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Diurnal Variation in Semen Characteristics of Red Sokoto Bucks Raised Under Tropical Conditions

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Abstract: The possibility of diurnal variation in semen characteristics of Red Sokoto bucks was investigated in a 60 day study. Semen was collected weekly by electroejaculation and was evaluated for colour, volume, sperm progressive motility, semen pH, viability, and morphology and spermatozoa concentration. Mean environmental temperature was found to be significantly higher in the evenings (35.52°C) than 30.07°C observed in the mornings. Best semen quality was observed in collections done in the morning than in the evening, where sperm viability was significantly higher ($P<0.05$) in the mornings with a difference of 4.08% from that obtained in the evening. The highest and the lowest values of, progressive motility and morphologically normal spermatozoa were observed in the mornings and evenings respectively. The percentage gross sperm motility and percentage morphologically normal spermatozoa for samples collected in the mornings were 59.88±5.84% and 97.01±1.63% respectively, which was significantly higher ($P<0.05$) than that obtained in the evening with 53.75±5.60% and 93.58±3.31 for percentage gross sperm motility and percentage morphologically normal spermatozoa respectively. In conclusion, diurnal variations in seminal characteristics do exist in Red Sokoto bucks raised in the sahel savannah. Highest values of percentage progressive sperm motility, viability and percentage morphologically normal spermatozoa which are indices of sperm fertilizing ability were recorded in the morning. The findings in this study therefore, necessitate the recommendation that semen intended for artificial insemination in Red Sokoto bucks are best collected in the morning.
Keywords: Diurnal, Red Sokoto Bucks, Semen, Stress, Temperature.

INTRODUCTION

The selection of a competent male is important for a successful breeding programme[1]. Evaluation of breeding bucks based on semen quality is important and provides the guideline to buck evaluation for reproductive performance [2]. The Red Sokoto is the most important goat breed in Nigeria, accounting for about 70% of the estimated 34.5 million goats in Nigeria [3]. They are better adapted to dry hot environments than other domestic animals and can therefore thrive and produce in these environments [4]. Despite the heat resistant characteristics of goats, those with production demands are often susceptible to heat stress and suboptimal productivity [5].

Stress has been reported to be one of the main factors that affect livestock productivity [6]. Environmental stress (changes in room temperature, humidity and moisture) and handling stress compromise the homeostasis of an animal which in turn has effects on the processes of reproduction [7, 8]. Environmental stress often results in low sperm quality in the male [6], which many cause low fertility in the females due to both or either low fertilizing rates and early embryonic mortality [9]. Dobson *et al.*[7] reported a decrease in fertilization rates in females following a temperature increase of 0.5°C of the uterus during hot days.

This study was aimed at investigating the effect of environmental temperature and time of collection on semen characteristics of Red Sokoto bucks with a view of establishing the best time for semen collection in this breed.

MATERIALS AND METHODS

Background of the Study Area

This study was conducted in Sokoto state, Nigeria which is geographically located to the northwestern part of Nigeria between the longitudes 4° 8`E and 6° 54`E, and latitudes 12°N and 13° 58N [10]. The climate is characterized by alternating wet and dry seasons with short cold and dry period of harmattan usually accompanied by dust- laden winds and fogs which starts from October and lasts through to February. The duration and intensity of annual rainfall range from 60- 160 days and 635- 1000mm (occurring

between May and October) respectively. The mean monthly temperature is generally high (20-38°C) with the highest temperature occurring in April [11].

Experimental Animals

Twenty apparently healthy Red Sokoto bucks of age range between 12 and 18 months were used. The bucks were conditioned for 14 days before commencement of the study. They were weighed using weighing balance and aged by their dental eruption and wearing patterns as described by Wosu [12].

The bucks were kept at the small ruminant section of Usmanu Danfodiyo University Veterinary Teaching Hospital city campus complex, Sokoto, Sokoto state, Nigeria in four pens, each measuring 12×10 feet area. The bucks were randomly divided into 4 groups of 5 bucks each and kept in separate pens. They were fed with hay, bean husk and wheat offal. Water was also provided *ad libitum*.

Environmental Temperature

The environmental temperature was measured using a room thermometer throughout the study period in the morning (6:30am to 8:30am) and evening (4:30pm to 6:30pm).

Semen Collection

Semen was collected weekly throughout the duration of the study by the same person with the aid of an electroejaculator (EE). Bucks were adequately restrained on standing position. Hairs around the preputial orifice were trimmed using a pair of scissors, then the prepuce was washed and dried using a paper towel.

