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RELATIONSHIPS BETWEEN SPERM MORPHOLOGY, SEMEN CHARACTERISTICS, TESTICULAR MEASUREMENTS AND BODY CONFORMATION TRAITS IN RED SOKOTO GOAT

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ABSTRACT
This study assessed the relationships between sperm morphology, semen characteristics, testicular measurements and body conformation in 31 Red Sokoto bucks. The body condition was scored on a scale of 1 to 5. The linear traits: {heart girth (HG), stature (ST), chest width (CW), withers height (WH), body depth (BD), body length (BL) and rump width (RW)} and testicular measurements:{testicular length (TL), testicular circumference (TC), were measured in centimeters using flexible tape while testicular width (TW) and testicular weight (TWT) were estimated using the appropriate formulae}. The semen characteristics: {semen volume, sperm motility, semen pH, sperm concentration and live and dead ratio}as well as sperm mophological traits {detached mid-piece and tail (DMT), detached head (DH), mid-piece droplet (MPD), coiled and bent tail (CBT), and acrosomal abnormality (ACR)} were accordingly determined. The results showed that, the correlations between semen and sperm morphological characteristics were generally negative and non-significant, except the correlation between CBT and some semen traits ($P<0.05$; r= -0.29 to -0.39) as well as the correlation between semen volume and some sperm morphological traits ($P<0.05$; r= -0.26 to -0.29), which were significant). Among the testicular measurements, TL recorded the only negative and significant correlation with MPD ($P<0.05$; r= -0.33). DMT was positively and significantly correlated with BD ($P<0.05$; r= 0.39) but negatively and significantly correlated with WH ($P<0.05$; r= -0.25). However, DH was positively and significantly correlated with WH and BD ($P<0.05$; r = 0.25 – 0.39) MPD also recorded positive and significant correlation with BW, HG and CW ($P<0.05$; r= 0.25 – 0.28), while CBT was observed to be negatively and significantly correlated with BW, BCS, HG and Stature ($P<0.05$; r= -0.25 to -0.39). The study revealed that bucks with good body size and higher semen volume and quality exhibited less sperm morphological defects, therefore semen traits such as semen volume, sperm concentration, Live /dead ratio; and body measurement such as BW, BCS, HG and HW could be used to estimate semen quality in bucks.

KEY WORDS: Bucks, Body condition, Body linear traits, Sperm morphology, Semen traits.

INTRODUCTION

Semen quality parameters such as motility, sperm number and sperm morphology are of value in identifying animals of low fertility in pastoral herds (Parkinson, 2004). Of the component of semen quality, spermatozoa morphology is of utmost importance in animals extensively reared in the tropics (Chacon, 2001). Abnormalities of the spermatozoa occur due to disorder of the seminiferous tubules, during ejaculation or in manipulation of the ejaculate including excessive agitation, over-heating to rapid cooling, mixture of water, urine or antiseptic in the semen (Hossain *et al*., 1990). Spermatozoa with abnormal morphology are the most important factor that has direct bearing on fertility of animals for successful AI programmes. Experiments revealed. that the proportion of morphologically abnormal spermatozoa in the semen correlates negatively with fertility results (Shamsuddin *et al*., 1993), whereas morphologically abnormal spermatozoa are unable to fertilize the oocytes (Shamsuddin and RodriguezMartinez, 1994). Recent studies have shown that the presence of 11% or more of head, mid-piece or tail abnormalities and 18% or more of total abnormalities of spermatozoa are associated with reduced fertility in ruminants (Sarder, 2004). Reddy *et al*. (1975) have reported significant negative correlation between abnormal spermatozoa and conception rate. Total sperm abnormalities has been reported to be positively and significantly influenced by abnormalities at head (r= 0.65) and midpiece (r=0.72) regions (Hazarika *et al*., 1988). The morphological characteristics of spermatozoa are influenced by several factors including the genetic make-up and physiological stage of the animal, nutrition, season, climatic factors, and disease (Dana *et al*., 2000; Dowsett and Knott, 1996; Barth and Oko, 1989). This implies that evaluation of sperm morphology should be performed along with clinical evaluations for different breeds at different ages and in different environments before using them for breeding. Previous studies have shown that sperm morphology has a close association with fertility (Soderquist *et al*., 1991). Poor sperm morphology is an indicator of decreased fertility in many species, including goats (Chandler *et al*., 1988).

