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Original Research Article

Caudal epididymal sperm morphology and body measurements relationships of the Gwembe dwarf bucks

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Although the Gwembe Dwarf (GD) is the most populous goat-breed in Zambia, not much reproductive information is available. In order to investigate sperm morphological characteristics, and their relationship to body and testicular measurements, 21 GD bucks were considered. The body length (BL), heart girth (HG), body weight (BW) and scrotal circumference (SC) were measured. Thereafter, semen was flushed from caudal epididymis and stained using the Eosin-Nigrosin stain. Stained spermatozoa were examined under light microscope at X40 and X100 magnification where 95.1% and 4.9% live and dead sperm cells were observed, respectively. 62.6% spermatozoa showed no morphological abnormalities while 1.25 and 29.0% had proximal and distal cytoplasmic droplets, respectively. Using a non-parametric Spearman's correlation, HG, BL and BW were significantly and positively correlated with SC (P<0.05). However, no significant correlation was noted between body measurements with spermatozoa abnormalities. The testicular weight (TW) was negatively and significantly correlated with the double bent tail abnormality (P<0.05). Caudal epididymal semen exhibited minimal morphological abnormalities not significantly correlated to body and testicular measurements. The HG, BL and BW can be indicators of good breeding selection criteria in the GD bucks.

Key words: Gwembe bucks, caudal epididymis, semen morphology, abnormality, body measurements

INTRODUCTION

Semen analysis is the initial and most important step of infertility evaluation and breed selection. Although the most important valid factor in the assessment of semen quality is the occurrence of viable pregnancies and normal off-spring, semen analysis is still considered to be a cornerstone of the laboratory evaluation of the buck's fertility potential without which breeding strategies could be compromised. Despite the fact that the GD bucks are believed to be prolific breeders, there is no scientific data to support this opinion. The extensive management systems that subject them to browse a wider range of plant species may play a role in the perceived breeding potential, hence the need to investigate their semen morphological properties. Semen sample could be collected through electro-ejaculation, artificial vagina or retrograde flushing from abattoir-derived testicles (Turri et al., 2011).

Normal semen morphology is believed to be one of the major elements that determine the semen ability to effect conception in the female, although many researchers have reported conflicting findings in this regard (Jasko et al., 1990; Love et al., 2000). Although there are no specific measurable properties in the spermatozoa that reflect the fertilizing capacity of the sperm that reach the fertilization site, accurate determination of morphological abnormalities could assist in eliminating males with low fertility potential in breeding herds (Rodríguez-Martínez

and Barth, 2007). Morphological characteristics of spermatozoa could be influenced by many factors such as season, nutrition, genetics and disease which could affect the ability to effect fertilization (Monteiro et al., 2011). Therefore, semen analysis is of high diagnostic value in assessing testicular and epididymal function, and should be considered a prerequisite for selecting superior breeding bucks. Earlier studies have revealed a negative correlation between the proportions of morphologically abnormal spermatozoa with fertility results, since morphologically abnormal spermatozoa cannot fertilize the oocytes (Shamsuddin and Rodriguez-Martinez, 1994).

Although goat production is an integral part of livestock production systems in Zambia, not much information is available about the production traits of the GD indigenous goat breed, raised under extensive management systems where plant shrubs are the main source of nutrition. Therefore, the aim of this study was to investigate semen morphology and its relationship with selected body measurements of the GD bucks as way of assessing their reproductive potential..

MATERIAL AND METHODS

Animals

The study was carried on 21 GD bucks raised on free range aged eighteen to twenty-four months, randomly selected based on phenotypic characteristics. Age was determined according to (Vatta et al. (2006). Animals were numbered with a permanent marker for easy identification and followed through the slaughter line for collection of testicles, which were equally marked as right and left. Immediately after slaughter, intact testicles were sliced off the animal and transferred into a cooler box containing ice-packs and transported to the laboratory for processing. No direct contact between testicles and ice-packs occurred. A total of 42 individual testes were considered.

