

Biometrical and histological studies on the epididymis of ram in chars of Pabna region

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Abstract

Objectives: The objectives of this research were to study gross anatomy including length, weight, size, shape, volume and circumference; and the microscopic structures of the epididymis with the special emphasis on the biometrical comparison between left and right epididymis.

Materials and Methods: For this purpose, epididymes of six adult rams were used and biometrical values were recorded. For microscopic studies, tissue sample were excised from the epididymis, fixed in 10% formalin solution and embedded in paraffin. Cross sections (6 μ m thickness) were stained with hematoxylin and eosin and evaluated the histological structures of the epididymal caput, corpus and cauda. Statistical analysis of the data was done by paired sample t-test using SPSS 16.0 programme.

Result: The mean values of length, weight, volume and circumference along with of the left epididymis were 19.58 \pm 0.18 cm, 11.65 \pm 0.17 gm, 13.65 \pm 0.01 ml and 14.27 \pm 0.11 cm and for right epididymis 19.53 \pm 0.15 cm, 11.41 \pm 0.13 gm, 13.65 \pm 0.00 ml and 14.22 \pm 0.07 cm, respectively. The mean value of caput weight, corpus weight and cauda weight for left epididymis were 4.05 \pm 0.06 gm, 1.61 \pm 0.02 gm and 5.99 \pm 0.09 gm, for right epididymis 3.97 \pm 0.05 gm, 1.58 \pm 0.02 gm and 5.87 \pm 0.07 gm respectively. The mean value of caput length, corpus length and cauda length for left epididymis were 9.35 \pm 0.171 cm, 8.450 \pm 0.096 cm and 1.783 \pm 0.069 cm, for right epididymis 9.35 \pm 0.171 cm, 8.417 \pm 0.09 cm and 1.767 \pm 0.047 cm, respectively. The ductus epididymis is lined with a pseudostratified stereociliated columnar epithelium surrounded by a small amount of loose connective tissue and circular smooth muscle fibers. Few sperm were found in the initial segment, but a large mass of sperm aggregates in the cauda.

Conclusion: Grossly the length, weight, volume and circumference of the left epididymis is higher than that of the right and histologically the ductus epididymis is lined with a pseudostratified stereociliated columnar epithelium surrounded by a small amount of loose connective tissue and circular smooth muscle fibers and a large mass of sperm aggregates in the cauda of the epididymis.

Keywords: Epididymis, Biometrical, Ram, Histology.

Introduction

The reproductive performance depends upon the normal structure and functions of the genital organs. The male reproductive system consists of testicles, which produce sperm and sex hormones, a duct system for sperm transport, accessory sex glands and the penis or male organ of copulation, which deposits semen in the female (Abebe, 2013). Though the sperm produce in testis but a duct leading from the testes, the epididymis serves to transport spermatozoa. In sexually active males the time involved in transport is, 13 to 15 days in rams, 9 to 11 days in bulls and 9 to 14 days in boars. Frequent ejaculation has been reported to speed transport by 10 to 20% (Hatem Atalla, 2010). This is highly coiled and tortuous so that in multiple cross sectional profiles of the same tube in a histological section are observed. The duct can be divided into three regions: the caput or head, the corpus or body and the cauda or the tail (Kwan, 2012). The ductus epididymis is lined by a pseudostratified epithelium, surrounded by a small amount of loose connective tissue and circular smooth muscle fibers, the number of which increases significantly toward the tail of the epididymis. Despite the epididymis are the important organs of male genital system, no comprehensive study has yet been undertaken in the ram of Bangladesh. The present study, therefore, was designed to determine the biometrical measurement and the histology, with the special emphasis on the cauda of the ram epididymis. This information of the present research will provide an insight into the understanding of existing male reproductive tract of our local ram population and will also draw special attention of the researchers. Therefore,

the present research work was undertaken for the following objectives:

1. To study the gross anatomy of the epididymis of rams.
2. To compare the gross anatomy between the left and right epididymis of rams.
3. To study the histology of the epididymis of rams.

Materials and Methods

The research work was carried out on epididymis both left and right of six rams for the gross and microscopic study, in the Department of Anatomy and Histology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur in the month of July to December, 2013.

