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Seasonal changes in steroid hormone profiles, body weight, semen quality, and the reproductive tract in captive African wild dogs (*Lycaon pictus*) in South Africa

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ABSTRACT

Characterization of reproductive seasonality in the African wild dog (*Lycaon pictus*) could assist reintroduction programs. Male wild dogs ($n = 14$) were assessed quarterly (January, mid-summer; April, late summer; August, late winter; November, early summer) for serum testosterone, body weight, testicular and prostatic volume, preputial gland measurement, and ejaculate characteristics. Bi-monthly fecal samples were collected from male ($n = 11$) and female ($n = 4$) wild dogs for analysis of fecal androgens and progestagens. Fecal androgens were higher in early summer (246.4 ± 14.5 ng/g) than in early winter (218.6 ± 13.4 ng/g). Serum testosterone was higher in mid-summer (1.4 ± 0.3 ng/ml) than in late winter (0.7 ± 0.1 ng/ml). Number of spermatozoa per ejaculate was greatest in late summer ($301.4 \pm 39.3 \times 10^6$). Other semen parameters peaked in mid-summer (pH: 7.4; progressive motility: 85.0 ± 0.1%; live spermatozoa: 81.0 ± 16%; normal morphology: 71.5 ± 8.2%). Total testicular and prostatic volume were greater during summer (testicular: 36.7 ± 4.2 cm^3; prostatic: 12.0 ± 1.9 cm^3) than winter (testicular: 25.2 ± 1.9 cm^3; prostatic: 5.8 ± 0.8 cm^3). Preputial pendulance also was greater in summer (7.1 ± 0.5 cm; $n = 9$) than winter (5.9 ± 0.2 cm). Baseline fecal progestagen metabolites were 6.2 ± 2.5 μg/g and peak fecal progestagen metabolites were 14.7 ± 2.8 μg/g. Copulations resulting in pregnancies ($n = 2$) occurred in late summer and gestation was 71 days. Female wild dogs were seasonally monoestrous with mating in summer and winter. In conclusion, wild dogs are reproductively seasonal with improvement in male reproductive variables during summer and a bi-phasic seasonal pattern to female receptivity.

1. Introduction

The endangered African wild dog (*Lycaon pictus*) is a cooperative breeder that lives in packs of seven to 28 dogs comprised of an unrelated alpha pair, other subordinate adults, and sub-adults [12,19,44].The wild dog formerly ranged throughout sub-Saharan Africa and the central Sahara [43,44]. However, wild dog populations have declined dramatically over the last 50 years, rendering this canid species the second most endangered African carnivore [33,51]. With the exception of wild dog populations in large protected areas in Tanzania and southern Africa, most populations are heavily fragmented and consist of less than 100 individuals [13,51]. Wild dog populations are declining due to predation by lion and hyena, exposure to infectious diseases, and, most significantly, habitat loss [13]. Due to competition with and avoidance of lions and hyenas, wild dogs require large territories (>500 km^2 per pack). Yet, human-induced habitat fragmentation has prevented the establishment of large, protected areas for wild dog conservation [50]. Despite their endangered status, wild dogs are robust breeders both in the wild and in captivity. There are approximately 300 wild dogs in captivity outside of Africa and at least 200 more animals in zoos and private collections within South Africa [33]. Captive populations of wild dogs are currently self-sustaining [18], although there is concern that these populations may lack the robust genetic variability many large free-ranging populations possess [20].

In 1998, a wild dog meta-population was established by the ongoing release of captive-bred wild dogs and periodic translocation of free-ranging wild dogs to several small (500–960 km^2) fenced reserves in South Africa [30,31,35]. Early in the reintroduction process, it was believed that such small reserves would require large prey populations due to the high hunting success rate of wild

Abbreviations: FT, fecal androgens; ST, serum testosterone; FP, fecal progestagens; SP, serum progesterone; L, length; H, height; W, width.

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dog [15] and their tendency to use fence lines, rivers and waterholes to easily kill prey [31,41]. However, a meta-analysis of 12 reintroduction sites and 18 release events in South Africa has demonstrated that the prey density does not have a significant influence on the long-term survival of reintroduced wild dogs [24].The only factors that positively influenced the success of reintroduction were the birth of pups while in the pre-release holding camp and a split of the pack after release [24,25]. Releases were conducted during the mating (22%), denning (45%) and other (33%) periods of the year [24]. Although the season of release did not impact reintroduction success [24], reproductive status prior to release and timing of the release to 3–4 months post partum are essential to successful reintroduction of wild dog.

Although gestation in the wild dog is well established as 69–73 days [11,14,16,32,36,49] and wild dogs are known to be monoestrous seasonal breeders [7], reports vary regarding the season(s) in which young are born. In free-ranging South African wild dogs, births have occurred during the summer [23,49] and the winter [34,48]. At The Ann van Dyk Cheetah Center in South Africa, captive-mating typically occurs from December to February (mid-summer) with pups born in March and April (late summer), but reports indicate that a secondary breeding season from June to August (winter) may occur in packs not bred in the summer [7]. To date, several studies of wild dog reproduction have been conducted, however, these studies were limited to small numbers of animals (1–7 dogs; [26,28,36]) or were conducted on wild dog populations at latitudes different to those of southern Africa [14,28,36]. Previous studies in North America and Australia have found increased fecal testosterone metabolites [36], increased testicular volume and more successful semen collection by electro-ejaculation [28] during the summer months. Comprehensive characterization of seasonal changes in the reproductive physiology of both male and female wild dogs in South Africa could help to understand the pack dynamics of the species and optimize the current reintroduction program through definitive determination of the breeding season in southern Africa.