Body measurements

Body measurements were done using a body weight flexible tape graduated in centimeters prior to slaughter and defined as follows:

Body Length: distance between the point of the shoulder and the ischium,

Heart Girth: circumference of the body at a point immediately behind the forelimbs and perpendicular to the body axis,

Scrotal Circumference: maximum dimension of testicular circumference while testes were firmly pressed into the scrotum (Akpa et al., 2006),

Body Weight: measured in kilograms as described by Pares et al. (2012) which is , $LW = 0.659GH - 17.467$, where HG is in cm.

Semen collection and processing

At the lab, testicles were removed from the scrotum and weighed while still covered in the vaginal tunica. Thereafter, tunica vaginalis was incised to expose the testes proper and the epididymis. The straight and convoluted part of the ducts deferens were separated from other structures until its attachment to the lower tip of the testis where the caudal part of the epididymis was severed at almost 90^0 angle to its outer contour. The seminal content of the caudal epididymis was collected by retrograde flushing method using the two step concentrated bovine semen extender (Continental Plastic Corp., Delavan, WI 53115-0902, USA) containing Tris 12.1g, Citric Acid 6.9g, Fructose 5.0g, and Glycerol 70 ml, to which egg yolk and antibiotic solution comprising Tylosin 60mg, Gentamycin 300mg, Spectinomycin 360mg and Lincomycin 180mg was added. Caudal epididymal semen content was collected into 5 ml conical tubes using 2ml of semen extender flush media warmed in a water bath to about 39^oC prior to flushing. The flushing was done using a 5 ml syringe to which was attached a 21G needle. Immediately after flushing one drop of semen content was put on warmed glass slide for motility examination.

Semen staining and evaluation

Spermatozoa were stained using the Eosin-Nigrosin staining solutions prepared as 1% solution in a 0.9% NaCl solution for Eosin, and 10% solution in 0.9% NaCl solution for Nigrosin. One drop of sperm was mixed with one drop of Eosin solution in a haemolysis tube and shaken for 30 seconds after which 2 drops of Nigrosin solution was added and mixed. A smear was then prepared and air-dried. Initial observation was done under X40 to determine the dead and alive spermatozoa. Thereafter, magnification was increased to X100, and individual sperm cell morphology was assessed under oil immersion and cover-slip. Use of cover-slip was to minimize abrasion of the sperm sample by the tip of the eye-piece objective. Live and dead spermatozoa were assessed based on their staining appearances as described by Hancock (1951), where dead and live sperm stained pink and colorless, respectively. Morphological characteristics of the epididymal sperm cells were assessed. A hundred spermatozoa were examined for each sample from which the abnormal sperm cells were identified and recorded such as distal or proximal droplets, detached head, narrow head base, pyriform, simple bent tail, coiled tail, swollen head, and dag defect.

Statistical analysis

Statistical analysis was done Using SPSS version 16.0. The

Table 1. Mean percentage values of morphological characteristic of the Gwembe Valley Dwarf goat bucks testicle samples (n=42)

Abnormalities	% Mean ± SE
Proximal cytoplasmic droplets	1.25 ± 0.37
Distal cytoplasmic droplets	29.0 ± 1.05
Detached head	0.48 ± 0.11
Narrow head base	0.02 ± 0.02
Pyriform shaped	0.02 ± 0.02
Other head defects (swollen head)	0.19 ± 0.10
Simple bent tail	0.61 ± 0.12
Double bent tail	0.71 ± 0.28

Percentage of normal spermatozoa (Live without any abnormality): 62.6%.
SE: Standard Error

Table 2. Showing mean body and testicular measurements/weight of Gwembe Valley Dwarf goat bucks (n=21: SC, BL, HG, BW); (n=42: TW)

Parameter	Mean ± Standard Error
Scrotal circumference	18.75 ± 0.23 cm
Body length	42.41 ± 0.36 cm
Heart girth	55.07 ± 0.97 cm
Body weight	18.75 ± 0.64 Kg
Testicular weight	60.70 ± 1.49 g

morphological characteristics from 42 flushed epididymal semen samples were summarized in percentage terms as in Table 1, while Table 2 shows descriptive statistics of the body and testicular parameters of GD bucks reported in mean percentage values with the standard deviations. The body length, body weight, heart girth and the scrotal circumference raw data was exposed to normality Shapiro-Wilk test and some values exhibited a non-normally distributed data ($P<0.05$). And hence, a non-parametric Spearman's correlation was used and significance reported at $P<0.05$. Data were considered for left and right testicles independently.