Sample collection

Six adult (n=6), sexually mature rams, aged from 14 to 18 months were selected in different chars of Pabna district, then the reproductive system of rams were collected through local butchers before the collection of epididymis. The age of each ram was 14 months (n=2; 18 kg, 18.5 kg), 16 months (n=2; 19 kg, 19.3 kg), 18 months (n=2; 20 kg, 19.6 kg) assessed by observing the pattern of teeth eruption (Rahman *et al.*, 2004) and by the history of birth of ram kid. All the experimental animals were examined general health status and the appearance of genitalia. The testes and epididymis were palpated and observed to their size, shape, free movement and position of the scrotum. The skin was observed for any kind of lesions.

After the physical examination the ram, the scrotal circumference was measured and then slaughtered the ram. The spermatic cord, vas deference and testis were dissected

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from the main part of epididymis to determine the biometrical values.

Gross anatomical study

The caput (head), corpus (body) and cauda (tail) three part of the epididymis was detected and marks. The circumference of epididymis along with testes was measured by encircling a graduated. The length of the left and right epididymis was measured along their long axis from head to tail at the maximum distance by a graduated. The epididymal volume was measured volumetrically using the Archimedes principles of water displacement in a measuring cylinder and result recorded.

The weights were measured by electric balance. The and epididymis was separated free of adhering connective tissues and fats. The left and right testes and epididymis were measured separately and their weight recorded. The epididymis was then divided into caput, corpus and cauda segment and all the segments were measured for weight.

Histological study

For histological study the samples were undergone through some process for permanent slide preparation. Immediately after the biometrical study, epididymis tissue was dissected out into small pieces for histological study. Tissues pieces were taken from three part caput, corpus and cauda of the epididymis. The retrieved tissue pieces were transferred from neutral buffered formalin solution to Bouin's fluid and kept for fixation for 24 hours. The fixed tissues were then dehydrated by transferring through submerging progressively more concentrated ethanol (70%, 80%, 90%, 95% and 100% absolute). The tissue was kept for 1hour in each grade of ethanol, and then transferred in a hydrophobic clearing agent, xylene in two changes to remove the ethanol. In both case, tissue were kept for 90 minutes. The tissues were then infiltrated with two changes in liquid paraffin in the oven for 2 hours. When the tissues were dehydrated, cleared and infiltrated, they were placed in liquid paraffin for embedding, which were then allowed for hardening. The hardened block containing the tissue sample were the sectioned at 6 μ m thickness using microtome (Mlcrom GmbH, type HM 325, Germany) and the sections were floated in a water bath at 45 $^{\circ}$ c. Then the sections were taken on cleaned slides using egg albumin and dried in slide warmer. The sectioned tissues were deparaffinized by transferring them through xylene for two changes; 15minutes in each case. Then these were transferred through descending grade of alcohol (100%, 95%, 90%, 80%, and 70%). Tissues were stained with hematoxylin and eosin (Gridly, 1960). The tissues then were dehydrated by transferring through baths of progressively more concentrated ethanol (70%, 80%, 90%, 95% and 100%) and passed through xylene. After the staining, cover slips were placed on the slide and mounted with Canada balsam.

Microscopic observation

The stained tissue sections of the epididymis of ram were studied under compound binocular microscope (Abbota Corporation, USA). The evaluation included tubular diameter and examining tubular morphology and the germ cell type present in the lumen of the section.

Photography

For histological study of the epididymis, images were captured from random areas. Photographs from the selected specimens were prepared and placed for better illustrations of the results.

Statistical analysis

The data recording the study of the left and right epididymis were tabulated and subjected to analysis. Statistical analysis of the data was done by paired sample *t*-test using SPSS 16.0 programme. The length, volume, weight and circumference between the left and right epididymis were compared by *t*-test.

Results and Discussion

Gross anatomy of the epididymis of ram

Topographic location of the epididymis of ram

Epididymis, the first external duct leading from the testis, was fused longitudinally to the surface of the testis and is encased in the tunica vaginalis with the testis. The single convoluted duct was covered with an extension of the tunica albuginea of testis. This finding was similar to that of Getty (1975), Islam (2001), Hafez (2000) and Mc Donald (1989) in ruminants.

Circumference of scrotum of ram

In the present study, the mean circumference in rams for the scrotum, left and right epididymis along with was 24.12 \pm 0.15 cm, 14.27 \pm 0.11 cm and 14.22 \pm 0.07 cm, respectively (Table 1). Siddiqui *et al.* (2005) found the circumference of adult ram as 13.45 \pm 2.95 cm for both left and right testis which is smaller than those in the present study. The scrotal circumference is remarkable higher in Balami ram that this value is 31.25 \pm 3.18 cm, in Uda ram it is 38.00 \pm 0.00 cm and in Yankasa ram it is 35.25 \pm 1.77 cm measured by Ibrahim *et al.* (2012).

