.

Semen collection was conducted in a dedicated surgical suite. An electroejaculator with a No. 4 rectal probe (1.6 cm diameter, 25.4 cm long; P.T. Electronic Model, P.T. Electronics, Boring, OR, USA) was used. Electroejaculation consisted of five sets of stimulations, with each set consisting of multiple on-off stimuli (~30 to 40), and a 5 min rest between sets. The voltage for each stimulus ranged from 2 to 5 V, with the voltage required for ejaculation varying among coyotes. Samples were collected in a prewarmed sterile glass tube.

2.3. Semen evaluation

Immediately after collection, the ejaculate was placed in a 37 °C water bath and volume and pH were recorded. The percentage of progressively motile spermatozoa was estimated by microscopic examination at (400 × magnification) on a prewarmed slide (37 °C), and a subjective assessment of progressive motility was recorded [22]. Sperm concentration was measured using a hemocytometer (Hausser Scientific, Horsham, PA, USA). A smear was stained with eosin-nigrosin [23] and sperm viability was estimated by viewing 200 spermatozoa under 1000 × magnification [23]. To evaluate morphologic and acrosomal abnormalities, a drop of each ejaculate was stained with Spermac® (Stain Enterprises, Wellington, South Africa) and 200 cells were examined at 1000× magnification [24]. Morphological abnormalities were visually classified as head, midpiece, and principle piece defects. Morphological characteristics were noted and the percentage of normal spermatozoa and of each abnormality was calculated. The Spermac® stain permitted differentiation of the acrosome (green) and the post-acrosome (pink), allowing for microscopic identification of acrosome damage, including partial or total acrosome removal.

2.4. Analysis of serum testosterone

Prior to induction of anesthesia, blood samples were obtained via cephalic venipuncture. After clot formation, samples were centrifuged (1200 × *g* for 15 min) and recovered serum was stored at −80 °C until analyzed. Serum samples were packed in dry ice and sent to the Colorado State University Animal Reproduction and Biotechnology Laboratory (Fort Collin, CO, USA) for analysis.

Serum testosterone concentrations were determined (single assay) by radioimmunoassay of 50 μL samples, as described by Berndston et al. [25]. The antiserum was reported to have <3.5% cross-reactivity with dihydrotestosterone and <2% crossreactivity with 30 other steroids. Sensitivity of the assay, defined as the least amount of hormone distinguishable from zero, was

13.1 pg/mL in a 50 μL sample. The intra-assay coefficient of variation of all duplicates was 8.29 ± 0.22%.

2.5. Statistical analysis

Data were analyzed using SAS (SAS Institute Inc., Cary, NC, USA). Influence of season on testicular size, seminal characteristics, and serum testosterone were analyzed using ANOVA; differences between mean values were determined using a Ryan–Einot–Gabriel–Welsch test. Spearman's correlation coefficients were also calculated between hormonal data, ejaculate traits, and testicular volume. Values were reported as mean ± S.E.M. and $P \leq 0.05$ was considered significant.

3. Results

3.1. Seasonal changes in testosterone concentration and testes size

There was a significant effect of season on serum testosterone concentration (Fig. 1a). Serum testosterone concentrations were highest in January (3.31 ± 0.9 ng/mL), and lowest in October (0.44 ± 0.7 ng/mL; difference between these 2 months, $P \leq 0.001$).

Mean testicular volume also had seasonal changes (Fig. 1b), with a peak in February (20.24 ± 5.4 cm^2), and a subsequent decline ($P \leq 0.001$), reaching a nadir in July (3.9 ± 0.7 cm^2). Testicular volume was positively correlated with serum testosterone concentration ($r = 0.413$, $P \leq 0.001$).