RESULTS

The morphological characteristics of 42 flushed epididymal semen samples were presented in Table 1. Only two abnormalities: i.e the proximal and distal cytoplasmic droplets had values above 0% in addition to the 4.9% dead-stained sperm cells. The pyriform shaped head and the narrow head base had the lowest mean percentage values. The morphological characteristics as analyzed included both the live and dead spermatozoa, where as 95.1 and 4.9% stained colorless and pink, respectively. A significantly higher mean percentage value of 29.0 ± 6.78 was observed in the distal cytoplasmic droplets in relation to other noted abnormalities. No abnormalities were observed in 62.6% of live spermatozoa analyzed.

The body and testicular parameters of GD bucks were presented in Table 2 and shows average values of 18.75 ± 0.23, 42.41 ± 0.36, and 55.07 ± 0.97cm of the SC, BL, and HG, as well as 18.75 kg and 60.70 ± 1.49 g of BW and TW of the studied GD bucks, respectively.

Table 3 shows relationship of body and testicular measurements with spermatozoa morphological abnormalities of the GD bucks which were mainly negative and not significant except for negative and significant correlation between the TW and the double bent tail abnormality ($P<0.05$).

Table 4 shows relationship among the body parameters and the scrotal circumference in the Gwembe valley goat bucks using nonparametric Spearman's correlation.

DISCUSSION

In this study, epididymal spermatozoa abnormalities were mainly observed in the cytoplasmic droplets, which are simply remnants of spermatid cytoplasm on the sperm cells occurring during the process of spermatogenesis, reflecting one of the most striking changes in spermatozoa physiology

Table 3. Relationship of body and testicular measurements/weight with morphological abnormalities of the spermatozoa

	DCP	PCD	SBT	DBT	DS	PSH	NHB	SH
BL	0.163	-0.220	0.046	-0.364	-0.002	-0.223	-0.284	-0.045
BW	0.262	-0.203	-0.005	-0.309	0.186	-0.385	0.243	-0.141
HG	0.262	-0.203	-0.005	-0.309	0.186	-0.385	0.243	-0.141
SC	0.288	-0.272	-0.170	-0.212	0.175	-0.142	0.203	0.017
TW	0.168	-0.474	0.160	-0.670*	-0.362	-0.221	0.397	0.064

= P<0.05, **Spearmen's correlation is significant at 0.05 level (2-tailed, BL: Body length, BW: Body weight, HG: Heart girth, SC: Scrotal circumference, TW: Testicular weight.DCP: Distal cytoplasmic droplets, PCD: Proximal cytoplasmic droplets, SBT: Simple bent tail, DBT: Double bent tail, DS: Dead sperm, PSH: Pyriform shaped head, NHB: Narrow head base, SH: Swollen head*

Table 4: Relationship among the body parameters and the scrotal circumference in the Gwembe valley goat bucks using nonparametric Spearman's correlation

Body Parameter	Scrotal Circumference (SC) (rho)	P-value
HG	0.56*	0.007
BL	0.45*	0.04
BW	0.56*	0.007