Shape of the epididymis of ram

The head is curves over the dorsal extremity and about a third of the way down the cranial border of the testicle. The body of the epididymis was a single; flatten duct extending along the longitudinal axis of the testis which becomes continuous with the cauda (tail). The tail is the continuation of body duct was highly coiled and tortuous.

Weight of the epididymis of ram

In the present study, the weight of the left and right epididymes was unequal and the left epididymis was significantly heavier in weight than the right one. The left epididymis was 11.65 \pm 0.17 gm and the right epididymis was 11.41 \pm 0.13 gm. The mean paired epididymal weight 23.06 \pm 0.30 gm was heavier than the 19.12 \pm 0.21 gm reported by Ahemen and Bitto (2007) for West African dwarf rams. This similarity may be attributed to genotype, as the WAD ram had been described as the smallest breed of indigenous sheep in Nigeria which is similar to the indigenous sheep breed in Bangladesh. However, study values were generally lower than the values reported by Besta (2006) as epididymal weight 33.85 \pm 2.15 gm in Doper rams of South Africa and the value 36.80 \pm 0.47 gm in Balami ram, 51.30 \pm 0.56 gm in Uda ram and 30.86 \pm 7.07 gm in Yankasa ram reported by Ibrahim *et al.* (2012). This differences may be attributed to genotype, as the WAD ram had been described as the smallest breed of indigenous sheep in Nigeria (Osinowo, 1990), while Balami and Uda are believed to be the larger breeds. Similarly, the values for the Doper rams suggest that the breeds are males of larger genotypes found in South Africa. The mean value of caput weight, corpus weight and cauda weight for left epididymis were 4.05 \pm 0.06 gm, 1.61 \pm 0.02 gm and 5.99 \pm 0.09 gm, for right

epididymis 3.97 ± 0.05 gm, 1.58 ± 0.02 gm and 5.87 ± 0.07 gm, respectively (Table 1).

Size of the epididymis of ram

The left epididymal size of ram was slightly longer than the right one in the present study. The mean length in rams for the left epididymis was 19.58 ± 0.18 cm and for the right epididymis these values were 19.53 ± 0.15 cm. The mean value of caput length, corpus length and cauda length for left epididymis were 9.35 ± 0.171 cm, 8.450 ± 0.096 cm and 1.783 ± 0.069 cm, for right epididymis 9.35 ± 0.171 cm, 8.417 ± 0.09 cm and 1.767 ± 0.047 cm, respectively. The variation between the length of left and right epididymis, the left one was significantly longer than the right one. The mean of left and right epididymal length 19.56 ± 0.16 cm is slightly smaller than the mean epididymal length of 22.12 ± 3.36 cm in Balami ram, but it is highly smaller than Yankasa ram is 31.37 ± 0.18 cm and Uda ram where the mean epididymal length is 26.00 ± 0.71 cm measured by Ibrahim *et al.* (2012). The mean paired epididymal length was 39.12 ± 0.32 cm (Table 1). These variations in the lengths of the epididymes were probably due to the breed and species variation.

Volume of the epididymis of ram

The epididymal volume of ram was very slightly difference between left and right epididymis. The mean epididymal volume of left and right is 13.65 ± 0.01 ml and 13.65 ± 0.00 ml respectively measured in the present study. The volume of the left epididymis was significantly larger than the right one. The epididymal volume is slightly lower than the 15.86 ± 5.46 ml in Balami ram, but the value is very smaller than the 18.50 ± 2.12 ml in Yankasa ram and 25.50 ± 1.41 ml in Uda ram reported by Ibrahim *et al.* (2012). The paired epididymal volume is 27.29 ± 0.01 ml and the mean volume of left and right epididymis is 13.65 ± 0.01 ml (Table 1).