An accurate morphological examination of spermatozoa thus enables the elimination of males with potentially low fertility prior to the preservation of their semen (Rodriguez-Martinez and Barth, 2007). Genetic improvements of farm animals rely on the intensive use of a few superior males either for natural mating or in artificial insemination programs. The knowledge on correlations of male reproductive traits especially sperm

morphological traits with other variables such as semenand testicular characteristics as well as body conformation traits might have important bearings to indicate the real producing ability of a male for sperm output and quality of semen. Therefore the study was aimed at determining the relationships between sperm morphology and variable such as semen characteristics, testicular measurement and body conformation traits in Red Sokoto goats.

MATERIALS AND METHODS

Study Location

The study was conducted at the Experimental and Research Farm of the Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria. The area is situated between latitude 11^0 and 12^0N and altitude of 640m above sea level (Encarta Encyclopedia, 2009 PC version). The area falls within the Northern-Guinea Savannah Zone having an average annual rainfall of 1100mm, which starts from late April or early May to mid-October. The peak rainy season is between June and September, followed by the harmattan period of cool and dry weather which last from October to January. This is then followed by hot-dry weather from February to April. The mean maximum temperature varies from 26^0C to 35^0C depending on the season, while the mean relative humidity during harmattan period and wet season are 21% and 27% respectively. Detailed description of Zaria was given elsewhere by Akpa *et al.* (2002).

Experimental Animals and their Management

A total of thirty-one Red Sokoto bucks were used for the study. The animals were under the management practices of the Department of Animal Science, Ahmadu Bello University, Zaria. The bucks were reared under semi-intensive system. The animals were released daily for grazing at 8.00am and another shift by 2.00 pm. Supplemental feed (concentrates) were provided. Animals received routine inspection and dipping (ectoparasite), as well as anti-helminthic drenching (deworming) and vaccination against endemic diseases. Drinking water was provided *ad libitum.* The experiment commenced when the bucks were 9 – 12 months of age in July 2011 and terminated when they were 21 – 24 months, in June, 2012.

Data Collection and Traits measurement

Body Weight measurement

The body weight of the bucks was measured in kilograms by following the procedure as described by Akpa *et al.* (1998). The weight of the observer was taken first, and then the body weight of each animal was taken by carrying the animal individually and standing on a weighing scale. The difference between this weight and that of the observer gives the weight of the animal. Weighing was done at the beginning of the study and subsequently on monthly basis. A total of 372 records were generated for body weight.

Body Linear Measurement

Measurement of linear conformation traits were taken on the day of measurements in centimeters (cm) using flexible tape as described by Alphonsus *et al.* (2009) and Boisot *et al*. (2002). The measurements were taken at the onset and subsequently on monthly basis. A total of 372 records were generated for each of the body linear measurements. The traits are described as follow:

Heart Girth (HG): This is the circumference of the body at a point immediately behind the fore limbs and perpendicular to the body axis.

The Stature (ST): This was measured from the top of the spine in between the hips to the ground.

Chest Width (CW): This was measured from the inside the surface between the top of the front legs.

The Wither Height (WH): This is the highest point over the scapular vertically to the ground.

Body Depth (BD): This is the distance between the top of the spine and the bottom of the barrel at the last rib.

Body Length (BL): This was measured from the point of shoulder to the ischium.

Rump Width (RW): This is the distance between the most posterior points of pin bones

Body Condition Score (BCS)

The body condition score (1-5) as described by Allen (1990) and Steele (1996) were employed to score the bucks. The buck's backbone, loin and rump areas were palpated and examined and then scored. These areas do not have muscle tissue covering them, hence, combination of skin and fat deposit account for any cover that were felt around these areas. Amount of fat deposit can be determined by the use of fingertip pressure which is exerted on the backbone, pin bone and hip bone respectively. The scoring as described by Allen (1990) and Steele (1996) is given below:

Score 1 (Very Thin)

Individual short ribs have a thin covering of flesh. Bones of the chine, loin and rump region are prominent. Hook and pin bones protrude sharply, with a very thin covering of flesh and deep depressions between bones. Bony structure protrude sharply and ligament prominent.