**= P<0.05, Correlation is significant at 0.05 level (2-tailed), HG: Heart Girth, BL: Body Length, BW: Body weight*

during the process of maturation (Robaire and Hermo, 1988). Earlier studies have indicated that high incidence of distal and proximal cytoplasmic droplets could have transient reduced fertility and poor *in* vitro fertilization rate outcomes, respectively (Datta et al*.,* 2010*)*. Datta et al*.* (2010) observed that while most spermatozoa from the corpus and cauda epididymis, and vas deferens possess distal droplets, those ones from cauda and vas deferens had less cytoplasmic droplets. This observation is consistent with our findings where only1.25 and 29.0% of spermatozoa from caudal epididymis had proximal and distal cytoplasmic droplets, respectively. The movement, alteration in fine structure and position of the cytoplasmic droplets during spermatozoan passage through the epididymis plays a key role for maturation of the male gametes (Datta et al., 2010). Since the occurrence of cytoplasmic droplets on the spermatozoa derived from the caudal epididymis through non-natural route such as retrograde flushing is a physiological process, there is need therefore to establish conditions and circumstances under which it could be regarded as an abnormality since their existence on the spermatozoa simply reflects a maturational phase prior to ejaculation (Turri et al., 2011). In addition, cytoplasmic droplets may have nutritive role on the spermatozoon as well as involved in the regulation of the cycle regeneration of seminiferous epithelium (Mann and Lutwak-Mann, 1981). No significant correlation was observed between the cytoplasmic droplets occurrence, and body and testicular measurements, and this simply implies that occurrence of cytoplasmic droplets is not influenced by the size of body or testicular parameters.

Apparently, significant correlations of the scrotal circumference with body length, heart girth and body weight were noted in this study implying therefore that BL, HG and BL has positive influence on testicular growth, hence increased scrotal circumference. However, no significant correlations between the body and testicular measurements were observed except for the correlation between TW and DBT which was negative and significant (P<0.05) (Table 3). It means therefore that TW may not be an indicator of the spermatozoa tail abnormality. This is in line with other earlier assertions that sperm morphological abnormalities are hardly correlated with testicular measurements (Ambali et al., 2013). It therefore means that an increase in testicular weight will lead to a decrease in tail bent abnormalities occurrence in the Gwembe Dwarf bucks, and the opposite could equally be true. According to Mekasha et al. (2007) sperm tail defects decrease with age or attainment of sexual maturity in domestic animals, and this could explain why very few morphological abnormalities were observed in this group of bucks aged 18 to 24 months. Also our findings showed that testicular weight gain or scrotal circumference increase is generally correlated with body development, which is age-dependent in a strict sense (Table 4). The observed low spermatozoa abnormalities in this study could also be in line with our hypothesis that probably the type of nutrition bucks are subjected to could positively influence spermatozoa development. Therefore, it be true that variability of plant species up-take could have medicinal effects on

spermatozoa development, hence observed low morphological abnormalities. While this may be true, it should also be noted that there are other underlying factors that may contribute to sperm tail abnormalities such as poor testicular thermo-regulation, testicular degeneration, hypo-osmotic conditions or failures in epididymal transit.

The live spermatozoa observed in this study was slightly higher than that of Tajik et al. (2007) where 86.2% live sperm cells were observed in the bovine. Furthermore, about 70% of the caudal epididymal spermatozoa analyzed in this study were devoid of cytoplasmic droplets, a figure much higher than the findings of Goavaerts et al. (2006) where only 41% spermatozoa had no cytoplasmic droplets in the bull. These variations could be due to a number of factors such as length of interval from slaughter to analysis, disease or pathological conditions, and even species specificity. The high values of live epididymal sperm cells with less cytoplasmic droplets is indicative of the near/attained-maturation and fertilizing potential of epididymal spermatozoa (Costa et al., 2011; Monteiro et al., 2011; Ringleb et al., 2011). Therefore more comparative studies on caudal epididymal sperm cells characteristics are needed in the Gwembe Dwarf bucks if meaningful comparative data has to be attained.

Conclusion

GD bucks aged 18 to 24 months have low semen abnormalities. Furthermore, no significant relationship between body measurements and sperm cells morphological abnormalities except the significant and negative correction between the testicular weight and bent tail abnormality, and that a high proportion of spermatozoa in the caudal epididymis had less to no cytoplasmic droplets, a sign of attaining maturity in readiness for ejaculation and subsequent fertilization of the ovum. The body measurements such as the heart girth, and body weight in the Gwembe Dwarf goat bucks can be an indicator of good selection criteria in breeding bucks as they were positively correlated with the scrotal circumference.

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