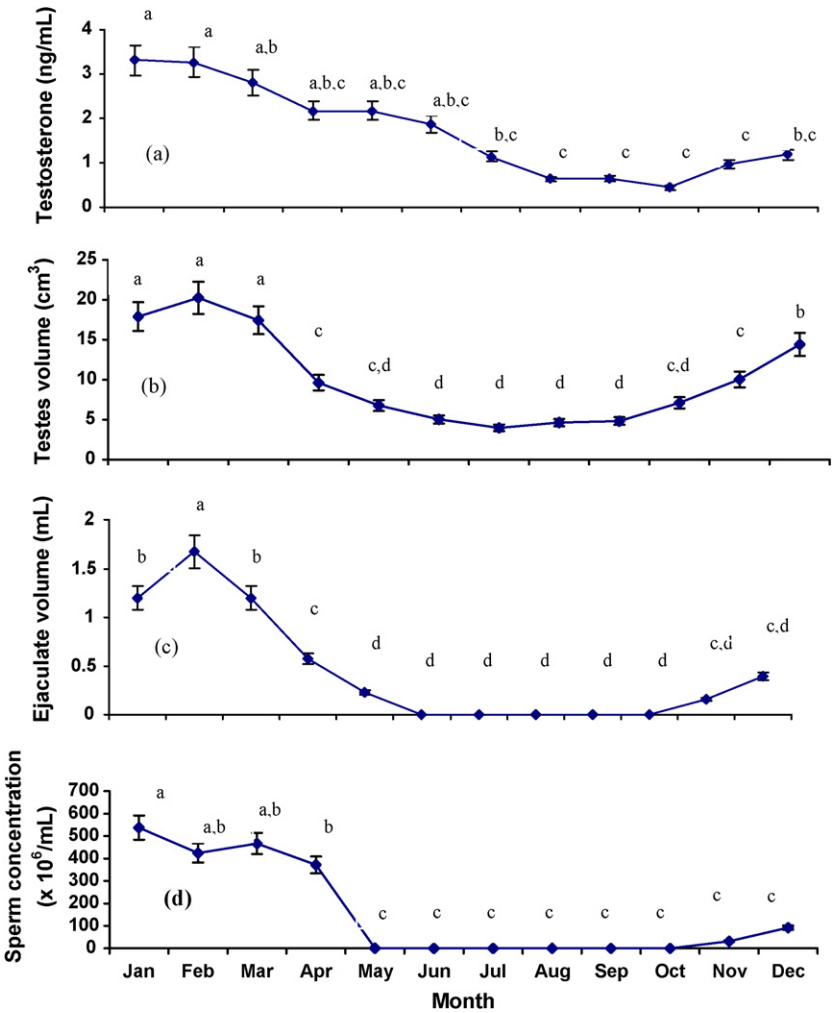

Fig. 1. Mean (±S.E.M.) seasonal variations in reproductive traits in male coyotes exposed to natural light: (a) serum testosterone concentration; (b) paired testes volume; (c) ejaculate volume; and (d) sperm concentrations. (a–d) Values without a common superscript differed ($P < 0.05$).

Table 1
Mean (±S.E.M.) characteristics of ejaculates collected from coyotes (*Canis latrans*)

Month	Characteristic			
	Spermatozoa ($\times 10^6$)/mL	Motility (%)	Viability (%)	Morphologically normal (%)
January	549.2 ± 297.7^{a}	90.4 ± 4.5^{a}	84.8 ± 4.1^{a}	78.0 ± 13.5^{a}
February	$445.1 \pm 71.8^{a,b}$	86.1 ± 3.4^{a}	81.9 ± 5.3^{a}	87.9 ± 2.9^{a}
March	$466.7 \pm 125.3^{a,b}$	82.0 ± 14.0^{a}	79.2 ± 7.1^{a}	83.0 ± 6.3^{a}
April	371.8 ± 149.2^{b}	78.6 ± 7.6^{a}	74.0 ± 7.7^{a}	83.2 ± 5.0^{a}
May	0.6 ± 1.8^{c}	4.6 ± 14.5^{b}	4.6 ± 14.5^{b}	8.0 ± 25.2^{b}
June	N/A	N/A	N/A	N/A
July	N/A	N/A	N/A	N/A
August	N/A	N/A	N/A	N/A
September	N/A	N/A	N/A	N/A
October	N/A	N/A	N/A	N/A
November	32.0 ± 64.9^{c}	23.6 ± 31.5^{c}	13.0 ± 22.4^{b}	31.8 ± 33.7^{c}
December	91.9 ± 94.9^{c}	56.4 ± 31.9^{d}	49.8 ± 27.9^{c}	61.9 ± 34.2^{d}

Within a column, values without a common superscript (a–d) differed ($P < 0.05$).

