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Testis and Epididymis of Indigenous Buffalo Bull: A Biometrical and Histomorphometrical Study

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ABSTRACT

Background and Objective: Testicular and epididymal parameters are important parameters utilized in breeding soundness evaluations in animals. The study was designed to examine the testicular and epididymal biometric and histomorphometric parameters of the indigenous buffalo bulls. **Materials and Methods:** Biometric values of testis and epididymis of experimental indigenous buffalo bulls (n = 5) were measured using electric balance and vernier-caliper. Histomorphometric values of the testis and epididymis were studied after staining the formalin-fixed tissue sections with routine hematoxylin and eosin stains. **Results:** The mean length, width and weight of testis were 10.94, 6.32 cm and 178.94 gm and epididymis were 18.88, 1.46 cm and 13.54 gm, respectively. The length and width of epididymis varied in head, body and tail regions. The mean thickness of tunica albuginea was 1503.33 µm and diameter of seminiferous tubules was 211.44 µm. A significant regional difference was observed in epithelial height and epididymal duct diameter among the different gross regions of the epididymis. The epithelial height was the highest in the head and the lowest in the tail region and the reverse was observed in the diameter of epididymal duct. **Conclusion:** Although the gross and histologic structures of testis and epididymis of indigenous buffalo bulls were almost similar to other domestic animals, values for the biometric and histomorphometric parameters were different from other domestic animals.

KEYWORDS

Biometry, histomorphometry, testis, epididymis, indigenous buffalo bull

INTRODUCTION

The buffalo (*Bubalus bubalis*) is a significant animal species that provides a valuable supply of milk and meat as well as superior labor productivity in challenging environments compared to cattle[1]. Its economic significance is mostly correlated with high milk production, which affects the dairy sector[2]. In Bangladesh, the current estimate of the buffalo population is 1.457 million[3]. Due to their great degree of adaptation to tropical and subtropical climates and their ability to live in places unsuitable for cattle and other domestic animals, buffalo species have recently attracted interest in terms of management and reproduction[4].

Understanding the fundamentals of reproductive organ morphometry is essential for evaluating domestic animal fertility potential and breeding soundness[5]. Since morphology and morphometry are the

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cornerstones of clinical practice in mammals, they are significant instruments in anatomical study[6,7]. To anticipate sperm production, storage potentials and the breeder male's ability to fertilize, a morphometric examination of the testes is required. The size of the testes is a reliable predictor of both current and future sperm production[8]. The epididymis, the excurrent duct of the male reproductive system, is grossly divided into three parts: the head (caput), body (corpus) and tail (cauda epididymis), but can be divided histologically into more segments[9]. Following spermiation in the testis, immature spermatozoa pass through the epididymal excurrent duct system and mature, gaining the ability to fertilize oocytes and to move[10,11].

The male reproductive organs of domestic animals have been the subject of several morphometric investigations published in Bangladesh: histomorphometrical observations on the testis of bull[12], ram[13] and buck[14], histomorphometrical studies on the epididymis of indigenous bull[15], the accessory sex glands of the black Bengal buck[16]. The morphometry of the testis and epididymis of domestic indigenous buffalo bulls, however, are poorly studied. In order to provide information that could be useful in the comparative anatomy of the male reproductive organs of domestic animals and, ultimately, an enhanced evaluation of buffalo bulls' reproductive potential and breeding soundness, the study aimed to examine the biometric and histomorphometric values of the testis and epididymis of the indigenous buffalo bulls.

MATERIALS AND METHODS

Animals and experimental design: The testicles and epididymis of native buffalo bulls (*Bubalus bubalis*) were used in this study for biometric and histomorphometric analyses. The study was carried out from March to September, 2023 at University of Rajshahi, Bangladesh. Animal experiments were conducted following the guidelines established by the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee of the University of Rajshahi, Bangladesh. Five adult indigenous buffalo bulls of similar age were selected in different local slaughterhouses at Rajshahi District in Bangladesh. Shortly after the animal was killed, the testicles and epididymis were removed. After that, the left testis and epididymis were preserved in 10% formalin solution for a study on histomorphometry, whereas the right testis and epididymis were employed for a study on biometry.

Biometric evaluation: Using an electric balance, the weight of the testicles and epididymis was determined and recorded in grams. The epididymis is anatomically divided into three anatomical regions, head (caput), body (corpus) and tail (cauda)[9]. Using a vernier-calliper, the length, width and each region of the epididymis were measured and the results were recorded against a centimeter scale.

