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ARTICLE

Diurnal Effects on Serum Testosterone and Spermiogram of the West African Dwarf (WAD) Sheep During the Early Rainy Season

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SUMMARY

Circulating serum testosterone and seminal ejaculate characteristics of eighteen sexually mature WAD rams were evaluated at three diurnal phases: in the morning (6-8am), afternoon (12-1pm) and evening (4-6 pm). The ambient, as well as the rectal temperature of the rams were recorded. Significant differences were observed in the mean ambient temperatures during the three diurnal phases ($p< 0.05$). However, there was no significant change in mean rectal temperature of the rams among the three diurnal periods ($p>0.05$).

Serum testosterone concentrations were 22.72 0.12 ng/ ml, 21.68 0.14 ng / ml and 21.15 0.10 ng/ml at 6-8am, 12noon-1pm, and 4-6 pm respectively. Significant differences were also observed during these three diurnal phases ($p<0.05$). However, seminal ejaculate characteristics such as concentration, percent live spermatozoa, and spermatozoa morphological defects did not vary significantly ($p>0.05$) between the diurnal phases. This study has shown that there are diurnal effects on serum testosterone concentrations. We therefore suggest that these effects be taken into consideration during natural breeding program(s) as sexual activity or increased libido is probably better expressed during the cooler periods of the day.

INTRODUCTION

The West African Dwarf (WAD) sheep breed is adapted to the hot humid tropical environment of the forest zone and so is the indigenous breed of Southern Nigeria (Olayemi *et al.*, 2000; Gbanbgoche *et al.*, 2011). Among the climatic adaptive factors of animals are adaptation to ambient temperature and relative humidity. The mechanism of adaptation to environmental temperature is insufficiently understood (e.g., sweating, panting, storage of water in body tissues) (Johnston *et al.*, 1963). It is generally accepted that high temperatures have a detrimental effect upon semen production in many species (Noakes *et al.*, 2001). Not only does a relatively short period of high ambient temperature and humidity raise testicular temperature with significant increases in abnormal spermatozoa ejaculated by bulls, rams and boars but, also affect sexual activity (Noakes *et al.*, 2001). Studies on the effect of cryptorchidism, scrotal insulation and elevated ambient temperature, demonstrated the thermolability of the steroidogenic function of the mammalian testis (Cheek and Mclachlan, 1998; Pearson *et al.*, 2005). It has been reported that elevated ambient temperature triggers an increase in rectal and testicular temperature and a decreased testicular endocrine secretion, in different species of

animal (Wetterman and Desjardins, 1979; Facemire *et al.*, 1995).

Due to the importance of diurnal influence on serum concentration of testosterone and sperm characteristics, this work was aimed at studying the diurnal effect on circulating serum testosterone concentration and seminal ejaculate characteristics of the WAD ram in its native environment in Nsukka area during the early rainy season.

MATERIALS AND METHODS

Eighteen clinically healthy rams aged between 2-3 years were used for this study. They were purchased from local markets in Nsukka and environs (latitudes 6° 30 and 7° 6 north and longitudes 6° 54 and 7° 54 east) (Madu, 2008). The ages were determined by dentition method as previously described by Chyne *et al.* (1974) and Heath *et al.* (1983). The rams were kept in the Faculty of Veterinary Medicine demonstration farm, university of Nigeria Nsukka. They were allowed to acclimatize for 2 weeks before commencement of the study.

During the period of acclimatization, they were dewormed using *albendazole* at adosage of15mg/15kg body weight and vaccinated against pestes des petites ruminants(PPR) using tissue culture rinderpest vaccine(VOM, Nigeria). Also during this period the rams were tethered out in open fields to graze between 06.00 am and 07.00 pm. The feeding (grazing and fresh grass cuts) was augmented with *bambara offals* at night. Water and salt licks were provided *ad libitum* .

The rams were randomly divided into three groups (A, B, C) of six animals each. Each group was assigned to a diurnal phase: Group A (6-8am), Group B (12-1pm) and Group C (4-6pm), while each animal in the groups was tagged for specific identification such as A_{1-6}, B_{1-6} and C_{1-6}.

Ambient temperature readings were recorded from the meteorological station of the Energy center, University of Nigeria, Nsukka. The rectal temperatures were taken with a clinical thermometer. Thereafter, blood and semen samples were collected in each group during the allotted diurnal phases for six weeks. Five ml of blood was aseptically collected weekly by jugular venepucture from each animal in a group. Each sample of blood was allowed to clot in a long test tube placed in a slanting position. Sera were separated and stored at -20°C until thawedfor testosterone assay by the enzyme immunoassay technique (Bosch *et al.*, 1978). Semen samples were collected once a week from each animal in the groups by electro-ejaculation and analyzed by conventional methods (Zemjanis, 1970; Noakes *et al.*, 2001).

Data were subjected to one way analysis of variance (ANOVA) and level of variation between means were tested by the least significant difference method of mean comparison at probability of 0.05 (Steel and Torrie, 1980).