3.2. Seminal traits

Ejaculate volume exhibited the same seasonal trend as testicular volume and serum testosterone concentration, ranging from a high of 1.67 ± 0.4 mL in February to a low of 0.0 ± 0.0 mL ($P \leq 0.001$) from June to October (Fig. 1c), when the coyotes had a period of aspermatogenesis. There were positive correlations between ejaculate volume and both testicular volume ($r = 0.679$, $P \leq 0.001$) and serum testosterone concentrations ($r = 0.368$, $P \leq 0.01$).

There were seasonal changes in sperm concentration (Fig. 1d), with a peak in January ($549.2 \times 10^6 \pm 297.7$ spermatozoa/mL), and a slow decrease throughout the breeding season until June ($P \leq 0.001$), when sperm concentrations were not calculated due to seasonal aspermatogenesis. There was a positive correlation between sperm concentration and both testicular volume ($r = 0.499$, $P \leq 0.001$) and serum testosterone concentrations ($r = 0.208$, $P \leq 0.05$).

Mean percentages of progressively motile and viable spermatozoa had a similar seasonal trend (Table 1) as semen volume and sperm concentration. Mean percentages of both progressively motile and viable spermatozoa were positively correlated with testicular volume ($r = 0.589$, $P \leq 0.001$; and $r = 0.586$), as well as with serum testosterone concentrations ($r = 0.167$, $P \leq 0.05$; and $r = 0.190$, $P \leq 0.05$).

Mean percentage of morphologically normal spermatozoa also varied seasonally, peaking in February ($87.9 \pm 2.9\%$), and then dramatically decreasing until sperm production ceased in June ($P \leq 0.001$). Mean percentage of morphologically normal spermatozoa was positively correlated with testicular volume ($r = 0.469$, $P \leq 0.001$), and serum testosterone concentrations ($r = 0.221$, $P \leq 0.01$). Specific sperm abnormalities, averaged across the breeding season (January–April) are shown (Table 2).

4. Discussion

These results represented the first detailed information characterizing seasonal changes in serum testosterone concentrations, testicular volume, and ejaculate quantity and quality in captive coyotes. There was an intricate relationship among testosterone concentrations, testicular volume, and the production of both functionally intact and morphologically normal spermatozoa. There were distinct seasonal changes in serum

Table 2
Abnormal structural and acrosomal morphology of coyote ($n = 10$) spermatozoa collected during the breeding season (January–April)

Morphological abnormality (%)	Mean (±S.E.M.)
Detached head	3.9 ± 2.9
Macrocephalic	0.04 ± 0.2
Microcephalic	0.05 ± 0.2
Bicephalic	0.1 ± 0.3
Malformed head	1.5 ± 1.8
Abnormal mitochondrial sheath	0.0 ± 0.0
Bent midpiece with cytoplasmic droplet	1.1 ± 1.0
Bent midpiece without cytoplasmic droplet	0.4 ± 0.8
Coiled flagellum	1.5 ± 1.6
Bent flagellum with cytoplasmic droplet	2.1 ± 1.9
Bent flagellum without cytoplasmic droplet	0.5 ± 0.7
Proximal cytoplasmic droplet	1.3 ± 1.0
Distal cytoplasmic droplet	0.8 ± 0.9
Biflagellate	0.01 ± 0.09
Acrosomal abnormality (%)	
Damaged acrosomal cap	1.7 ± 1.3
Partial acrosome removal	1.6 ± 1.2
Total acrosome removal	0.6 ± 0.7

testosterone concentrations, testicular volume, and ejaculate traits, with highest values occurring January through March (breeding season for coyotes in this geographic location).

Testicular volume in the coyote exhibited seasonal variation, with peak volume in February, corresponding with the breeding season. These findings agreed with previous results reported for this species [14]. The pattern of regression and recrudescence of testicular volume in these coyotes was associated with changes in serum testosterone concentration and in spermatogenic capacity, including overall ejaculate volume, sperm concentration, percentage of both progressively motile and viable spermatozoa, and morphologically normal spermatozoa. Adult males of many seasonal breeding animals have annual cycles of testicular involution and recrudescence. This strategy is advantageous to the coyote, allowing males to minimize energy expended on reproduction, and redirect that energy to hunting and caring for young [26].