Based on this information, we formulated the following objectives for this study: 1) assess year-round male reproductive activity through fecal testosterone metabolites, serum testosterone, body weight, total testicular and prostatic volume, preputial gland measurements, and ejaculate analysis; 2) characterize mating periods and gestation using fecal progestagens and behavioral observations.

2. Materials and methods

2.1. Animals, husbandry, and experimental design

This study was approved by the Research and Animal Care and Use Committees of the University of Pretoria (Protocol number V025/05). For fecal steroid assessment, 14 male and four female wild dogs housed in male–female pairs at The Ann van Dyk Cheetah Centre, Hartebeespoort, South Africa were utilized (Table 1). Eighteen male wild dogs housed in mixed-sex and male-only social groups of two to six animals were immobilized on a quarterly basis for evaluation of serum testosterone (ST; ng/ml), testicular and prostatic volume, and a semen analysis (Table 1). Opportunistic assessment of 15 female wild dogs for serum progesterone (SP; ng/ml), vaginal cytology, and vulvar appearance was conducted (Table 1). To determine social hierarchy, packs of four or more dogs in the study were observed for one hour per day for four days every other week. All study dogs were housed in fenced outdoor enclosures partially covered with indigenous bush and had visual, olfactory, and auditory contact with dogs in neighboring enclosures. Dogs whose reproductive parameters were assessed quarterly were fed either raw meat (0.5 kg per dog), whole chicken (one chicken per dog), or two cups of pelleted Iams Adult Canine diet (The Iams Company, Dayton, Ohio, USA) once daily. To differentiate between male and female fecal samples, male dogs in the fecal steroid study were fed raw meat (0.5 kg per dog) injected with 5 ml of green food coloring (Robertson's Apple Green Food coloring, South Africa) the day prior to fecal sample collection days. By coloring the meat the day before, it ensured that the feces defecated 24 h later was colored green. Cohabitant female dogs were fed raw meat (0.5 kg per dog) with no colorant. Water was available *ad libitum*.

2.2. Behavioral observations and social dominance

Twice weekly for a two hour period each time, three packs of two or more dogs were assessed for intra-pack behavior. At the beginning of each two-hour observation period, several pieces of meat were tossed into the enclosure. The meat served to stimulate situations in which dominance or subordination might be more easily assessed and to increase the overall dog-to-dog interaction frequency. Behaviors were categorized into the following types: submissive (e.g., ears back and head down, licking another dog's mouth), aggressive (e.g. challenge for meat, fight), vocalizations (e.g., alarm call, whine), sexual (e.g., smell vulva, mate), bodily functions. (e.g., urination, defecation, regurgitation). Dogs were assigned rankings within their sex (male or female). For female dogs, the size and appearance of the vulva was noted. Dogs that demonstrated aggressive behaviors, were never submissive, and always won fights or challenges were assigned alpha male dogs status in each group. Dogs that demonstrated submissive behaviors, were never aggressive and never won fights or challenges were assigned gamma in each group. All other dogs were assigned beta status. Such behavioral categories were used to assess the social dominance hierarchy and were based on previously published techniques [14,15].

2.3. Fecal sample collection and processing for steroid metabolites

Every other week, two to three fecal samples per week were collected from each dog for 12 consecutive months (males) and eight consecutive months (females) (Table 1). Samples were collected within three hours of defecation and stored at −20 °C until processing. Prior to fecal extraction, samples were lyophilized until devoid of moisture, pulverized, mixed, and 0.2 g of pulverized material was weighed out into a 15 ml glass tube. Samples were extracted with a protocol similar to one developed by Brown et. al. [10]. Briefly, 5 ml of 90% aqueous ethanol was added to each 0.2 g sample tube, after which the tube was spiked with 100 µl (~1000 cpm) of either ^{3}H-Testosterone (Testosterone, [1,2,6,7-^{3}H(N)], Perkin Elmer, Waltham, Mass, USA) or ^{3}H-Progesterone (Progesterone, [1,2,6,7-^{3}H(N)], Perkin Elmer). Tritium-spiked samples were boiled at 80–90 °C for 20 min after which samples were centrifuged for 10 min at 500 g, and the supernatant was decanted into clean 15 ml tubes. The extraction was repeated with another 5 ml of 90% aqueous ethanol and the second supernatant was combined with the first. The combined supernatants were dried under a flow of nitrogen gas in a water bath at 37 °C, after which one ml of methanol was added and the sample was vortexed for one minute [10]. All extracts were diluted 1:10 (v:v) with a phosphate buffer (0.09 M NaH_2PO_4, 0.12 M Na_2HPO_4, 0.075 M NaCl, pH 7.0) and stored at −20 °C until analysis. Extraction efficiency was >85% for all samples.

2.4. Radioimmunoassay

Fecal androgens (FT) were assessed from fecal extracts at a 1:10 (v:v) dilution with phosphate buffer using a double antibody DSL ^{125}testosterone radioimmunoassay (RIA) kit (DSL-4100, Beckman Coulter Inc., Brea, CA, USA) previously validated for FT measurement in male African wild dogs [36]. This assay has the following significant