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Histomorphometric study of seminiferous tubules and epididymis in adult ram

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Abstract

This research set out to examine the epididymis and seminiferous tubules of adult male Rams, use both histological and histomorphometric methods. For this purpose, ten paired of testis of healthy adult local rams were taken from local abattoir in Fallujah. Tissue was removed and cut into tiny pieces from different parts of the testicles before being put in Bouin's solution. The tissue samples were processed by common paraffin technique then stained with routine H & E stain as well as Masson's trichrome stain. According to the results of the present histological analysis, the ram's testis is enclosed by a capsule of thick irregular connective tissue, and its lobules are separated by trabecular and interlobular septa, with seminiferous tubules and interstitial tissue making up each lobule. Spermatogenic cells and Sertoli cells border the seminiferous tubules. Spermatozoa develop from spermatogenic cells. Spermatogonia are the earliest stages of spermatogenic cells; they are tiny, spherical cells with black, spherical nuclei that sit on the basement membrane. Primary spermatocytes, which are bigger cells with often-distinct chromatin, are generated during mitosis in the spermotogonia. Because of their rapid second meiotic division and subsequent formation of haploid spermatids, secondary spermatocytes are seldom seen. Clustered near the seminiferous tubule's lumen, the spermatids are spherical cells with pale nuclei. There are less Sertoli cells (sustentacular cells) than spermatogenic cells. The nucleus is oval or triangular in shape and pale in colour, with a large nucleolus. Leydig cells are found in the connective tissue between neighbouring tubules. Epithelium that is pseudostratified lines the epididymis. The stereocilia are long cytoplasmic structures that extend into the lumen. Circular smooth muscle is present in varying amounts, and the epithelium is supported by a connective tissue lamina propria.

In conclusion, the current study concluded that the thickness of the epithelium of epididymis was decrease toward the tail while the thickness of the smooth muscle layer was increase toward the tail.

Keywords: seminiferous tubules, spermatogenic cells, epididymis, ram, histomorphometric

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I. INTRODUCTION

The testicles are tubular organs that have both endocrine and cytogenic functions (Hafez & Hafez, 2000). These functions are necessary for the production of spermatozoa and testosterone. Most animals, including stallions, rams, bulls, llamas, vicuna, deer, and boar, have testicles that are complicated tubular structures surrounded by a thick capsule of dense irregular connective tissue called the tunica albuginea. White, reticular, and elastic fibres, as well as fibroblasts and a small number of blood vessels, are abundant in this capsule.

Mesothelium and a connective tissue layer underneath it formed the tunica vaginalis, which subsequently fused with tunica albuginea (Rodrguez et al.,1999; Kuwar et al., 2006; Odabaş and Kanter, 2008; Bacha and Bacha, 2012; Mahmud et al., 2015). The boar testicular capsule has three layers: the outside instinctual tunica vaginalis, the middle tunica albugenia, and the inner tunica vasculosa (Reddy et al., 2016).

White and reticular fibres, myoid cells, various phases of spermatogenic cells, and Sertoli cells all made up the exterior lamina propria of the seminiferous tubule in domestic animals (Ahmed and Sinowatz, 2005). Histology of testes is important for understanding the problems of infertility and sex development and growth. Studies had indicated that testicular dysfunctions are associated with histological variations (Sailaja and Vasanthi, 2016). Fewer tall columnar cells with irregular and visible nuclei radiating from the basal laminae to the seminiferous tubule lumen were characteristic of Sertoli cells, which were otherwise easy to identify. Since tubules stretch all the way through the seminiferous epithelium, Sertoli cells provide a structural framework for them. Regularly dividing and differentiating into sperm-forming cells. Primordial germ cells, which originate in the yolk sac and eventually colonise the gonadal ridges, are responsible for this process. Layers of spermatogenic cells form between neighbouring Sertoli cells, however these layers are not well characterised (Gofur et al., 2008).

