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Research Article

MORPHOLOGICAL AND HISTOLOGICAL STUDIES ON THE EPIDIDYMIS OF PRE-PUBERTAL, PUBERTAL AND ADULT INDIGENOUS BULL (*BOS INDICUS*) OF BANGLADESH

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ABSTRACT

The experiment was conducted with an aim to compare the gross and histomorphology of the epididymis of pre-pubertal, pubertal and adult indigenous bulls (*Bos indicus*) of Bangladesh. The tissue samples (testes with epididymes) were collected from nine (09) bulls of three age groups namely pre-pubertal (1.5 to 2 years), pubertal (2 to 3 years) and adult (above 3 years) from the Govt. slaughter house, Sylhet, Bangladesh immediately after slaughter and were grossly examined, measured for weight or length and processed for histology. Grossly, three major segments of the epididymis were revealed namely the head, body and tail. The oval shaped head was connected to the U shaped tail by a narrow slender body of the epididymis. The length and weight of left epididymis was slightly larger than the right epididymis and epididymal tail was the largest of the three segments in all three studied groups. Histological observations revealed that the epididymis was characterized by epididymal ducts separated by connective tissue and surrounded by the peritubular smooth muscle layer. Luminization started from the head of the prepubertal stage. A variety of cell types were observed in the lining epithelium extending into lumen including the principal cells, basal cells and apical cells. The head was characterized by tall pseudo-stratified columnar epithelium with apical cells extending into lumen, but no sterocillia was observed in that part of epididymis. The lining epithelium in the body was low columnar epithelium with short narrow cells and stereocilia. The amount of spermatozoa was comparatively higher in the lumen of the body and tail region respectively. Histomorphometric result showed that there was a gradual reduction in epididymal epithelial height of the tail compared with the head of the right and left epididymis. It was concluded that the studied animals exhibited interesting morphological features of epididymis in three age periods of indigenous bulls of Bangladesh. These basic morphological studies can be important for further studies related to reproductive biology and breeding.

INTRODUCTION

The mammalian epididymis is a highly convoluted tubule between the testis and the ductus deferens which performs a variety of important functions like sperm storage, maturation and transport (Mahmud *et al.,* 2015). When the spermatozoa leave the testis, they pass through the efferent ducts into the epididymis. Along with the passage of spermatozoa through the epididymis a remarkable morphological, physiological and maturational changes of spermatozoa have been recognized (Orgebin, 1967). By secreting important components of semen, the epididymis serves a critical function in preparing the male germ cells for fertilization (Joseph *et al.,* 2009). While leaving the testis the spermatozoa remain as immature, immovable and cannot fertilize an oocyte (Yanagimachi *et al.,* 1985; Flesch and Gadella, 2000), where special proteinaceous compound released from the epididymal epithelium forms a very complex environment surrounding the spermatozoa within the intraluminal compartment (Hermo *et al.,* 1994; Sullivan, 2004). The spermatozoa remain in the epididymal luminal area until ejaculation and prepares for fertilization through the availability of necessary supplements in terms of temperature, oxygen tension, pH and an available energy substrate (Dacheux *et al.,* 2005). Several morphological, biochemical, physiological and functional changes to the structures of the spermatozoa takes place in the epididymal duct, consequently the spermatozoa convert into fertilization-competent cells (Toshimori, 2003; Gatti *et al.,* 2004; Flesch and Gadella, 2000).

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The epididymal epithelium is considered to be composed of diverse cellular populations with wide range of functional capacity allowing sequential changes in the composition of the luminal fluid throughout its length of epididymis and transforming the immature testicular sperm into mature sperm (Cornwall *et al.,* 2007; Sostaric *et al.,* 2008).

The epididymis is considered to be comprised of three main parts namely, head or caput, body or corpus and tail or cauda (Olugbenga and Babalola, 2006). The head and tail are the enlarged portions while the body is the thinner part, found in between head and tail (Zemjanis, 1977). The caudal portion or tail of the epididymal duct acts as reservoir for the sperm, which possesses a wide lumen having a high concentration of spermatozoa (Roberts, 1971; Amann, 1981).

Histological and histochemical studies on the epididymes have been reported in various domestic, laboratory animals and subhuman primates (Nicander, 1957; Hamilton, 1975; Donald and Alan, 1978), though anatomical and histological studies on epididymis of local domestic ruminants are not available, except few references that are available in the textbook on ruminants in general (Dyce *et al.,* 2002). Ruminants play a vital role in the national economy of Bangladesh, although literature about their reproductive biology is remarkably scarce. There is practically no study regarding histology of epididymis of bull at different ages. For implementing appropriate reproductive management, understanding basic anatomy and reproductive physiology of epididymis is a must. Therefore present study on the gross histology of the epididymis in the indigenous bull at different ages was carried out. The information will provide the basis for future research on influence of endogenous and exogenous factors of this important part of reproductive tract.