Serum testosterone concentrations peaked during January and February, corresponding to the breeding season, and were positively correlated with testicular volume. Serum testosterone concentrations in this study were within ranges previously reported in coyotes [11], gray wolves [27], and domestic dogs [28,29]. Several studies have shown serum testosterone concentration influenced size and functionality of the epididymis and thereby testes size and the maturation and survival of spermatozoa during epididymal transit [30–33]. It also appears that testosterone concentration played a fundamental role in preventing apoptotic cell death of testicular tissue [34]. There was an inverse relationship between testosterone concentrations and proliferation in testicular parenchyma and the level of apoptosis in both brown hares and roe deer [34]. This cycle of spontaneous degeneration of spermatogenic cells and spermatogonial proliferation seemed to be common in seasonal breeding mammals [35]. As with many seasonally breeding mammals, the increased testosterone concentrations reported in this study were accompanied by increased sperm concentration, with improved sperm morphology and motility [36–39].

Ejaculate volume and sperm concentration both peaked during January and February, consistent with peak breeding season. Our results seemed similar to those previously reported using electroejaculation in the coyote [11–13] and red wolf [21], and within range of those previously reported using manual stimulation in the domestic dog [28]. Both ejaculate volume and sperm concentrations were positively associated with testicular volume and serum testosterone concentrations, and suggested a functional role of testosterone for normal spermatogenesis in the male coyote [40]. This information was consistent with work in bonnet monkeys [41] and domestic rabbits [42]. In these studies, deprivation of testosterone led to arrest of meiotic division of primary spermatocytes to spermatids, effectively terminating sperm production.

Seasonality not only effected serum testosterone concentrations, testicular volume, and spermatozoa quantity, but also sperm quality. Mean percentages of both progressively motile and viable spermatozoa and morphologically normal spermatozoa fluctuated seasonally, peaking during the breeding season and subsequently declining, leading to a period of aspermatogenesis. That all of these ejaculate traits were positively associated with testicular size and serum testosterone, we inferred that high concentrations of circulating testosterone were a prerequisite for normal spermatogenesis and the production of functionally intact and morphologically normal spermatozoa.

The information attained from this study, while increasing the knowledge of male coyote reproductive biology, could assist development, implementation, and seasonal targeting of gamete-based contraceptive vaccines. In that regard, vaccines developed towards sperm antigens could induce infertility in both males and females [43]. This has the advantage of not only rendering sperm within the male genital tract incapable of fertilization before entry into the female, but also of inactivating sperm within the female genital tract. Immunocontraception potentially offers the most effective method for management and long-term population control of vertebrate pest species, including the coyote. These methods are not new and have resurfaced in recent years [17]. In the early 1960s, investigators examined the use of chemical sterilants to limit the reproductive capacity of animal populations [44]. Although these methods were effective, they resulted in chemical castration. Consequently, production of key sex hormones was suppressed, interfering with the normal social structure of target populations; an undesirable effect that can lead to breakdown of intricate social hierarchies such as those maintained by coyotes [6]. Till and Knowlton suggested that reproductive control in coyotes would be effective at reducing depredation of small ruminants in their breeding pair hypothesis [45]. They indicated that many depredation problems caused by coyotes arose from territorial adults providing for their young. These adult coyotes switch from feeding principally on small and medium prey to killing larger species such as lambs. The information obtained from this study could serve in

the development and seasonal targeting of a chemical sterilants that does not affect the hormonal system, and could be delivered effectively, which would sterilize coyotes, modify their predatory behavior, while concurrently leaving their social behavior intact.

In conclusion, the coyote maintained spermatogenic activity during breeding season, with peaks in testicular volume, serum testosterone concentration and ejaculate quantity and quality occurring during the months of January and February. Testicular volume was positively correlated with serum testosterone concentration and each ejaculate characteristic was positively associated with both testicular volume and circulating serum testosterone concentration. The information obtained from this study serves to further increase knowledge of male coyote reproductive biology and could be utilized to improve the application of chemosterilants for controlling coyote populations.

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