Large, polygonal, eosinophilic, and generally containing lipid droplets, Leydig cells (interstitial cells) are common. In addition to the rod-shaped crystals of Reinke that are characteristic of this cell type's cytoplasm, lipofuscin pigment is typically seen in these cells as well (Pawlina and Ross, 2018). The dynamic mammalian epididymis relies on androgens produced in the testes to keep its epithelium in a differentiated condition. Including the lengthy, coiled ductus epididymidis, there are anywhere from eight to twenty-five ductuli efferentes. The epididymis may be visually broken down into a "head," "body," and "tail." It is protected by the visceral layer of tunica vaginalis and the thick tunica albuginea of dense irregular connective tissue. Stallions' tunica albuginea is mostly thick connective tissue with a smattering of smooth muscle cells. There is a pseudostratified columnar epithelium lining the ductus epididymidis, some slack connective tissue, and circular smooth muscle fibres, with the latter increasing in density as one moves towards the epididymis's tail. The epididymal epithelium's height and the muscular layer's innervation density show yearly variation in seasonal breeders like the camel (Eurell and Frappier, 2013). This research set out to examine the testes and epididymis of adult Rams, focusing specifically on their histological characteristics and histomorphometric measurements.

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II. MATERIALS AND METHODS

Ten pairs of testicles from mature, locally-reared rams were used in the study. All of the samples were collected just after the animals were killed at the Fallujah slaughterhouse. Tissue was removed from the testicles and cut into small pieces before being preserved in Bouin's solution for a week at room temperature. After fixation, washing in tab water, dehydration in a graded series of alcohol, clearing in xylene, infiltration, embedding, and sectioning at 5 m thickness using a sliding microtome, and finally mounting on slides, the tissue samples underwent standard paraffin processing (Weiss et al., 2010). H & E as well as Masson's trichrome stains were used for regular preparation of sections for histological investigation.

Histomorphometric measurements:

We used a light microscope (Kruss- Germany) and an adapter digital camera (Optica - Italy "4083.B5") to perform histomorphometry on micro-photographs acquired from our slides. A Photographic analysis was performed by Optica view 7 image analysis. This is progressing micro-imaging software program for image processing and estimation that permits you to seamlessly achieve, process, and measure images, to create valuable data and reports. The digital camera was calibrated on the four objective lens of the light microscope (4x, 10x, 40x and 100x) by using a stage micrometer The image processing program developed according to (Ballesteros *et al.*,2012).

Statistical analysis

All data presented as mean ± standard deviation. Using SPSS version 20, and setting the significance threshold to ($P > 0.05$), we calculated the statistical significance of the mean differences.

III. RESULTS AND DISCUSSION

The histomorphometric measurement of the diameter of seminiferous tubules, lumen of seminiferous tubules, spermatogonia, primary spermatocytes and Sertoli cells were recorded in table 1.

The histomorphometric measurement of the thickness of the epithelium of the epididymis showed that there were a significant differences between the head, body and tail of the epididymis as we mentioned in table 2, while, Table 2 shows no statistically significant variations in epididymal diameter among the three sections.

A capsule of thick irregular connective tissue contained a vascular bed that supplied blood and removed waste from each ram's testis (Fig. 1 -A), as seen under a microscope. According to the findings of one study (De Almeida, 2013), the tunica vasculosa is sometimes considered to be a distinct structure and may be rather deep in animals such as the boar and the stallion.

Microscopic analysis of the testis revealed a network of trabecular and interlobular septa within the parenchyma (Fig. 1-B), with no evidence of unusual condensation. The stroma of the testes is partitioned into lobules by septa. The testis is divided into lobules, and inside each lobule are seminiferous tubules and interstitial tissue (Fig. 1).