Experimental Section

The experiment was conducted in the laboratory of the Department of Anatomy and Histology, Sylhet Agricultural University, Bangladesh. The tissue samples (testes with epididymes) of indigenous bulls of Bangladesh were collected aseptically from the Govt. slaughter house, Sylhet, Bangladesh immediately after slaughter. Samples were collected from nine (09) bulls of three age groups namely prepubertal (1.5 to 2 years, n=3), pubertal (2 to 3 years, n=3) and adult (above 3 years, n=3). The age was determined based on tooth eruption patterns (Getty, 1975). The epididymis was then separated from the testis. After washing with normal saline, the tissue pieces from the epididymes were fixed in Bouin's fluid for the histological examinations.

Following Bouins fixation the epididymal tissue was dehydrated submerging in progressively higher concentrated alcohol (70%, 80%, 90%, 95%, 100%) keeping 3 hours in each grade. The dehydrated tissue samples were then cleared using two changes of xylene and infiltrated through 2 changes of liquid paraffin in the micro oven taking 2 hours in each step. When the tissues were dehydrated, cleared and infiltrated, they were placed in liquid paraffin for embedding, which were then allowed for hardening. This was achieved by cooling in the room temperature. The hardened tissue blocks were sectioned serially at 5 µm thickness and the sections were stained with the Haemotoxylene and Eosin (H&E) Stain after placing in permanent slides.

Tissues were stained with Hematoxylin and Eosin (Gridly, 1960) following deparaffinization through two changes of xylene and hydration through retrograde alcohol series. After staining the tissue was dehydrated by transferring through baths of progressively higher concentrated alcohol and passed through xylene and was mounted in Canada balsam under a cover slip. Each section was examined under light microscope to study the histological characteristics of epididymis region of testis.

Briefly, the total diameter of the epididymal duct, the luminal diameter, the epithelial height and the muscular thickness were taken using the ocular and stage micrometer and the obtained data were expressed as Mean ± SEM (Standard Error of Mean) and subjected to statistical analysis. One-Way Analysis Of Variance (ANOVA) at 95% confidence interval (CI) was used to determine level of significant difference in mean values of the data. Values of (P=0.05) were considered significant.

RESULTS AND DISCUSSION

The epididymes of the indigenous bull of Bangladesh are shown on figure 1 as an elongated and convoluted tube lying firmly attached lateral to the testis. The convoluted tube projects from the proximal border to the distal border of the testis and was macroscopically divided into three main segments; caput (head), corpus (body) and cauda (tail). The proximal head was irregularly flat and oval shaped was connected with the short U shaped distally located tail by a long slender and cylindrical body of the epididymis. These were similar to the general patterns as described earlier observations in mammals (Nickel *et al.,* 1979), African Dwarf Buck (Raji *et al.,* 2015), in goat (Sharma *et al.,* 2014), in African giant rat (Oke, 1988), and in rabbits (Holtz, 1972).

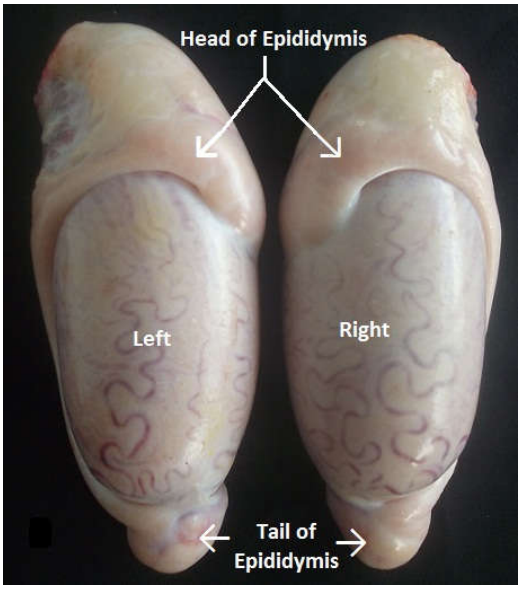

Figure 1 Testis and epididymes of the indigenous bull of Bangladesh

The mean weight and length of the right and left epididymis were presented in table 1. The weights and lengths of the left and right epididymis of indigenous bulls were unequal and the left one was heavier than the right, and interestingly the values of weight and length increased from pre-pubertal to adult gradually. There was similarity in the findings of Adhikary *et al.,* (2015) in indigenous bull regarding the weight, length and

volume of testis and epididymis, whereas in African Dwarf Buck (Raji *et al.,* 2015), the right epididymal structure were heavier than the left one, although, Bitto and Egbunike (2006) and Ugwu (2009) reported close values for the weight of epididymis in African Dwarf Buck.

