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Testicular histo-morphometry and semen parameters of West African Dwarf bucks

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Abstract

This study evaluates the gross and histo-morphometry of the testes as well as the semen parameters of West African Dwarf (WAD) goats bred and raised in Abeokuta, Ogun State, Nigeria. Five apparently healthy WAD bucks were used for this study were electro-ejaculated once weekly for two weeks. Testicular weight, gonadosomatic index, seminiferous tubular diameter and seminiferous epithelial height were measured using standard techniques. Semen parameters which included volume, colour, motility and concentration as well as scrotal circumference were also determined using standard procedures. All data were recorded as mean ± standard deviation, subjected to descriptive statistics and Pearson's correlation analysis at $p < 0.05$. Our findings revealed that the testicular gross morphometric values significantly correlate with the histometric values with normal testicular histo-architecture suggestive of typical spermatogenesis. Furthermore, the scrotal circumference showed positive relationship with both the gross and histometric parameters of the testis. However, there was no significant correlation between scrotal circumference and semen parameters. This information could improve understanding of reproduction in the WAD buck.

Keywords: Semen evaluation, Spermatogenesis, Testicular morphometry, Reproduction, WAD goat.

Introduction

The West African Dwarf (WAD) goats are small, hardy ruminant animal that possess the ability to make maximum use of roughage (Olurode, 2002). They are very common in the western part of Nigeria and as a non-seasonal breeder, adjudged one of the most prolific in the world in terms of young produced per doe per year (Bitto & Egbunike, 2006). These qualities have accounted for their successful contribution to all season meat supply in the south-western parts of Nigeria (Bitto & Egbunike, 2006). However, in spite of these genetic potentials, their productivity is still low due to infertility and reduced reproductive efficiency among other factors. Therefore, improvement in their reproductive efficiency particularly through the selection of highly fertile buck is of utmost importance (Mickelsen & Memon, 2007).

The basis for a sound knowledge of the male reproductive biology in any species lies in the detailed morpho-functional study of its reproductive organs (Segatelli *et al.*, 2004; Adebayo *et al.*, 2015). Morphometric analysis on the testes of any species

or breed is necessary in predicting not only sperm production, but also the storage potentials and fertilizing ability of the male (Adebayo *et al*., 2009). In particular, testicular size (which is represented either by testicular weight or volume) has been reported to be a good indicator of the present and future sperm production in different animal species (Togun & Egbunike, 2006; Adebayo *et al.*, 2009). According to Mickelsen & Memon (2007), testicular size correlates, not only with spermatozoa production, but also with scrotal circumference. Although some testicular measurements has been documented in some animal species (Willis, 2001), there is dearth of information on testicular measurements and their application in the prediction of good sire goats (Bitto & Egbunike, 2006)

Therefore, this study was carried out to evaluate the testicular histo-morphometry and semen analysis in the WAD goat, thereby providing additional information on the possible relationship between testicular size, and semen production in the WAD buck.

Material and Methods

Animals and management

Five post-pubertal WAD bucks aged between 10 and 18 months, with body weight that ranged from 10-13 kg were used for the study. They were purchased from local markets in Abeokuta and housed in the Experimental Unit of the Department of Veterinary Public Health and Reproduction, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta. At the time of purchase, the bucks were examined for physical defects especially at the testicular region to ensure normal descent of the two testes. The bucks were fed with cassava peels and grasses, in addition to supplemented concentrates. Clean water was provided *ad libitum* in water troughs.

Semen collection and evaluation

Semen was collected from the bucks once weekly for two weeks using electro-ejaculation (EE) method. The semen was collected into graduated tube which was immersed in a warm water bath at 40^0C and the volume reading recorded.

Sperm concentration

Sperm concentration was evaluated by mixing a drop of semen with 49 drops of formol saline. The spermatozoa were counted by haemocytometer method using improved Neubauer Chamber described by Pant & Srivastava (2003).

Sperm motility

The sperm motility was estimated by mixing a drop of semen with few drops of 2.9% sodium citrate and fixed volume was delivered onto a warm glass slide and covered with cover slip. The preparation was then examined at x400 magnification under the light microscope.

Percentage livability

The proportion of live/dead spermatozoa in each ejaculate were counted and differentiated in smear preparations, stained with Eosin-Nigrosin stain and examined at x400 magnification.

Sperm morphology

On a clean warm glass slide, a drop of semen was placed with 2 drops of 2.9% sodium citrate buffer and a drop of Eosin-Nigrosin stain. The diluted semen and stain were thoroughly mixed together with a smear made on another clean and warm slide. The smear was air-dried and observed using the light microscope starting with low power to high magnification; at least 200 spermatozoa were observed at x100 magnification of the objective lens with oil immersion.