Epithelium composed of spermatogenic cells and Sertoli cells that lines the seminiferous tubules. Spermatozoa develop from spermatogenic cells. Small, ovoid cells called spermatogonia have dark, rounded nuclei and are seen in close proximity to the basement membrane (figures 2 and 3). Similar findings may be seen in (Pawar and

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Wrobel, 1991 ; Fukuda et al., 2001). This study's microscopic analysis of the ram's testis indicated that spermotogonia were the first cells in the spermatogenic lineage. Primary spermatocytes, which are bigger cells with often-distinct chromatin, are generated during mitosis in the spermotogonia. The initial meiotic division produces secondary spermatocytes from the larger primary spermatocytes. Because of their rapid second meiotic division and subsequent formation of haploid spermatids, secondary spermatocytes are seldom seen. Clustered near the seminiferous tubule's lumen (figures 2 and 3), the spermatids are spherical cells with pale nuclei. (Al-Hamery, 2008) also described the structural basis of spermatogenesis and spermiogenesis in the goat.

The quantity of spermatogenic cells much outnumbers the number of Sertoli cells (sustentacular cells). Their nuclei are pale and oval or triangular in shape, with a large nucleolus and irregular cleft-like infoldings. They are long cells that reach all the way from the tubule's basement membrane to its lumen, but their borders are difficult to make out in standard histologic preparations (figures 2 and 3). The findings jived with those of (Gofur et al., 2008), who found that Sertoli cells could be identified by their smaller size and distinctive morphology (fewer tall columnar cells with irregular and visible nuclei).

Tubules are connected by connective tissue that includes polyhedral interstitial (Leydig) cells. Particularly high levels of these may be seen in male stallions and boars. Their acidophilic, frequently foamy cytoplasm (see Figure 4) and tiny, spherical nucleus are telltale signs. Leydig cells (interstitial cells) were found to be big, polygonal, eosinophilic cells, which was consistent with the results of (Pawlina and Ross, 2018).

The epididymis may be split into a "head," "body," and "tail" at the macroscopic level. The visceral layer of the tunica vaginalis covers it and protects it from the surrounding tunica albuginea of dense irregular connective tissue (figure 5) .

The epididymis is a single, long, coiled duct coated with pseudostratified epithelium, where sperm develop and become motile. Stereocilia are long, thin projections of the cytoplasm that extend into the lumen. Epithelium is supported by lamina propria, a layer of connective tissue, and circular smooth muscle is present in varying amounts. As the epididymal tail reaches the ductus deferens (figures 6 and 7), the latter swells.

The head to tail regions of the epididymis have different structural characteristics. There is some smooth muscle surrounding its pseudostratified columnar epithelium, which has stereocilia and is thickest in the head area. Lower smooth muscle mass and thinner epithelium are seen in the body (mid) region. There is the largest abundance of surrounding smooth muscle and the thinnest pseudostratified epithelium in the epididymis tail area (fig. 8 & 9). According to the findings of (Eurell and Frappier, 2013), the pseudostratified columnar epithelium that lines the epididymidis is encircled by circular smooth muscle fibres and a small amount of loose connective tissue, the number of which increases noticeably towards the epididymis' tail.

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Table 1: diameter of seminiferous tubules, diameter of spermatogonia, diameter of primary spermatocytes and Diameter of sertoli cells

Parameters of seminiferous tubules	Range (µ)	Mean
Diameter seminiferous tubules	175.705 – 382.5	273.4
diameter of lumen of seminiferous	59.577 - 134.091	102.68
Diameter of spermatogonia	4.353 - 7.1	5.521
diameter of primary spermatocytes	5.12 - 8.207	7.024
Diameter of Sertoli cells	6.1 - 9.266	7.58

Table 2: diameter and thickness of the wall of head, body and tail of epididymis.