Histomorphometric evaluation: Samples obtained from the left testis and epididymis, from the head, body and tail regions of epididymis were fixed and processed for histomorphological study. According to Gofur *et al.*[12], sections were cut at a thickness of 5 µm using a sliding microtome (Thermo, Germany) and then stained with standard hematoxylin and eosin stain. The testicular and epididymal stained sections were closely inspected under 10 and 40 magnification using compound microscopes. The thickness of tunica albuginea and diameter of seminiferous tubules of testis, the diameter of epididymal duct and the epithelial height of three different regions of epididymis were measured (at 10×) using a calibrated scale by oculometer (Erma, Japan). The images of the stained tissue sections were taken using a photographic microscope system (Digital camera model: C-B5, OPTIKA, Italy) equipped with a microscope (Model B-293PLi, OPTIKA, Italy).

Statistical analyses: All values were presented as Mean±SE. Differences in biometrical and histomorphometrical values among the epididymal regions were evaluated by One-way Analysis of Variance (ANOVA), followed by Turkey HSD *post-hoc* analysis according to Gofur *et al.*[17]. Significant differences were defined as p-values of 0.05 or less.

RESULTS

Biometry of testis and epididymis of indigenous buffalo bulls: To study the biometry, length, breadth and weight of the testis and epididymis of adult indigenous buffalo bulls were measured. The representative figure of testis and epididymis of indigenous buffalo bulls (*in situ*) is presented in Fig. 1 and their biometrical values (length, breadth and weight) are presented in Table 1. The average length, width and weight of testis were 10.94 cm, 6.32 cm and 178.94 gm and of epididymis were 18.88 cm, 1.46 cm and 13.54 gm, respectively. The length and width of epididymis varied in head, body and tail regions.

Histomorphometry of testis and epididymis of indigenous buffalo bulls: The testis was covered by a connective tissue capsule known as tunica albuginea and the testicular parenchyma consisted of seminiferous tubules scattered throughout the interstitial tissue or stroma (Fig. 2a-b). Most of the tubules were convoluted. Sertoli cells and spermatogenic cells make up the complex stratified epithelium lining the walls of seminiferous tubules, along with the basement membrane and lamina propria. All types of cells of the spermatogenic lineage, including spermatozoa, were found in the seminiferous tubules (Fig. 2c). A tiny number of detached, deteriorated, or apoptotic cells might be seen in the lumen of a few seminiferous tubules. For histomorphometrical study, the tunica albuginea thickness and seminiferous tubule diameter were measured. The average tunica albuginea thickness was 1503.33 μm and the seminiferous tubule diameter was 211.44 μm (Table 2).

The epididymis parenchyma was made up of the ductus epididymides, or epididymal ducts, which were surrounded by a few layers of smooth muscle and lined by pseudostatified epithelium made up of short basal cells and tall columnar principal cells that were dispersed throughout the stroma (Fig. 2d-f). Cilia were observed at the apical surface of epithelium in all three regions. Sperm were observed in the ductal lumen in all three regions (Fig. 2d-f). The height of epithelium and diameter of epididymal duct of different regions of epididymis were measured. A significant regional difference was observed in epithelial height and epididymal duct diameter among the different gross regions of the epididymis, namely: Head, body and tail regions. The height of lining epithelium of epididymal duct was significantly ($p<0.05$) different in each region of epididymis in indigenous buffalo bulls (Table 2 and Fig. 2d-f). The epithelial height was the highest in the head and the lowest in the tail region. The reverse was observed in the diameter of epididymal duct among the epididymal regions. Regarding regional difference, the greatest diameter was always observed in the tail and the smallest in the head ($p<0.05$), whereas body was not different from head (Table 2 and Fig. 2d-f).

Table 1: Biometric values (Mean±SE) of testis and epididymis of adult indigenous buffalo bulls

Organs	Length (cm)	Width (cm)	Weight (gm)
Testis	10.94±0.26	6.32±0.138	178.94±7.79
Epididymis			
Head	4.76±0.11[a]	2.47±0.044[a]	13.54±0.35
Body	9.74±0.18[b]	0.97±0.028[b]	
Tail	4.38±0.12[a]	2.24±0.037[a]	

Significant variances ($p<0.05$) in length and width among different regions of epididymis are indicated by values in a column with different superscripts

Table 2: Histomorphometrical parameters of testis and epididymis of adult indigenous buffalo bulls

Parameters	Thickness of tunica albuginea (μm)	Diameter of seminiferous tubules (μm)
Testis	1503.33±66.82	211.44±13.45
Region	Epithelial height (μm)	Diameter of epididymal duct (μm)
Epididymis		
Head	71.75±4.25[a]	295.2±14.67[a]
Body	47.13±3.51[b]	337.5±20.51[a]
Tail	22.13±1.67[c]	418.3±27.24[b]

Significant variances in ductal diameter ($p<0.05$) and epithelial height ($p<0.01$) among different regions of epididymis are indicated by values in a column with different superscripts