Gross and histo-morphometric evaluation

Following successful collection and evaluation of the semen, the scrotal circumference was measured prior to orchidectomy of the bucks using open castration technique. Open castration was carried out to measure the actual size and weight of the testes of the WAD bucks. The goats were physically restrained, lidocaine was infused intradermally, thereafter, 3cm long pre-scrotal incision was made. The underlying fascia was dissected bluntly and the left testis was forced out through the incision by pressure over the scrotum. The tunica vaginalis was cut though to expose the testis and isolate the spermatic cord. Three artery forceps was used to clamp the spermatic cord at three successive points leaving a small gap between each forceps. The spermatic cord was then ligated at the gaps between the forceps (double ligation) using chromic catgut size1. Following the ligation the spermatic cord was transected at the outer most forceps (the forceps closest to the testis). A double ligature was then placed at the base of the gubernaculums testes after which it was also transected and the testis was

removed. The second testes (right) was grasped and pushed towards the incision point, where it was further milked out through the incision. The tunica vaginalis was also incised and the same procedure conducted for the left testis was repeated on the right. 1ml of penicillin-streptomycin was infused into the scrotal sac. The testicles were examined grossly for abnormalities. Testicular weight and length were also determined.

Tissue sample of each testis were fixed in Bouin's solution for 24-48 hours. The paraffin blocked sample was sectioned at 5µ thickness, routinely stained with haematoxylin and eosin (H & E) and viewed at x100 magnification with oil immersion. Histometric parameters evaluated included Sertoli cell count, seminiferous tubular diameter, seminiferous epithelium height, germ cell counts.

The tubular diameter of the seminiferous tubule and the height of the seminiferous epithelium were measured at x100 magnification using an ocular micrometer calibrated with a stage micrometer. Ten tubular profiles that were round or nearly round were randomly selected and measured for each animal. All germ cell nuclei and Sertoli cell nucleoli were counted in the cross-sectional view of 10 round or nearly-round seminiferous tubules randomly selected. The counts determined were corrected for section thickness and nucleus or nucleolus diameter according to Abercrombie (1946) and modified by Amann (1962). The following germ cells were counted: type A1 spermatogonia, pachytene primary spermatocytes, and round spermatids. The following ratios were calculated using the corrected counts:

- pachytene spermatocytes/type A1 spermatogonia, to estimate the coefficient of efficiency of spermatogonial mitosis;
- round spermatids/type A1 spermatogonia, to obtain the overall rate of spermatogenesis;
- round spermatids/pachytene spermatocytes, to obtain the rate of germ cell loss during meiosis (meiotic index);
- round spermatids/Sertoli cell nucleoli, to estimate the Sertoli efficiency;
- total number of germ cells/Sertoli cell nucleoli, to obtain the total support capacity of each Sertoli cell.

Some definitions:

- Spermatogonia is the cell of the germinal epithelium of the seminiferous tubule.
- Coefficient of efficiency of spermatogonia l mitosis is the ratio of the Pachytene spermatocytes to type A1 spermatogonia.
- Overall rate of spermatogenesis is the ratio of round spermatids to type A1 spermatogonia.
- Meiotic index is the rate of germ cell loss during meiosis and measured as the ratio of round spermatid to pachytene spermatocyte.
- Estimated Sertoli efficiency is the ratio of the round spermatids to Sertoli cell nucleoli.
- The total number of Sertoli cells was determined from the corrected counts of Sertoli cell nucleoli per seminiferous tubule cross-section and the total length of seminiferous tubules according to the method described by Hochereau-de-Reviers *et al*. (1978).

Statistical analysis

All data were recorded as mean ± standard deviation and subjected to descriptive statistics and Pearson's correlation analysis.

Results

The testes showed normal histo-architecture suggestive of typical spermatogenesis (Plate I).

Table 1 shows the result of testicular morphometry of the WAD bucks. The cell counts done (Table 1) shows that the coefficient of efficiency of spermatogonia l mitosis was 1.24; the overall rate of spermatogenesis was 1.15. The meiotic index was 0.924. It was also noted that the estimated Sertoli efficiency was 1.94. Table 2 shows shows the result of the semen parameter of the WAD bucks including semen colour, semen volume, sperm motility, sperm concentration, percentage livability and sperm morphology. Table 3 shows the correlation coefficients of semen parameters comprising semen volume, sperm motility, total sperm count and sperm morphology as well as the scrotal circumference of WAD bucks indicating weak and insignificant correlation. Table 4 reveals the correlation coefficients of testicular parameters. It is shown from Table 4 of the result of statistical analysis that the testicular weight is strongly correlated with the seminiferous tubular diameter ($r= 0.967$; $P < 0.05$) and seminiferous epithelial height ($r= 0.977$; $P<0.05$). There is also a strong positive correlation between Seminiferous tubular diameter and epithelial height ($r= 0.961$; $P< 0.05$). In the same vein, there is strong positive correlation between the scrotal circumference, testicular length and seminiferous epithelial height. It was equally observed that there is a strong but statistically insignificant relationship between the scrotal circumference, testicular weight and seminiferous tubular diameter. However, it was observed that