Group	Thickness of epithelium (mean ± Std. E)	Diameter (mean ± Std. E)
Head of epididymis	794 ± 8 A	55.2 ± 1.7 A
Body of epididymis	368 ± 5 B	50.2 ± 2.1 A
Tail of epididymis	336 ± 3 B	49.3 ± 1 A

Different litters: Significant at P<0.05, Similar litters: Non significant at P<0.05

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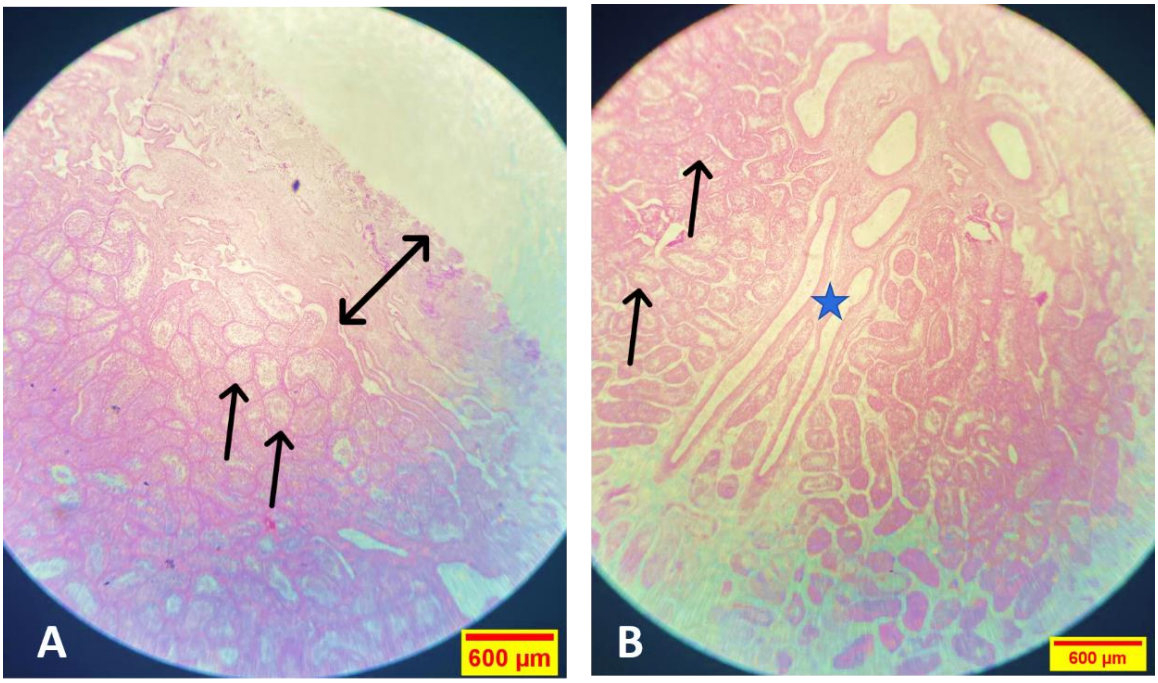

Figure 1: The histological section of testis of adult Ram showing the dense irregular connective tissue capsule (double head arrow), interlobular septa or trabecule that divide the testis into lobules (blue star), each lobule of the testis there composed of seminiferous tubules (black arrows). H&E stain.