Score 2 (Thin)

Individual short ribs can be felt but are not prominent. Each rib is sharp to touch but have a thicker covering of flesh. Short ribs do not have as distinct an over-hanging shelf effect. Individual bone is the chine, loin and rump regions are not visually distinct but easily distinguishable by touch. Hook and pin bones are prominent but the depression between them is less severe. Area below tail head and between pin bones is somewhat depressed but the bony structure has some covering of flesh.

Score 3 (Moderate)

Short ribs can be felt by applying slight pressure. Altogether, short ribs appear smooth and the over-hanging effect is not so noticeable. The backbone appears as a rounded ridge, firm pressure is necessary to feel individual bones. Hook and pin bones are rounded and smooth. Area between pin bone and around tail head appears smooth without sign of fat deposit

Score 4 (Fat)

Individual short rib is distinguishable only by firm palpation. Short ribs appear flat or rounded, with no overhanging shelf effect.

Ridge formed by backbone in chine region is rounded and smooth. Loin and rump region appear flat. Hooks are rounded and the space between them is flat. Area of tail head and pin bones is rounded with evidence of fat deposit

Score 5 (Obese)
Bony structures of backbone, short ribs and hook and pin bones are not apparent; subcutaneous fat deposit very evident. Tail head appears to be buried in fat tissue.

Testicular measurement:
These were done at the onset and subsequently on weekly basis before semen collection. A total of 1488 records were generated for each of the measurement. The measurement were as follows:

Testicular Length (TL)
This was measured in centimeter with a flexible measuring tape as the distance along the caudal surface of the scrotum, from its point of attachment to the tip of the scrotum as described by Akpa *et al*. (2012) and Bratte *et al*. (1999).

Testicular Circumference (TC)
This is the maximum dimension around the pendulous scrotum after pushing the testes firmly into the scrotum (Akpa *et al*. 2006). It was measured in centimeters (cm)

Testicular Width (TW): This was taken as the division of Testicular Circumference by two.

Testicular Weight (TWT): This was determined using Bailey *et al.* (1996) formulae as given below;

$TWT = 0.5533 \times TL \times TW^2$

Where; TWT = Testicular weight
TL = Testicular length
TW = Testicular width

Semen Collection and Evaluation
Semen samples were collected from each animal at the onset and thereafter on weekly basis for 52 weeks using an electro-ejaculator and and were labelled accordingly. This was done in the morning hours throughout the duration of the experiment. The sampled semen samples were evaluated immediately for colour, volume, motility and pH as describe by Zemjanis (1970). Smear of each semen sample was prepared; air dried, labelled and kept for further examination, vis determination of sperm concentration using formaldehyde and determination of live/ and dead ratio using eosin nigrosin. A total of 1488 records were generated for each of the observed characteristics. Determination of Sperm concentration, Live and dead ratio and sperm morphology are given below:

Semen Concentration
The concentration of the spermatozoa was determined using the Red Blood Cell counting chamber of a haemocytometer that were crossed with microscopic grids containing 25 large squares with each containing sixteen smaller squares. The total number of smaller squares on the haemocytometer is 400. Sperm cells were counted diagonally from top left to the bottom right and from top right to the bottom left in five large squares or a total of 80 smaller squares (Rekwot *et al*., 1997). Prior to counting, formaldehyde were used as a dilution reagent. A drop of semen was taken from each sample using automatic pipette and diluted with formaldehyde at 1:100.

The haemocytometer was mounted into the microscope and an absorbable tube and O-no pette was used to pipette a drop of the solution into the haemocytometer chamber. The absorbable tube and the O-no pette were blown before pipette to avoid air bubbles in the O-no pette. After appropriate counting in the 5 large squares, the number obtained was multiplied with 100 (dilution factor), 16 (the number of smaller squares in a larger square and the volume of the semen sample collected, multiplied by 10^6). The result obtained was recorded as the sperm cell concentration for the sample.