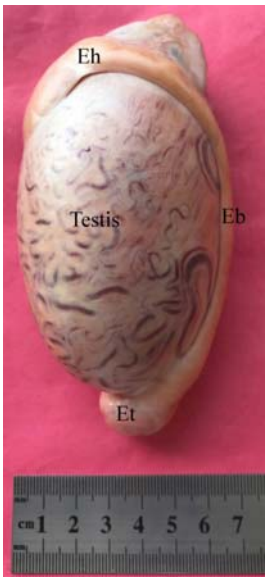

Fig. 1: Macroscopic images of testis and epididymis of adult indigenous buffalo bulls
Eh: Head, Eb: Body and Et: Tail of epididymis

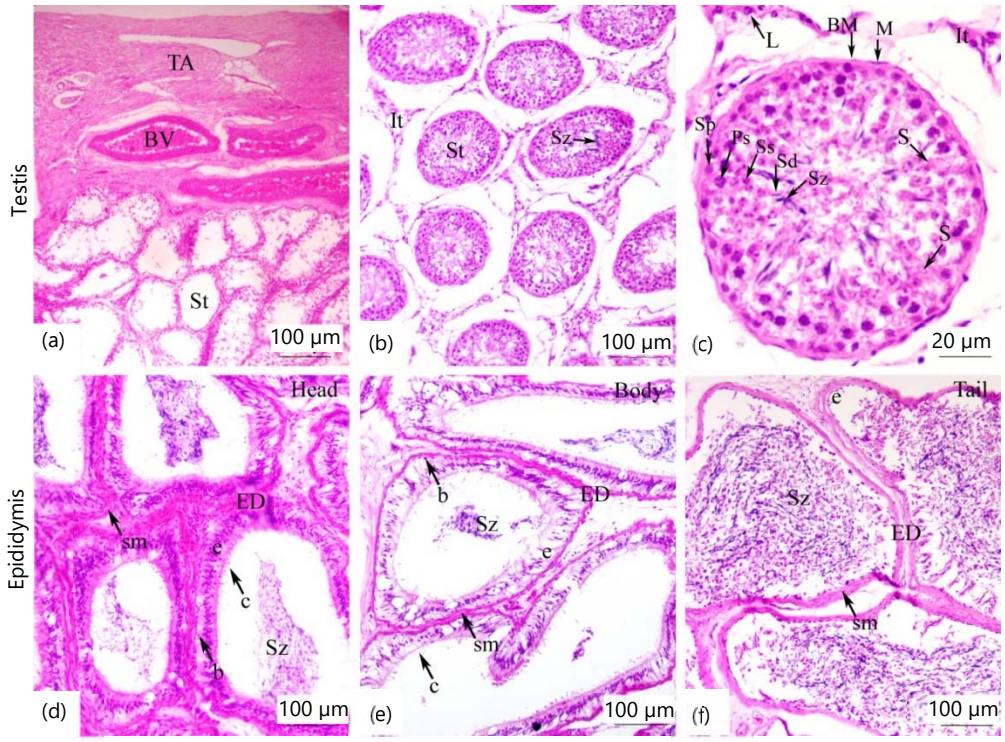

Fig. 2(a-f): Histological images depicting the microscopic structure of testis and epididymis of adult indigenous buffalo bulls
TA: Kunica albuginea, BV: Blood vessel, St: Seminiferous tubules, It: Interstitial tissue, S: Sertoli cell, Sp: Spermatogonia, Ps: Primary spermatocytes, Ss: Secondary spermatocytes, Sd: Spermatids, Sz: Spermatozoa, M: Myoid cell, L: Stromal cells including leydig cells, BM: Basement membrane, ED: Epididymal duct, e: Epithelium, b: Basal cell, c: Cilia and sm: Smooth muscle

DISCUSSION

A buffalo bull's breed, physical confirmations, libido and mating powers have to be considered while choosing one for breeding. Moreover, both external and internal genitalia are examined and its semen quality needs to be taken into consideration. When evaluating a breeding animal andrologically, biometric analyses are crucial measurements. Okwun *et al.*[18] reported that males who have larger testicles typically

generate more sperm. Testicular measurements and the alterations that take place during testicular growth from birth to maturity have been extensively studied in bulls[12], rams[13] and bucks[14]. The testicular weight of indigenous buffalo bulls was 178.94 gm which was almost similar to that of Mediterranean Italian buffaloes (168.99 gm)[2], but much higher than that of Egyptian water buffalo bulls (33.3 gm)[1]. The average thickness of tunica albuginea was 1503.33 µm in indigenous buffalo bulls which is much thicker than that of indigenous bull (950.35 µm)[12], black Bengal buck (288.13 µm)[14] and indigenous ram (312.38 µm)[13] of Bangladesh. The species difference is the basis of the variation in tunica albuginea thickness.