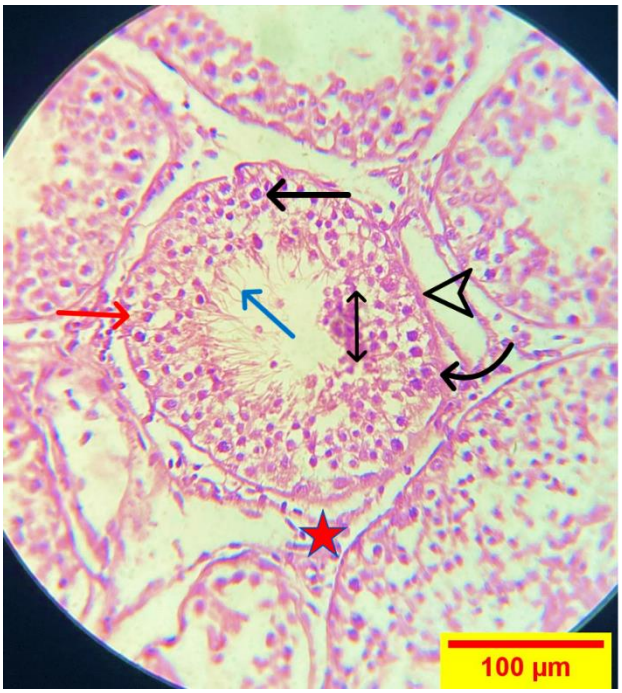

Figure 2: The histological section of testis of adult Ram showing the epithelium of the seminiferous tubules with various stages of spermatogenesis rest on basal lamina (arrow head), spermatogonia (curves arrow), primary spermatocytes (black arrow), cluster of spermatids (double head arrow) sperms in the lumen (blue arrow), presence of Sertoli cell (red arrow), and the red star referred to the interstitial tissue. H&E stain.

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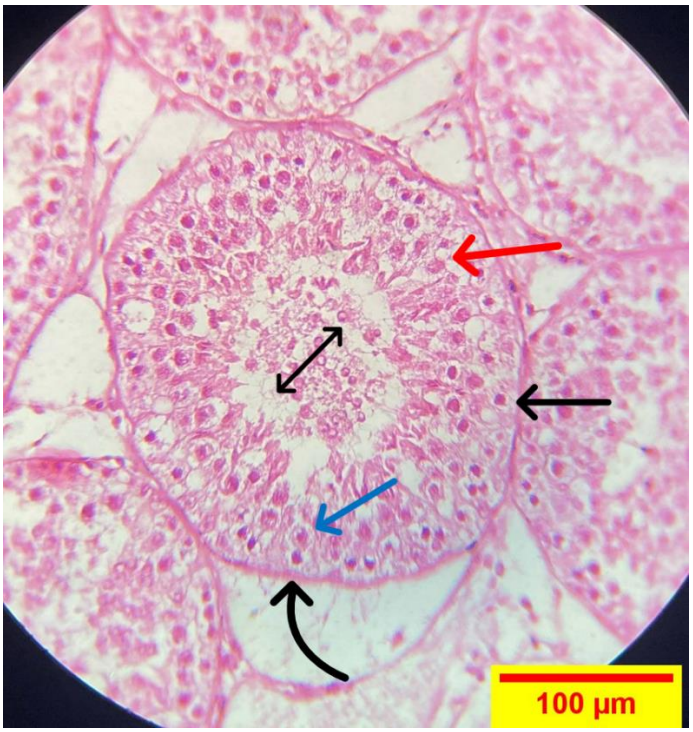

Figure 3: The histological section of testis of adult Ram showing the spermatogonia (black arrow) resting on basal lamina (curved arrow), primary spermatocytes (blue arrow), cluster of spermatids with sperms in the lumen (double head arrow), presence of Sertoli cell (red arrow). H&E stain.