RESULTS

The mean diurnal ambient temperature (°C), rectal temperature (°C), serum testosterone concentration (ng/ml), percent live spermatozoa, sperm concentration (x 10^9), and sperm morphological defects (%) among the three groups are presented in table1. The mean ambient temperatures were 23.881.03° C (6-8am), 25.750.96°C (12-1pm), and 29.201.80°C (4-6pm) and differed significantly during the diurnal phases ($p<0.05$). The mean rectal temperatures of the rams were 38.860.33°C (6-8am), 38.980.15°C (12-1pm), 39.201.80°C (4-6pm) with no significant variation during the diurnal phases ($p> 0.05$) whereas, the

circulating serum testosterone concentration of the rams were 22.720.12ng/ml (6-8am), 21.680.14 ng/ml (12noon-1pm), 21.150.01 ng/ml (4-6pm) and varied significantly between the diurnal phases ($p<0.05$). The mean percent (%) live spermatozoa 84.202.13(6-8am), 83.125.10(12-1pm), 85.102.44(4-6pm), the sperm concentration 2.49 0.29(6-8am), 2.22 0.51(12-1pm), 2.30 0.60(4-6pm) and sperm morphological defects 3.331.56 (%) (6-8am), 3.211.40 (%) (12-1pm), 3.111.37(%) (4-6pm), were all not significantly different among the three diurnal phases studied.($p>0.05$). The sperm morphological defects recorded in this study were minor defects according to the classification of Bloom (1973).

TABLE 1. Mean± SD of ambient and rectal temperatures (^{0}C), testosterone concentration (ng/ml), percent live spermatozoa, sperm concentration and sperm morphological defects (%).

Diurnal phase	6-8am	12noon-1pm	4-6pm
Ambient temperature(^{0}C)	23.88±1.03^c	25.75±0.96^b	29.20±1.80^a
Rectal temperature(^{0}C)	38.86±0.33	38.98±0.15	39.30±0.55
Testosterone concentration (ng/ ml)	22.72±0.12a	21.68±0.14b	21.15±0.01c
Percent (%) live spermatozoa	84.20±2.13	83.00±5.10	85.00±2.24
Sperm concentration. (x109/ml)	2.49±0.29	2.22±0.51	2.30±0.60
Sperm morphological defects (%)	3.331±0.56	3.211±0.40	3.111±0.37

DISCUSSIONS

The mean ambient temperature during the period of the study varied significantly whilst the mean rectal temperature of the rams did not vary significantly between the diurnal phases. This agrees with the general notion that homeotherms maintain their body temperatures within a narrow range. The ability to maintain a constant core body temperature is a measure of adaptation of these breeds to their native environment in the humid tropical conditions. The mean serum testosterone of the rams varied significantly during the diurnal phases. The current findings agree with previous reports which showed that there is a greater probability of finding peaks of testosterone at certain times of the day rather than at others (Boyar *et al.*, 1972, 1973; Lincoln *et al.*, 1977; Ortavant *et al.*, 1982; Mattern *et al.*, 1993). Even though a true diurnal rhythm in the release of LH or testosterone has not been demonstrated (Katangole *et al.*, 1974; Wilson and Lapwood, 1978), peaks of LH or testosterone are likely to be found at particular periods of the day (Ortavant *et*

al., 1982). Ortavant *et al.*, (1982) demonstrated that in sheep the release of LH and testosterone varies in a non-random way as a function of day time and that there is higher probability of finding peaks of LH and testosterone at one part of the day than at another. It is known that testosterone is secreted episodically in response to pulsatile releases of LH (Sandford *et al.*, 1974).

In the present study the highest concentration of testosterone occurred at 6-8am when the ambient temperature was lowest while the lowest concentration of testosterone occurred at 4-6 pm when ambient temperature was highest. Previous studies have shown that factors such as light (Ortavant *et al.*, 1982), locomotor activity (Boyar *et al.*, 1972, 1973; Lincoln *et al.*, 1977) and timing of daily meal in take (Mattern *et al.*, 1993) influenced the diurnal rhythm of testosterone secretion. Since there was significant variation in ambient temperature during the diurnal phases, ambient temperature may have influenced testosterone concentration indirectly through a regulatory effect on the release of LH. Boyar *et al.*, (1972, 1973) related maximal release of gonadotrophin in pre-pubertal boys with sleep and Lincoln *et al.* (1974) associated minimum density of LH peak, with maximal activities and vice versa. These show the diurnal influence on LH release. By extension, this could lead to increased secretion of testosterone and thus its concentration in serum. This probably was the mechanism by which high ambient temperature (at later part of the day, 4-6 pm) induced lowered concentration of serum testosterone and vice – versa in this study.

Seminal ejaculate characteristics such as % live spermatozoa, concentration and spermatozoa morphological defects did not significantly ($p>0.05$) vary during the diurnal phases. This probably was because high ambient temperature during the study period did not result in higher core body, and scrotal temperature to have adversely affected testicular function. Maintenance of reproductive function is also a measure of adaptation to prevailing environmental conditions. Sperm morphology is of interest because of its influence on fertility. In this study the sperm morphological defects seen were minor defects which were previously shown not to significantly affect fertility (Blom, 1973).

CONCLUSION

The results of this work indicate that high quality seminal ejaculate can be expected from the WAD ram at any time of the day in its native environment.

Thus it is hoped that this information would be useful in the planning of breeding programs for the WAD sheep in the tropics.

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