Table 1 Weight and length (Mean±SE) of left and right epididymis in pre-pubertal, pubertal and adult indigenous bulls of Bangladesh

Animals	Weight (gm)		Length (cm)	
	Left	Right	Left	Right
Pre-pubertal	22.38±0.22	21.92±0.19	11.97±0.17	11.42±0.19
Pubertal	26.89±0.34	25.17±0.33	12.32±0.25	11.79±0.23
Adult	31.97±0.19	30.09±0.11	13.98±0.33	13.06±0.22

The mean weight of the epididymal of indigenous bull obtained in this study was close to the findings of Ibrahim *et al*., (2012) in Nigerian Uda ram and whereas Ahemen *et al.,* (2007) reported lower mean weights of epididymis in West African dwarf ram. The difference may account for the genetic build up. The West African dwarf ram is known as the smallest of indigenous sheep breed in Nigeria Osinowo *et al.,* (1990).

Histological observations revealed that all three segments of the epididymis were characterized by epididymal ducts separated by connective tissue and surrounded by the peritubular smooth muscle layer. Histologically, the epididymes was lined by epithelium resting on basement membrane, followed by submucosa containing interstitium, circular and longitudinal muscle layer and finally the outermost serosa. A variety of cell types were observed in the lining epithelium including the principal cells, basal cells and apical cells. Centrally the lumen was observed filled with the sperms (Figure 2). The principal cells were pseudostratified columnar epithelium with steriocillia extending from the basal lamina to the lumen (Figure 2 a,b,c). Their nuclei were oval to elongate in shape and were positioned within the basal half of the cell. Triangular and flat basal cells having elongated or round shaped nuclei were found in the base of the epithelium in close association with the principal cells. At the luminal border, apical cells were seen with few microvilli and apically located nucleus. While studying the comparative histology of epididymis of prepubertal, pubertal and adult indigenous bulls of Bangladesh a distinct variation was noticed in the sections of head, body and tail of epididymis.

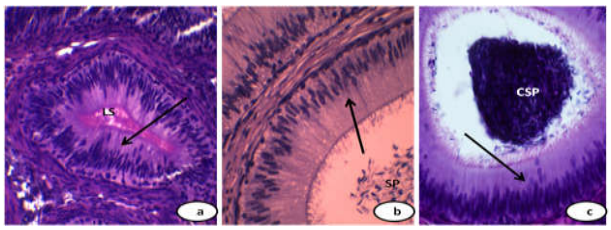

Figure 2 (abc): The head of epididymis showing a variety of cell types like pseudostratified ciliated epithelium (PSE) arrowhead with elongated Nucleus, basal cell, luminization started (LS) in the prepubertal (a), spermatozoa (SP) in pubertal (b) and condensed spermatozoa (CSP) in the lumen of adult (c) bull. HE 40X

In prepubertal stage, the lining epithelium of the epididymis was found as pseudostratified columnar epithelium. The epithelium in the head of the epididymis showed having elongated nucleus and elongated apical cells. In body and tail region the nucleus of the columnar epithelium and apical cells were less elongated when compared to the head. Basal cells were found oval or somewhat rounded.

Luminization started from the head of the prepubertal stage, but no sterocillia was observed in that part of epididymis (Figure 2a). Lumen in the body and tail was found slightly developed than the head and steriocillia started to develop. Throughout the head, body and tail no spermatozoa was seen in the lumen during the prepubertal stage (Figure 3a). The epithelial layer was found thickest and connective tissue layer and smooth muscle layer were found thinnest in the head of the epididymis. Gradually the thickness of the epithelium diminishes along the body and tail and the thickness of the smooth muscle layer increases over the course of the epididymis from head to body to tail (Figure 4a).

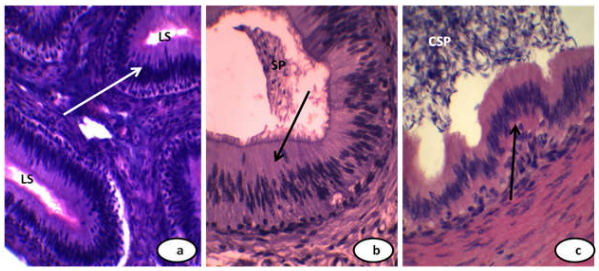

Figure 3 The body of epididymis showing pseudostratified ciliated epithelium(PSE) arrowhead with elongated nucleus, luminization started (LS) in the prepubertal (a), spermatozoa(SP) in pubertal (b) and condensed spermatozoa (CSP) in the lumen of adult(c) bull. HE 40X

In pubertal stage, the columnar epithelium cells with nuclei and apical cells were found comperatively shorter than the prepubertal stage. Basal cells were found rounded to flattened (Figure 2b). There was well developed lumen with the presence of sterocilia and spermatozoa. The epithelial layer was comperatively less thicker and connective tissue and smooth muscle layer were found comperatively thicker than the prepubertal stage in the body and tail of epididymis (Figure 3b and 4b).