The probe of the electro-ejaculator was inserted into the rectum after lubrication with K-Y jelly. The stimulation was done rhythmically by switching the EE on intermittently, the voltage application was in grading order (i.e. in increasing magnitude) which lasted between 5 and 10 seconds. Semen samples were collected into a calibrated test-tube via a funnel held under the preputial orifice, once semen began to flow out, the voltage was held steady for collection of the ejaculate. The collected semen sample was observed and evaluated for color, volume, motility, pH, viability and concentration.

Color was assessed by visual observation while the semen was in the calibrated test tube and color observed was noted and recorded, the color ranges from white, milky to yellowish. Volume was measured using the calibration of the collection vial and recorded in milliliters.

Gross sperm motility was assessed by placing a drop of the raw undiluted semen on a clean dry glass slide and covered with coverslip and viewed at x10 magnification under a light microscope. The motility was subjectively rated based on speed of the sperm swirls and the intensity of the dark linear concentration of the spermatozoa; its motility was assessed and recorded in percentage.

The pH was determined using a pH indicator paper. The pH indicator paper was dipped in a drop of raw undiluted semen placed on a clean glass slide. The resultant change in color was compared with the standard and the reading recorded.

The Improved Neubeur haemocytometer was used to determine the semen concentration as described by Anzar *et al.* [13] with slight modifications.

Percentage of viable spermatozoa was determined by gently mixing a drop of raw undiluted semen with a drop of eosin-nigrosin stain. A thin smear of the mixture was made and air dried. The live sperm cells appeared white while the dead spermatozoa appeared dark. Two hundred sperm cells were counted in 10 fields.

Morphological characteristics were observed by the use of light microscope at ×1000 magnification. Morphology of spermatozoa in eight fields, with more emphasis in each field on normal spermatozoa, proximal cytoplasmic droplet, distal cytoplasmic droplet, detached head, coiled tail, bent tail, microcephaly and macrocephaly was observed. Percentage was computed for each and then recorded to the corresponding defect.

STATISTICAL ANALYSES

All data collected were expressed as mean ± standard deviation (Mean±SD). Significance of differences of variables between groups were analysed at $P \leq 0.05$ using t-Test. Analysis was conducted using the Graphpad Instat software version 3.05, 2000.

RESULTS

Environmental temperature

Environmental temperature was observed to vary at different periods of the day. Mean temperature measured in the evening was 35.52ºC which was significantly higher than 30.07ºC recorded in the morning ($p<0.001$; table 1).

Semen characteristics

Environmental temperature and time of semen collection had no significant effect on semen characteristics such as volume, concentration and pH between the semen collected in the mornings and evenings except sperm motility, viability and normal morphology.

The data showed that the semen collected in the evenings had higher mean values for volume (0.98ml), sperm concentration (208.15×10^6 sperm cells/ml) and ph (8.66) when compared with samples

collected in the mornings with 0.90ml, 207.60×10^6 sperm cells/ml and 8.53 for volume, sperm concentration and pH respectively. However, the differences observed were not statistically significant ($p>0.05$; table 1).

Significantly higher percentage of progressively motile spermatozoa was observed and recorded in the morning than evening with 59.88±5.84% and 53.75±5.60% respectively ($p<0.01$; table 1). Environmental temperature was also observed to have an effect on the viability of spermatozoa where sperm cell viability from semen collected in the morning was 79.39±8.17% which was significantly higher statistically than 74.59±8.57% recorded for sperm cells in samples collected in the evening ($p<0.01$; table 1).

Significant positive correlation ($p<0.01$) was observed to exist between sperm viability and motility for both samples collected in the morning and evening ($p<0.01$; table 3; table 4).

Table-1: Mean±SD environmental temperature and semen characteristics of samples collected from Red Sokoto bucks in the mornings and evenings

PARAMETERS	MORNINGS	EVENINGS
Environmental Temperature (°C)	30.07±3.36	35.52±4.90***
Volume (ml)	0.90 ± 0.52	0.98 ± 0.70
Concentration($X10^6$)	207.60 ± 8.42	208.15 ± 6.34
Progressive motility (%)	59.88 ± 5.84**	53.75 ± 5.60
pH	8.53 ± 0.81	8.66 ± 0.75
Viability (%)	79.39 ± 8.17**	74.59 ± 8.57

** $p<0.01$, *** $p<0.001$

Sperm morphology

Statistical analysis on the morphology of semen collected in the mornings and evenings indicated that there is a significant variation in the percentage normal spermatozoa of the two samples.

Average normal spermatozoa in the mornings was 97.01±1.63 % and that in the evening was 93.58±3.31% and the difference is considered to be statistically ($p<0.05$) significant (table 2). Even though the respective mean percentage of spermatozoa with proximal cytoplasmic droplet (1.8±1.10%), detached head (1.61±1.26%), bent tail (1.07±0.67%), microcephalus (1.10±0.95%) and macrocephalus(0.95±0.53%) was higher in semen samples collected in the morning than evening with 0.71±0.51%, 1.51±1.55%, 0.94±0.97%, 0.94±0.77% and 0.90±0.45%, the differences were not greater than was expected by chance ($p>0.05$; table 2). Coiled tail was not observed in both groups.