Live and Dead Ratio
The live and dead ratio was estimated by the preparation of a smear of individual semen sample using eosin-nigrosin stain immediately after collection. A drop of semen was diluted and placed on a clean glass slide using automatic pipette. A drop of the eosin-nigrosin solution was placed alongside the semen on the slide. A gentle circular turning of the slide was done to allow a uniform mixture of the two samples. A one-quarter of the part of another clean slide was placed on top of the first sample and the two slides were gradually and carefully drawn apart to prepare a thin smear on the first slide. This was allowed to dry and thereafter labelled. This was done for each sample and they were later mounted on the microscope for counting the live and dead sperm cells. The principle is that the dead sperm cells accept the stain and appear stained while the live sperm cells reject the stain and remain unstained. The procedure above was developed by Hancock (1951)**.**

Sperm Morphology
The same slides prepared for live and dead ratio were used for the morphology studies. Live and abnormal spermatozoa percentages were counted using hand counter. Fifty spermatozoa were examined for each sample. The total number of abnormal cells were counted and recorded. Types of abnormality observed were: detached mid-piece and tail, detached head, mid-piece droplet, coiled and bent tail, and acrosomal abnormality (acrosome membrane detached, acrosome outlines and acrosome cap defect). Acrosomal abnormalities were determined by using smears made from the raw semen and stained by Giemsa stain according to Watson (1975).

STATISTICAL ANALYSIS
Correlation analysis procedure of SAS (2002) was used to assess the relationship between the measured characteristics. The weekly data (1488 records) on sperm morphology were used for estimating their relationships with seminal traits and testicular measurements. However, to estimate the relationship of sperm morphology with body weight and body conformation traits, their weekly observations were averaged for each month to get a comparative value to the monthly body measurements.

RESULTS
Table 1 shows the correlation analysis between semen and sperm morphological characteristics in Red Sokoto buck. The correlations between semen and sperm morphological characteristics were generally negative and not significant except the correlation between CBT and some semen traits ($P<0.05$; r= -0.29 to -0.39) as well as the correlation between semen volume; and some sperm morphological traits ($P<0.05$; r= -0.26 to -0.29) which were significant. However the only positive and significant correlation was observed between ACR and live and dead ratio. Other correlations were close to zero or not significant.

TABLE 1: Correlated Relationships between Semen and Sperm Morphological Characteristics in Red Sokoto bucks

	Volume	Motility	pH	Concentration	Live and dead ratio
DMT	-0.26*	-0.24	0.04	-0.17	0.13
MPD	-0.18	-0.11	-0.11	0.06	0.13
DH	-0.26*	-0.24	0.08	-0.17	-0.13
CBT	-0.29*	-0.24	0.13	-0.29*	-0.39*
ACR	0.09	0.04	0.19	-0.15	0.27*

* =P<0.05, MPD: Midpiece droplet, DMT: Detached midpiece and tail, DH: Detached head, CBT: Coiled and bent tail, ACR: Acrosomal abnormality

TABLE 2: Correlated Relationships between Testicular Measurements and Sperm Morphological traits in Red Sokoto bucks

	TL	TC	TW	TWT
DMT	0.02	0.02	0.02	-0.00
MPD	-0.33*	0.17	0.17	-0.09
DH	0.02	0.02	0.02	-0.00
CBT	-0.08	-0.02	-0.02	-0.05
ACR	-0.12	0.05	0.08	-0.01

* =P<0.05, MPD: Midpiece droplet, DMT: Detached midpiece and tail, DH: Detached head, CBT: Coiled and bent tail, ACR: Acrosomal abnormality, TL: Testicular length, TC: Testicular circumference, TW: Testicular width, TWT: Testicular weight

TABLE 3: Correlated Relationships between Body Conformation and Sperm Morphological traits in Red Sokoto Bucks