In adult, the columnar epithelium cells with nuclei and the apical cells were also found comperatively shorter than the pubertal stage. Basal cells were found more rounded and flattened than the pubertal stage. There was well developed lumen that reveals exteded sterocilia and condensed spermatozoa. Sterocilia formed brush border in this stage (Figure 2c). The epithelium layer was found comperatively thinner and connective tissue and smooth muscle layer were found comperatively thicker than the pubertal stage. In the adult bulls, of the spermatozoa was more condensely found in the tail of the epididymis (Figure 4c).

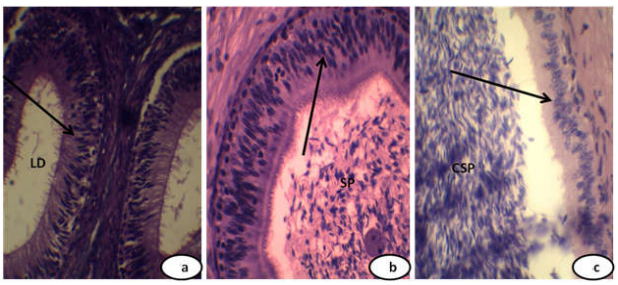

Figure 4 (abc): The tail of epididymis showing pseudostratified ciliated epithelium (PSE) arrowhead with elongated nucleus, luminization developing (LD) in the prepubertal (a), Spermatozoa (SP) in pubertal (b) and condensed spermatozoa (CSP) in the lumen of adult (c) bull. HE 40X

The histological features were similar to previous reports on the epididymis in goat (Sharma *et al.,* 2014), in greater cane rat (Adebayo and Olurode, 2010), in Northern Great Grey Kangaroo (Khamas *et al.,* 2014); and in West African Dwarf Buck (Raji *et al.,* 2015).

The different histomorphometric results (Mean±SE) of the epididymis (µm) of pre-pubertal, pubertal and adult indigenous bulls of Bangladesh were presented in table 2. There were remarkable differences in the mean of luminal diameter and total diameter of epididymal duct and in the epididymal epithelial height and muscle thickness among the pre-pubertal, pubertal and adult indigenous bulls of Bangladesh. The luminal diameter gradually increased over age and maximum diameter was recorded in the tail region of epididymis. On the other hand the lining epithelial height was more during pre-pubertal period and over the time the height gradually decreased. Among the three segments the epithelium in head region were found as the tallest.

Table 2 Histometric parameters (Mean±SE) of epididymis (µm) of pre-pubertal, pubertal and adult indigenous bulls of Bangladesh

Component/ Region		Pre-pubertal	Pubertal	Adult
Total diameter	Head	524.29±6.76c	752.13±6.72 b	803.54±2.89 a
	Body	459.93±7.67 c	695.83±5.84 b	754.37±9.76 a
	Tail	779.13±4.12 c	911.48±6.54 b	998.30±4.39 a
Lumen diameter	Head	212.75±1.89 c	480.39±4.91 b	553.86±7.94 a
	Body	223.77±1.61 c	403.15±2.92 b	500.04±3.29 a
	Tail	387.12±5.77 c	705.46±5.54 b	738.01±7.89 a
Epithelial height	Head	243.11±3.28 c	112.89±1.35 b	98.69±5.25 a
	Body	168.12±3.37 c	104.60±4.46 b	74.35±6.15 a
	Tail	119.93±2.52 c	99.87±5.53 b	67.38±3.12 a
Muscle thickness	Head	26.66±1.99 c	36.59±1.49 b	56.60±1.96 a
	Body	28.60±1.37 c	36.64±1.47 b	62.63±3.06 a
	Tail	27.41±1.72 c	34.84±1.71 b	57.66±2.13 a

[a, b, c] Means within the same row without the same superscript letters are significantly different (p=0.05) from each other.

There was paucity of available related studies and so adequate comparison on histometry of epididymis was hindered. However, some histomorphometric results obtained in this experiment were higher than previously recorded values in Malabari goats (Harshan *et al.,* 1978) and camel (Tingari and Moniem, 1979). The mean values of epididymal luminal diameter, total diameter and epithelial height recorded in this study were more or less similar in the head and body region of the epididymis of West African dwarf buck, but the mean values for the tail region of the epididymis of West African dwarf buck were recorded having more values (Raji *et al.,* 2015). The mean values of epididymal luminal diameter, total diameter and epithelial height were recorded smaller in Northern Great Grey Kangaroo (Khamas *et al.,* 2014); and in African sideneck turtle (Olukole *et al.,* 2014).

It was concluded that the studied animals exhibited interesting morphological features of epididymis in three age periods of indigenous bulls of Bangladesh. The basic morphological studies done in this experiment can be important for further studies related to reproductive biology and breeding.

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