Table 1: Testicular histo-morphometry of the West African Dwarf goat

Testicular parameters	**Mean± SD**
Body weight (kg)	8.40 ±1.14
Testicular weight (g)	48.44 ± 11.63
Testicular length (cm)	7.80 ± 0.71
Scrotal circumference (cm)	15.75 ± 0.79
Gonadosomatic index (%)	0.583 ± 0.02
Seminiferous Tubular diameter (µm)	182.15 ± 12.25
Seminiferous Epithelial height (µm)	55.65 ± 6.27
Spermatogonia A1	18.46 ± 3.54
Spermatocyte	22.90 ± 2.81
Round spermatid	21.16 ± 5.51
Sertoli cell nucleolus	10.88 ± 2.56
Cell counts:	
Spermatogonia A1	18.46 ± 3.54
Spermatocyte	22.90 ± 2.81
Round spermatid	21.16 ± 5.51
Sertoli cell nucleolus	10.88 ± 2.56

Table 2: Mean of Semen parameters of West African Dwarf goat

Parameters	Mean ± SD
Semen colour	Creamy/milky white
Semen volume (ml)	0.40 ± 0.07
Sperm motility (%)	74.80 ± 6.08
sperm concentration ($x10^9$/ml)	5.06 ± 0.20
Livability (%)	82.00 ± 2.00
Normal sperm morphology (%)	81.80 ± 9.7313

Table 3: Correlation coefficients between semen parameters and scrotal circumference

		Semen Volume	Sperm motility	Sperm concentration	Sperm morphology	Scrotal circumference
Semen Volume	Pearson Correlation	1	-.015	.097	-.690	.056
	Sig. (2-tailed)		.982	.877	.197	.929
Sperm motility	Pearson Correlation	-.015	1	.280	-.104	-.117
	Sig. (2-tailed)	.982		.648	.868	.852
sperm concentration	Pearson Correlation	.097	.280	1	-.397	-.099
	Sig. (2-tailed)	.877	.648		.508	.874
Sperm morphology	Pearson Correlation	-.690	-.104	-.397	1	.634
	Sig. (2-tailed)	.197	.868	.508		.251
scrotal circumference	Pearson Correlation	.056	-.117	-.099	.634	1
	Sig. (2-tailed)	.929	.852	.874	.251	

n = 5

there is generally a weak and insignificant correlation between the scrotal circumference and semen parameters measured in this study.

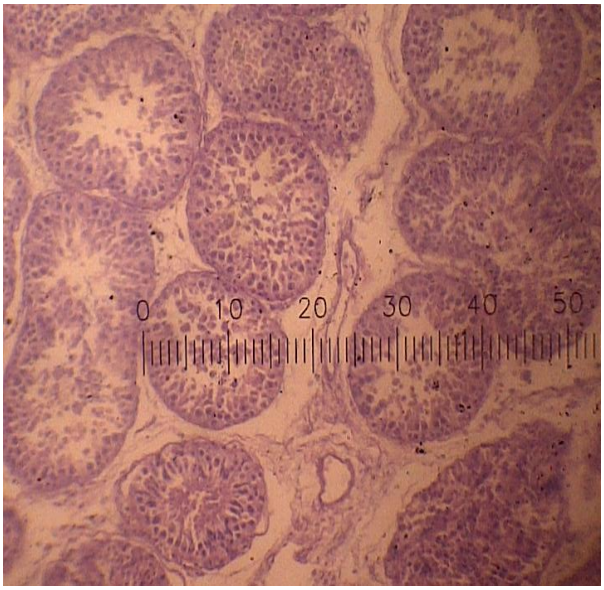

Plate I: Photomicrograph with ocular micrometer showing normal architecture of testis and spermatogenic cycle of WAD buck (H&E; x100)

Discussion

This work presents information on basic morphometric values of the testes of indigenous West African Dwarf buck found in Abeokuta. The semen colour obtained in this study is similar to the findings of earlier researchers (Bearden & Fuquay, 1997; Oyeyemi *et al.,* 2011). Also, mean percentage livability observed in this study is in agreement with the findings of Oyeyemi *et al*. (2008) who worked on the same breed of goat. The value of scrotal circumference observed in this work agrees with the observation of Ugwu (2009) who reported a value of 17.25 ± 0.76 cm. Ogwuegbu *et al*. (1985) reported higher scrotal circumference value (21.1cm) for Red Sokoto, a breed of larger phenotype found in the savannah belt of Nigeria.