Testicular parenchyma was primarily made up of seminiferous tubules. The wall of tortuous seminiferous tubules consisted of lamina propria, basement membrane and a lining of complex stratified epithelium which consisted of sertoli cells and all cells of spermatogenic linage, similar to other domestic animals[12-14]. A clear species difference in the diameter of seminiferous tubules was observed among domestic mammals. The seminiferous tubules diameter was 211.44 µm in indigenous buffalo bulls which is much larger than that of indigenous bull (191.69 µm)[12], black Bengal buck (190.89 µm)[14] and indigenous ram (176.22 µm)[13] of Bangladesh. Regarding breed difference of buffalo, the diameter of seminiferous tubules of indigenous buffalo bulls is almost similar to that of Mediterranean Italian buffaloes in non-mating season (211.77 µm) but a little lower than that of in mating season (243.19 µm)[2].

The ductus epididymis, a single, long duct that is extremely convoluted and divided into many segments separated by connective tissue septa, is what makes up the epididymis[11,19]. The head (caput), body (corpus) and tail (cauda) are the three anatomical regions that make up the epididymis macroscopically[9]. The epididymal weight of indigenous buffalo bulls was 13.54 gm which is much lower than that of Mediterranean Italian buffaloes (32.26 gm)[2] but a little higher than that of Indian bulls (10.84 gm) reported by Saurabh *et al.*[20]. The variation in epididymal weight is due to breed difference. The epididymis parenchyma was composed of epididymal ducts (ductus epididymides), lined by pseudostratified epithelium consisting of tall columnar principle cells and short basal cells, scattered within stroma and surrounded by few layers of smooth muscle, similar to other domestic animals. Regarding histomorphometry of epididymis, a significant regional difference was observed in epithelial height and epididymal duct diameter among the different regions of the epididymis. The epithelial height was the highest in the head and the lowest in the tail region. The reverse was observed in the diameter of epididymal duct among the epididymal regions. A similar trend in epithelial height and diameter of epididymal duct is also reported in Mediterranean Italian buffaloes though the values are different[2]. Pilutin *et al.*[21] also reported a similar trend in epithelial height and ductal diameter in rat epididymis. Bedford[22] reported that while the tail has low epithelium and a very wide lumen packed with spermatozoa, the head has tall epithelium with long stereocilia, the body is characterized by cytoplasmic vacuoles and mass collections of spermatozoa in the lumen. The observations in the present study were almost similar to the above. Regarding the progressive reduction in the epithelial height of the epididymal duct from the head through body to the tail, the current study confirmed earlier findings in several species[23-25]. The steady decline of the epithelial height downwards the epididymal duct may mechanically facilitate the sperm's transit toward the tail. High epithelium in the head may, however, indicate a more absorptive power of the epithelium in this region. The sperm may be mechanically assisted in their travel toward the tail by this progressive reduction in epithelial height distal wards the epididymal duct. However, high epithelium in the head would suggest that the epithelium of head region has a higher absorptive capacity. According to James *et al.*[26], the head of the epididymis absorbs more than 90% of the fluids that enter the epididymal duct in bulls. Furthermore, Turner[27] reported that, in relation to fluid loss, there is net resorption of sodium ions in rats between the head region of the epididymis and the rete testis. The findings of the present study will help to recognize the developmental anomalies as well as to identify pathologic or toxicological changes in the testis and epididymis of indigenous buffalo. This research only studied the buffaloes just reached puberty. However, it is necessary to study involving different age groups of buffaloes for detailed understanding.

CONCLUSION

Biometric and histomorphometric values of testicular and epididymal parameters of indigenous buffalo bulls are worthwhile indicators when choosing bulls for breeding. Indigenous buffalo bulls have a unique value of testicular and epididymal parameters in biometric and histomorphometric views, though the gross and microscopic architecture was similar to other domestic animals.

SIGNIFICANCE STATEMENT

Understanding the fundamentals of reproductive organ morphology and morphometry of buffalo is essential to evaluate fertility potential and breeding soundness and ultimately to upgrade buffalo production. The study was designed to examine the testicular and epididymal biometric and histomorphometric parameters of indigenous buffalo bulls. Indigenous buffalo bulls of Bangladesh have a unique value of testicular and epididymal parameters in biometric and histomorphometric views, though the gross and microscopic architecture was similar to other domestic animals. A significant regional difference was observed in length and width of epididymis as well as in epithelial height and epididymal duct diameter among the different gross regions of the epididymis. However, it is necessary to study involving different age groups of buffaloes for detailed understanding.

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