	DMT	MPD	DH	CBT	ACR
BW	-0.10	0.28*	0.10	-0.39*	0.02
BCS	0.10	0.04	-0.10	-0.25*	0.14
Heart girth	-0.11	0.28*	-0.11	-0.39*	0.03
Stature	0.20	0.09	0.20	-0.26*	-0.18
Chest width	0.11	0.25*	-0.11	0.01	0.30*
Wither height	-0.25*	0.11	0.25*	-0.01	-0.07
Body depth	0.39*	-0.00	0.39*	0.10	-0.11
Body length	0.18	0.23	0.18	-0.21	-0.02
Rump width	0.08	0.00	0.08	-0.03	0.16

* =P<0.05, BW: Body weight, BCS: Body condition score, MPD: Midpiece droplet, DMT: Detached midpiece and tail, DH: Detached head, CBT: Coiled and bent tail, ACR: Acrosomal abnormality.

The result of the correlation analysis between testicular measurements and sperm morphological traits is presented in Table 2. The correlations between testicular measurements and sperm morphological traits were generally low and not significant except the correlation between TL and MPD which was negative and significant (P<0.05; r= -0.33). Although not significant (P>0.05), the correlated relationships between TWT and morphological traits (r= -0.00 to -0.09) as well as the relationship between CBT and testicular traits (r= -0.02 to -0.08) were low and negative.

The correlation analysis between body conformation and sperm morphological traits is shown in Table 3. DMT was positively and significantly correlated with BD (P<0.05; r= 0.39) but negatively and significantly correlated with WH (P<0.05; r= -0.25). MPD was positively and significantly correlated BW, HG and CW (P<0.05; r= 0.25 – 0.28). ACR was observed to have a positive and significant correlation with CW (P<0.05; r= 0.30). DH only recorded positive and significant correlation with WH and BD (P<0.05; r= 0.25 – 0.39), while CBT was observed to be negatively and significantly correlated with BW, BCS, HG and Stature (P<0.05; r= -0.25 to -0.39). Other correlations were close to zero or not significant.

DISCUSSION

Semen volume showed a negative but significant correlation with DMT, DH and CBT indicating that the higher the semen volume the lower the level of these abnormalities in spermatozoa. The present results are in accordance with the findings of Kealey *et al*. (2006) who reported negative correlation of (r= -0.38) between semen volume and abnormal midpiece and (r=-0.05) between semen volume and CBT, respectively in Hereford bull. The negative but significant correlations between some semen characteristics and CBT indicate that CBT is not an indicator of sperm concentration and sperm live and dead ratio. Sperm morphological traits are hardly correlated with testicular measurement. However MPD was observed to be negatively correlated with testicular length. This signified that an increase in the length of testis will lead to a decrease in the occurrence of mid piece droplet and vice versa.

As has been reported earlier (Hossain *et al*., 1990) that, abnormalities of the spermatozoa could occur due to disorder of the seminiferous tubules or germinal epithelium, which is directly or indirectly related to the body conditions of the bucks. The negative correlation observed between CBT and BCS suggests that bucks with good BCS may likely not record appreciable casesof CBT abnormality in spermatozoa.CBT was also observed to be negatively related to BW, HG and Stature. This indicates that bucks with good body size may show lesser abnormality relating to CBT in the spermatozoa of the bucks. According to Barth and Oko (1989), sperm tail pathologies (bent or coiled) may result from failures in thermoregulation, testicular degeneration, hypo-osmotic conditions or failures in epididymal transit. Under normal conditions, sperm tail defects decrease against the increasing age gradient, which indicates sexual maturity of domestic animals (Mekasha *et al*. (2007).
However, positive and significant relationship recorded between MPD; and BW, HG and CW may not be unconnected to high ambient and scrotal temperatures. Occurrence of the mid piece spermatozoa abnormality has been traced to the period of storage in the epididymis (Oyeyemi and Babalola, 2006). The positive and significant correlation between BW and MPD was supported by the findings of Jalal, (2008) who reported the same trend.

CONCLUSION

Bucks with good body size and higher semen volume and quality exhibited less sperm morphological defects. Therefore, semen traits such as semen volume, sperm concentration and Live and dead ratio; and body measurement such as BW, BCS HG and HW could be used to estimate semen quality in bucks, thereby ensuring the elimination of unqualified bucks before being used for breeding purposes.

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