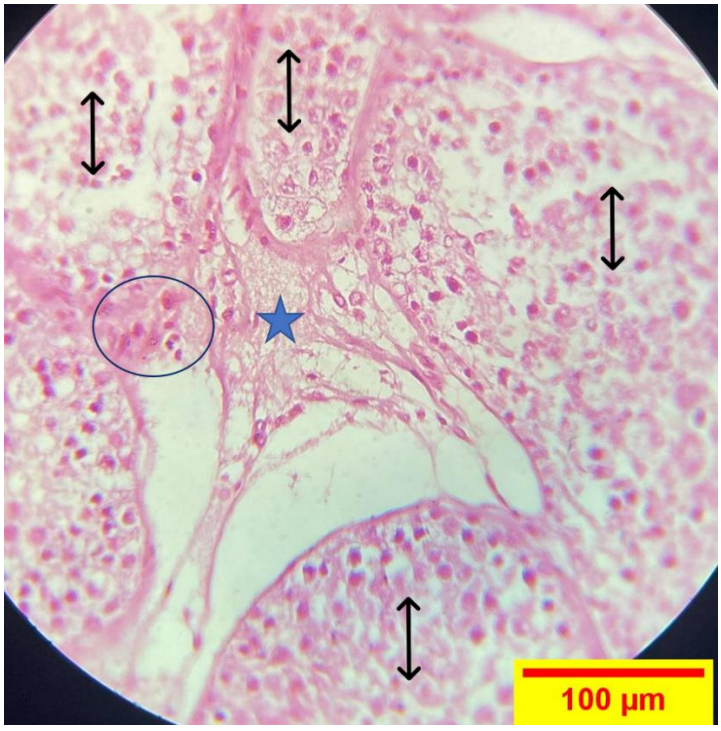

Figure 4: The histological section of testis of adult Ram showing the interstitial tissue (blue star) occupied by connective tissue and Leydig cells (circle), the double head arrows represent the seminiferous tubules. H&E stain.

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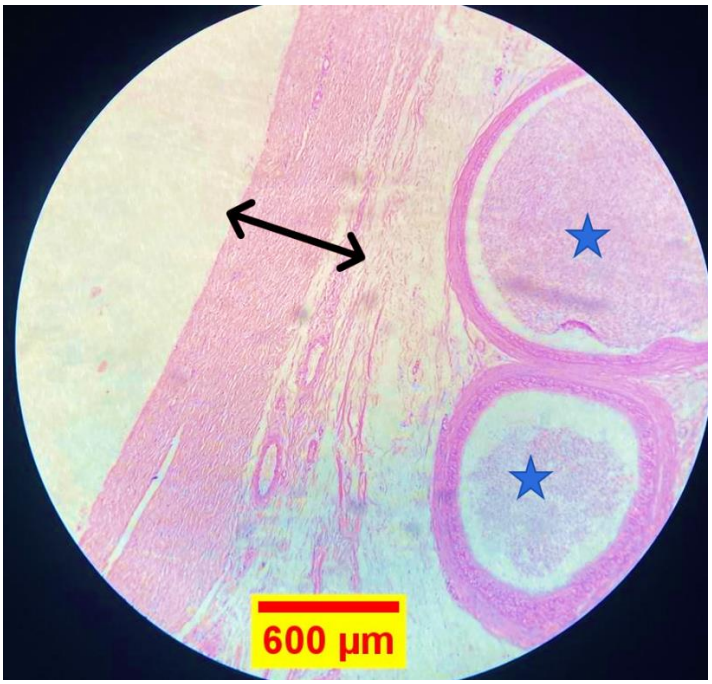

Figure 5: The histological section of head of epididymis of adult Ram. The double head arrow represents the dense irregular connective tissue that surround the epididymis, blue stars referred to the lumen of epididymis. H&E stain.