Histology of the epididymis of ram

The ductus epididymis is lined with a pseudostratified stereociliated columnar epithelium surrounded by a small amount of loose connective tissue and circular smooth muscle fibers. There are layers of smooth muscles outside the epithelium. In the head region, the layer is circumferential (Figure 2) which was support the findings of Kwan (2012). The thickness of the epididymal epithelium varied with the thickest portion in the proximal caput and the thinnest in the caudal region. Conversely, the luminal diameter and the thickness of the peritubular smooth muscle increases from the proximal to the distal regions (Figure 2, 3 and 4). Few sperms were found in the initial segment, but a large mass of sperms aggregates were located in the cauda (Figure 4). This observation is similar to Wrobel and Dellmann (1992), Kwan (2012) and the report of www.wikipedia, the free encyclopedia (2013). Within the interstitial spaces found the loose connective tissue.

Table 1. Measurement of parameters for the gross study of epididymis of Ram: (n=6), mean (\pm s.d.) length, weight, volume, circumference

Epididymis	Length	Weight	Volume	Circumference
Left	19.58 ± 0.18	11.65 ± 0.17	13.65 ± 0.01	14.27 ± 0.11
Right	19.53 ± 0.15	11.41 ± 0.13	13.65 ± 0.00	14.22 ± 0.07

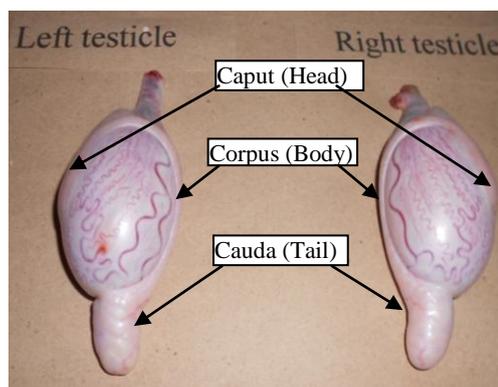


Figure 1. Left and right testis and epididymis of ram.

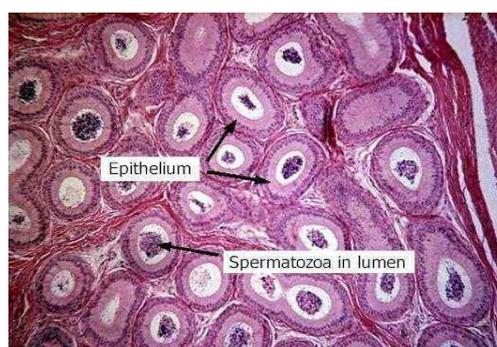


Figure 2. Caput (Head) of Epididymis of ram (10x, H&E stain).

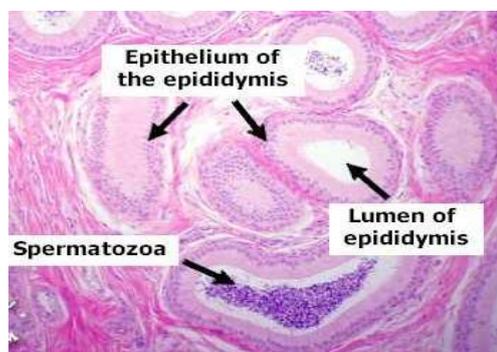


Figure 3. Corpus (Body) of Epididymis of ram (10x, H&E stain)

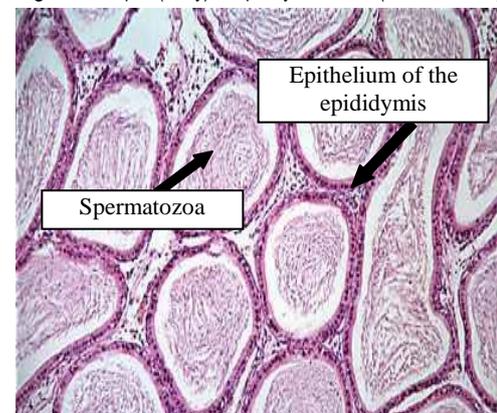


Figure 4. Cauda (Tail) of Epididymis of ram (10x, H&E stain)

Conclusions

The weight of the left and right was unequal and the left epididymis was heavier in weight than the right one. The left

epididymal was slightly larger in volume than right one. The ductus epididymis is lined with a pseudo stratified stereociliated columnar epithelium surrounded by a small amount of loose connective tissue and circular smooth muscle fibers. There are layers of smooth muscles outside the epithelium. The thickness of the epididymal epithelium varies with the thickest portion in the proximal caput and the thinnest in the caudal region. Conversely, the luminal diameter and the thickness of the peritubular smooth muscle increases from the proximal to the distal regions. Few sperm are found in the initial segment, but a large mass of sperm aggregates are located in the cauda. Within the interstitial spaces found the loose connective tissue.

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