Table-2: Mean±SD diurnal sperm morphological characteristics of Red Sokoto bucks

PARAMETERS	MORNINGS	EVENINGS
Normal (%)	97.01±1.63**	93.58±3.31
PCD (%)	0.71 ± 0.51	1.8 ± 1.10
DCT (%)	1.03 ± 0.82	1.03 ± 0.59
Detached Head (%)	1.59 ± 1.55	1.61 ± 1.26
Bent Tail (%)	0.94 ± 0.97	1.07 ± 0.67
Coiled tail (%)	0.00 ± 0.00	0.00 ± 0.00
Microcephaly (%)	0.94 ± 0.77	1.10 ± 0.95
Macrocephaly (%)	0.90 ± 0.45	0.95 ± 0.53

** $p<0.01$

KEY: PCD= Proximal Cytoplasmic Droplets; DCD= Distal Cytoplasmic Droplets; SD= Standard Deviation

Table-3: Correlation coefficient of semen characteristics of semen samples collected in the mornings

	Volume	*Concentration*	*Motility*	*pH*	*Viability*
Volume	1				
Concentration	0.183535	1			
Motility	0.08718	0.016975	1		
pH	-0.04469	-0.01149	0.040728	1	
Viability	0.165155	0.017561	0.831068**	0.039613	1

** $p<0.01$

Table-4: Correlation coefficient of semen characteristics of samples collected in the evenings

	Volume	*Concentration*	*Motility*	*pH*	*Viability*
Volume	1				
Concentration	-0.0347	1			
Motility	-0.01569	0.001783	1		
pH	0.05701	0.029596	0.003793	1	
Viability	-0.01391	-0.03779	0.838015**	0.006888	1

** $p<0.01$

DISCUSSION

The results obtained in this study showed that there are significant variations in environmental temperatures at different times of the day with those of the evenings being highest, which may alter physiologic activities of biological systems as reported by Seebacher and Franklin [14]. The significant variation in temperatures observed in the mornings and evenings agrees with the findings of Amadi *et al.* [15] who reported that environmental temperature varies within a day in a meteorological study. Higher temperature values recorded in the evenings than mornings are attributed to the heat emitted by the sun and absorbed by surrounding structures which later dissipate it gradually in the night leading to a cooler morning.

The lack of significant variation of semen volume (0.83±0.59 ml and 0.93±0.87 ml), sperm concentration (206.97±10.04 and 209.06±7.27) and semen pH (8.53.±) suggests that environmental temperature variation has no effect on spermatogenesis because the testis was not subjected to direct heat, as direct exposure of the testes to high temperature had been reported to cause changes in some critical stages of spermatogenesis thereby affecting the quality of the ejaculate [8, 16]. On the other hand, changes in environmental temperature trigger thermoregulatory mechanisms to maintain homeostasis [6, 17].

The significant ($p>0.05$) variation observed in the progressive sperm motility (59.88±5.84% and 53.75±5.60%) and viability (79.39±8.17% and 74.59±8.57%) of spermatozoa in semen samples collected in the mornings and evenings respectively is attributed to the variation in environmental temperature earlier observed in this study, which most likely affected semen handling especially when temperatures were higher leading to a decline in these parameters. It has been reported by Al-Badry [18] and El-Kelawey *et al.* [19] that semen handling may affect the fertilizing ability of spermatozoa. Heat stress readily affects semen quality and may be manifested as loss of motility and viability of spermatozoa. The significant variation ($p<0.05$) in the percentage of morphologically normal spermatozoa (97.01± 1.63 and 93.58±3.31) between the two collections is attributed to environmental temperature differences observed at the different times. High environmental temperature have been reported by Li *et al*. [20] to alter the sperm morphology leading to spermatozoa with free head and cytoplasmic droplets which was also observed in this study.

CONCLUSIONS AND RECOMMENDATION

Diurnal variations in environmental temperature have no effect on spermatogenesis due to lack of significant differences in sperm concentration and volume of semen, but rather have effect on the fertilizing ability of sperm cells as indicated by the significant differences in the progressive sperm motility, sperm morphology and viability of sperm cells collected in the mornings and evenings. Semen collected for artificial insemination in the field is prone to losing its fertilizing capacity due variations in temperatures during the day.

Therefore, we recommend that the best time for semen collection in areas where significant diurnal variations exist is in the morning due to low morphological abnormalities, higher percentage progressive sperm motility and percentage viable sperm cells in the semen collected at that time.

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