In this study, it was observed that while there is a strong relationship between scrotal circumference and testicular morphometric parameters. There was no significant relationship between the scrotal circumference and semen parameters. The report of Osinowo *et al.* (1981) had earlier established a high correlation between scrotal circumference and testicular weight in bull. This work confirms this observation in the WAD goat. According to Macchado-Junior *et al.* (2011), scrotal morphology

Table 4: Correlation coefficients between testicular parameters and scrotal circumference

		Testicular weight	seminiferous tubular diameter	gonadosomatic index	Seminiferous epithelial height	Scrotal circumference
Testicular weight	Pearson Correlation	1	.967**	.105	.977**	.801
	Sig. (2-tailed)		.007	.867	.004	.103
seminiferous tubular diameter	Pearson Correlation	.967**	1	.131	.961**	.723
	Sig. (2-tailed)	.007		.834	.009	.168
gonadosomatic index	Pearson Correlation	.105	.131	1	-.001	-.421
	Sig. (2-tailed)	.867	.834		.999	.480
Seminiferous epithelial height	Pearson Correlation	.977**	.961**	-.001	1	.872
	Sig. (2-tailed)	.004	.009	.999		.054
scrotal circumference	Pearson Correlation	.801	.723	-.421	.872	1
	Sig. (2-tailed)	.103	.168	.480	.054	

* * indicates significant ($p < 0.05$) correlation

n = 5

and morphometry affect testicular structure and spermatogenic efficiency in cross-bred goats. Similarly, Ugwu (2009) had also reported a relationship between scrotal circumference and gonadal and extragonadal sperm reserves. While scrotal circumference may provide a good indication for spermatogenic efficiency, it may not necessarily be a good reflection of the semen quality and quantity which may largely depend on the method of semen collection. This fact may be responsible for the no significant relationship between scrotal circumference and semen parameters observed in this study.

In the same vein, the seminiferous tubular diameter and epithelial heights observed in this work are similar to the findings of Oke *et al.* (1984) in WAD goat. There is a strong positive correlation of the testicular weight with the seminiferous tubular diameter and the epithelial height in this study. According to Machado-Junior *et al*. (2012), testicular weight and volume can only be a sure index of spermatogenic efficiency and a reflection of daily spermatic production if it is accompanied by greater number of germ and Sertoli cell per section of the seminiferous tubule. This was observed in this work.

According to Johnson (1991) Germ cell loss can be estimated by comparing the ratios between germ cells counted during specific steps of spermatogenesis. We did not perform an investigation specifically to count the total germ cells, but the results found for the ratio of primary spermatocytes to type-A spermatogonia, investigated suggest that at least 5 generations of differentiated spermatogonia are present in the goats studied. The ratio of spermatids to primary spermatocytes show that 30% of germ cell loss occurred in the WAD goat during meiosis (Table 1). This value is similar to that observed for most mammalian species investigated (França & Russell, 1998). Also, assuming that 5 generations of differentiated spermatogonia are present in the WAD goat, the ratio of round spermatids to type A1 spermatogonia observed in this species indicate that 75% of germ cell loss took place during spermatogenesis. Previous research reports indicate that Sertoli cell number is directly related to the sperm production rate of an animal and it was found that spermatogenic efficiency correlated positively with the number of germ cells supported by each Sertoli cell (Sharpe, 1984; Franca & Russell, 1998).

In conclusion, this work has provided valuable information on the testicular morphometry and reproductive parameters of the WAD goat in Abeokuta thereby contributing to the general knowledge of the reproductive biology of this economically-viable small ruminant.

References

Abercrombie M (1946). Estimation of nuclear population from microtome sections. *Anatomy Records*, **94**(2): 239-247.

Adebayo AO, Oke BO & Akinloye AK (2009). Characterizing the Gonadosomatic Index and its Relationship with Age in Greater Cane Rat (*Thryonomys swinderianus*, Temminck). *Journal of Veterinary Anatomy*, **2**(2): 53-59.

