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Effects of the environment on dog semen parameters and testosterone concentration

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ABSTRACT

Whether a mammal reproduces seasonally or continuously depends mostly on the environment and its effects on the endocrine pattern. Although the dog was the first species to have been domesticated, little information is available on the changes in reproduction that have occurred since. In this study, we evaluated whether environmental stimuli can act as modulators of male gonadal activity in the dog at the latitude of Bari (Italy). Therefore, for 1 year, serum and seminal testosterone (T) concentrations, together with seminal parameters, were recorded monthly and evaluated in relation to environmental variables such as temperature, humidity, and photoperiod. We found that, in temperate regions, the annual serum T profile is not affected by environmental conditions, whereas seminal T profile peaks in October and reaches its nadir in April. The percentage of progressive motile spermatozoa is also dependent on environmental cues. The results support the intuitive idea that recorded data require a proper analysis to be meaningful. In fact, we found that, in the dog, environmental changes appear to affect male gonadal physiology, and this is clear when recorded data are analyzed monthly; in contrast, pooling data into seasonal groups hides monthly environmental variations.

1. Introduction

The dog is a monoestral polyovulatory nonseasonal species [1]. As a result, bitches spontaneously ovulate only once or twice per year, with ovulation occurring at any time. Although the physiology and reproductive biology of the bitch have been studied for a long time, information about reproductive patterns in the dog is still scarce and limited. In recent years, several studies in the dog have focused on the stimuli responsible for activating the male gonadal response, which in turn produces androgens. Testosterone (T) plays an important role in sperm production and sexual behavior [2], thus providing an indirect measure of male testicular function. For animals whose reproduction is regulated by annual variations in environmental conditions, the pattern of reproductive hormone secretion from stage to stage changes within the season and is in part responsible for the successive changes in behavior. Advances in our knowledge of the physiologic and anatomic relationships between the brain and the pituitary gland provide insights into the mechanisms by which external stimuli can reflexly cause changes in hormone secretion, even in nonseasonal breeding species [3]. In 1996, Herndon et al. [4] reported that in captive male rhesus monkeys, T levels fluctuated seasonally even without exposure to female monkeys, thus suggesting that environmental stimuli alone might be sufficient to activate male gonadal response. In a more recent article by Svartberg et al. [5] it was reported that, even in humans, total T has a bimodal seasonal variation correlated with body weight and fat distribution in relation to seasonal differences, with a small peak in February and a more prominent peak in October and November. Although dogs are successful breeders in almost all kinds

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of environments, significant seasonal variations in the spermiogram are reported in domestic dogs [6,7]; because there are differences in the precise timing of the variations observed, it is likely that there is a correlation with different environmental and climatic conditions. It has been reported in fact that, in the tropics, seasonal variations influence ejaculate volume, total sperm count, and motility in stray dogs [8] and serum T [9]. Regarding temperate regions, there are insufficient data about variations in dog seminal characteristics and T concentration in relation to environmental modifications during the year. To our knowledge, a comprehensive seasonal profile of serum [T_s] and seminal [T_{sem}] T concentration and of the sperm cell kinetics related to environmental characteristics has not been described in the dog; moreover, there are no published guidelines for timing of semen collection relative to environmental conditions and sexual activity. Among mammals, the first species to be domesticated was the dog [10], but there appears to be little information on the changes in reproduction that have occurred in this species: indeed whether a mammal reproduces seasonally or continuously depends mostly on the environment, e.g., food availability, rainfall, light, temperature.

The general objective of this study was to elucidate new knowledge regarding the reproductive biology of male dogs. The specific objectives were to characterize seasonal changes, at latitude and longitude of Bari-Italy (41° 7′ 7.32″ N, 16° 51′ 7.20″ E), in [T_s] and [T_{sem}] over a one-year period; moreover, a detailed analysis of the modifications occurring in the kinetics of sperm cells regarding gross motility (GM) and progressive motility (PMS) was also assessed, with the aim of highlighting a possible role played by environmental stimuli as modulators of male gonadal activity, even in temperate regions.

2. Materials and methods

2.1. Animals

Experiments were conducted over a 12-month period (from January to December) on adult mixed-breed dogs housed at the local kennel that has an arrangement with the University of Bari "Aldo Moro", Italy. Subjects were chosen among males that had been living at the kennel for at least six months. Dogs were housed individually in a large area (270 m^2) with 30 runs separated by cement block partition walls (2.50 m high), concrete floors, and an exposed rafter ceiling (2.50 m high). During the day, the environment was illuminated with a combination of natural and fluorescent light. In addition, dogs were given daily exercise (approximately 40 minutes per day) in an exercise area inside the premises.

Semen samples (first and second fractions only) were collected using digital manipulation once a month for 12 months from 12 fully grown dogs (aged 3–6 years; 10–40 kg body weight). Males were stimulated for 1 minute before attempts to collect semen, by allowing them to sniff swabs impregnated with proestrus or estrus bitch vaginal discharge. In this study, only dogs that showed normal seminal parameters were enrolled. At the same time, peripheral blood samples were also obtained. Because T has a daily pulsatile pattern, blood samples were always taken at 9 AM beginning each month with the same subject. For all dogs, blood and semen samples were collected by two operators, each of whom performed the same task throughout the year. Blood was always sampled first and semen collected later. The selected male dogs were housed in an area of the kennel far from where the bitches were kept, to avoid any influence of the female dogs on the male hormonal pattern. All subjects were fed a daily ration of complete commercial food for adult dogs, with water provided *ad libitum*. The experiments were conducted according to the protocols approved by the Italian Ministry for Scientific Research.

2.2. Semen evaluation

Immediately after collection, the ejaculate was placed in a 37 °C water bath and volume recorded. Samples were quickly transferred to the laboratory for evaluation. Sperm concentration and percentage of spermatozoa with GM and PMS were estimated using computer-assisted sperm analysis with the Hamilton Thorne IVOS (IMV Technologies, Piacenza, Italy) equipment set for dog parameters on a prewarmed (37 °C) Leja 4 analysis chamber slide, 20 μm in depth (Leja Products B.V., The Netherlands). Morphologic abnormalities of spermatozoa were visually classified as head, midpiece, and principle piece defects, and the percentage of morphologically normal spermatozoa was calculated for each sample.

2.3. Seminal T concentration

Each sample of semen, after assessment of GM and progressive motile spermatozoa (PMS), was centrifuged at 1000 × *g* and seminal plasma recovered and frozen at −80 °C until T evaluation. Seminal T concentration was determined using enzyme-linked immunosorbent assay (ELISA, Radim, Pomezia, Rome, Italy) of 300-μL samples. The antiserum was reported to have 16% cross-reactivity with dihydrotestosterone, <0.8% cross-reactivity with androstenedione, and 0% cross-reactivity with seven other steroids. The sensitivity of the assay was 0.075 ng/mL. The intra-assay coefficient of variation of duplicates was 4.6%, whereas the interassay value was 7.5%.

2.4. Serum T concentration

Blood samples were obtained by cephalic venipuncture and collected in serum tubes. After clot formation, samples were centrifuged at 1500 × *g* for 15 minutes at 4 °C, serum was recovered and stored at −80 °C until T determination using the same procedure described in section 2.3 for [T_{sem}].

2.5. Environmental indicators

For each day of sampling, mean values of light-dark photoperiod were retrieved from the Sun Maps at the National Center of Meteorology and Climatology of San Marino, Italy, and mean values of temperature (°C) and humidity (%) were retrieved from the Bari-Palese weather station.