Adebayo AO, Akinloye AK, Olukole SG, Oyeyemi MO, Taiwo VO, Ihunwo AO & Oke BO (2015). Gross, histological and ultra structural features of the bulbourethral gland in the greater cane rat (*Thryonomys swinderianus*). *Anatomia, Histologia Embryologia*, **44** (1): 59-65.

Amann RP (1962). Reproductive capacity of diary bull III: The effect of ejaculation frequency, unilateral vasectomy, and age of spermatogenesis. *American Journal of Anatomy*, **110**(1): 49-67.

Bearden HJ & Fuquay JW (1997). *Applied Animal Reproduction*. Fourth edition. Prentice Hall, New Jersey. Pp 158-169.

Bitto II & Egbunike GN (2006). Seasonal variations in sperm production, gonadal and extragonadal sperm reserves in pubertal West African dwarf bucks in their native tropical environment. *Livestock Research and Rural Development*, **18**(9): 134-134.

Franca LR & Russell LD (1998). *The Testis of Domestic Animals*. In: *Male Reproduction* (J Regadera, F Martinez-Garcia, editors). Churchill Livingstone, Madrid. Pp 197–219.

Hochereau-De Reviers MT, Courot M, Perreau C & Pisselet C (1978). Sertoli cells and development of seminiferous epithelium. *Annales de biologie animale, biochimie, biophysique*, **18** (2B): 573-583.

Johnson L (1991). Seasonal differences in equine spermatogenesis. *Biology of Reproduction*, **44**(2): 284-291.

Machado Júnior AAN, Assis Neto AC, Sousa Júnior A, Menezes DJA, Alves FR, Sousa AL & Carvalho MAM (2011). Daily sperm production and testicular morphometry in goats according to external scrotal conformation. *Animal Reproduction Science*, **127**(1): 73–77.

Machado Júnior AAN, Oliveira LS, Assis Neto AC, Alvesa FR, Miglino MA & Carvalho MAM (2012). Spermatogenesis in goats with and without scrotum bipartition. *Animal Reproduction Science*, **130**(1): 42-50.

Mickelsen WD & Memon MA (2007). Infertility and diseases of the male reproductive organs of bucks. In: *Current Therapy in Large Animal Theriogenology,* Second edition (RS Youngquist, WR Threfall, editors). Saunders Elsevier, Missouri, Columbia, USA. Pp 519-523.

Ogwuegbu SO, Oke BO, Akusu MO & Aire TA (1985). Gonadal and extragonadal sperm reserves of the maradi (Red Sokoto) goat. *Bulletin of Animal Health and Production* **33**: 139-141.

Oke BO, Ugwuegbu SO & Akusu MO (1984). Mophometric studies of the testis of the West African Dwarf goat. *Bulletin of Animal Health and Production* South Africa, **32**:57-

Olurode SA (2002). *Andrological Studies and Pathology of the West African Dwarf Goat*. Unpublished MVSc Theriogenology thesis, Department of Veterinary Surgery and Reproduction, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. Pp 1.

Osinowo EO, Molokwu EC & Osori DC (1981). Growth and testicular development in Bunaji bulls. *Journal of Animal Research*, **16**: 55-67.

Oyeyemi MO**,** Olukole SG & Esan O (2008). Sperm morphological studies of West African Dwarf bucks treated with Pumpkin Plant (Cucurbita pepo). International Journal of Morphology, **26**(1): 121-126.

Oyeyemi MO, Olukole SG, Ajadi TA & Adeniji DA (2011)**.** Semen characteristics and sperm morphological studies of the West African Dwarf Buck treated with *Aloe vera* gel extract. *Iranian Journal of Reproductive Medicine* **9**(2): 83-88.

Pant N & Srivastava SP (2003). Testicular and Spermatotoxic effects of quinaphos in rats. *Journal of Applied Toxicology*, **23**(4): 271-274.

Segatelli RA, William JD &Wilbank JN (2004). Fertility and Infertility in Domestic Animals. *Animal Reproduction*, **1**: 122-128.

Sharpe RM (1984). Intratesticular factors controlling testicular function. *Biology of Reproduction,* **30**(1): 29-49.

Togun VA & Egbunike GN (2006).Seasonal variations in the sperm production characteristics of Zebu (White Fulani) cattle genitalia in the humid tropical environment. *Middle East Journal of Science Research*, **1**(1): 87-95.

Ugwu SOC (2009). Relationship between scrotal circumference, in situ testicular measurements and sperm reserves in the West African dwarf bucks. *African Journal of Biotechnology*, **8**(7): 1354-1357.

Willis BM (2001). Breeding Policies in Developing Regions. In*: Dalton's Introduction to Practical Animal Breeding, Fourth edition*). Oxford, Blackwell Science, Pp 1328.