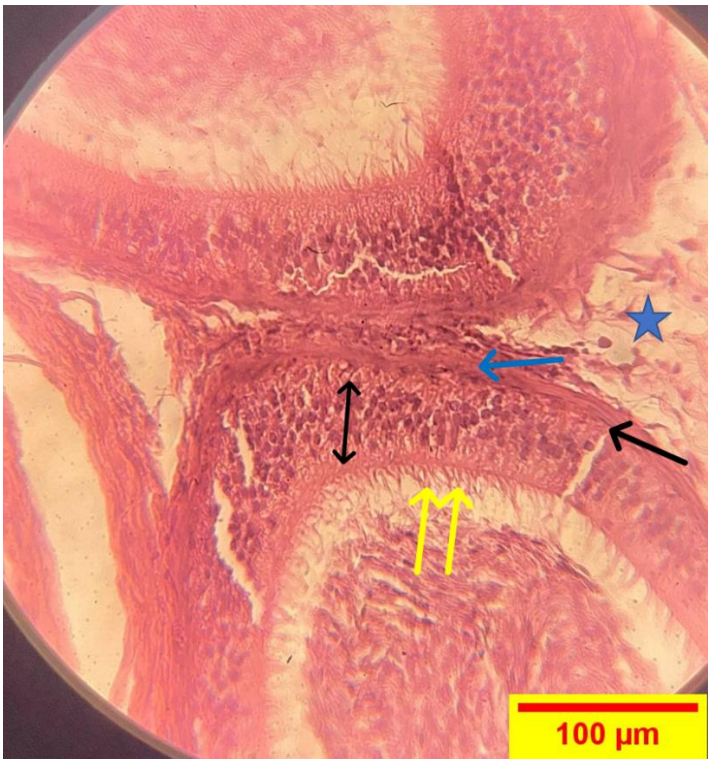

Figure 6: The histological section of head of epididymis of adult Ram. The lining epithelium of the epididymis are pseudostratified epithelium (double arrow) that rests on a connective tissue lamina propria (black arrow) and

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there is a variable amount of circular smooth muscle (blue arrow). Long cytoplasmic processes, the stereocilia, project into the lumen (yellow arrows). The blue star represents the vascular connective tissue. H&E stain.

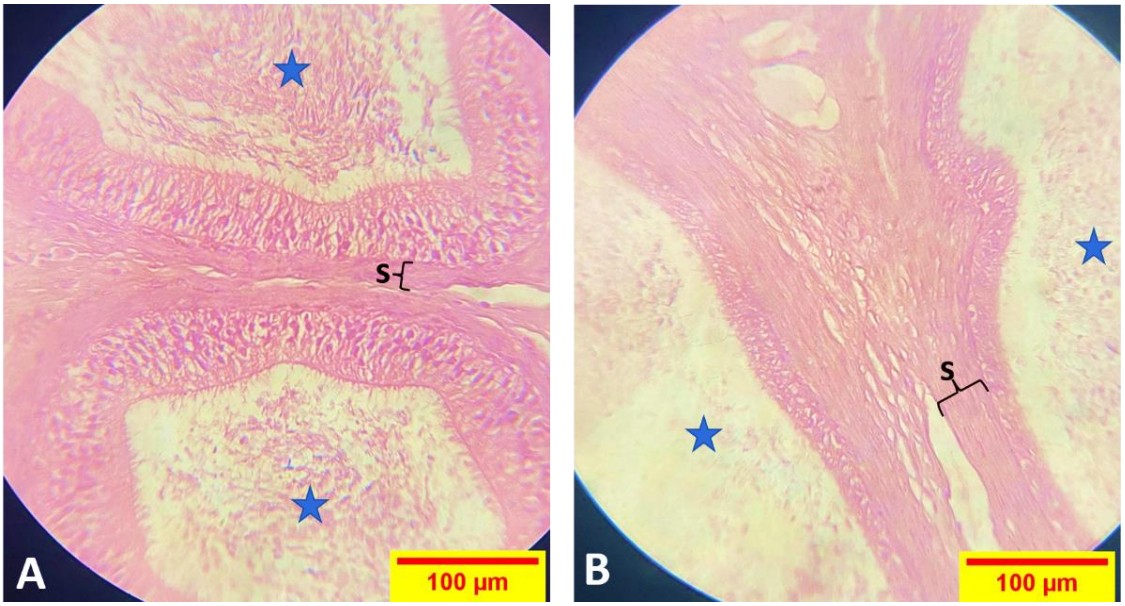

Figure 7: The histological section of body of epididymis (A) and tail of epididymis (B) of adult Ram. Showing the differences in the thickness of the smooth muscle layer between body and tail(**s**). The blue stars represent the lumen of epididymis. H&E stain.

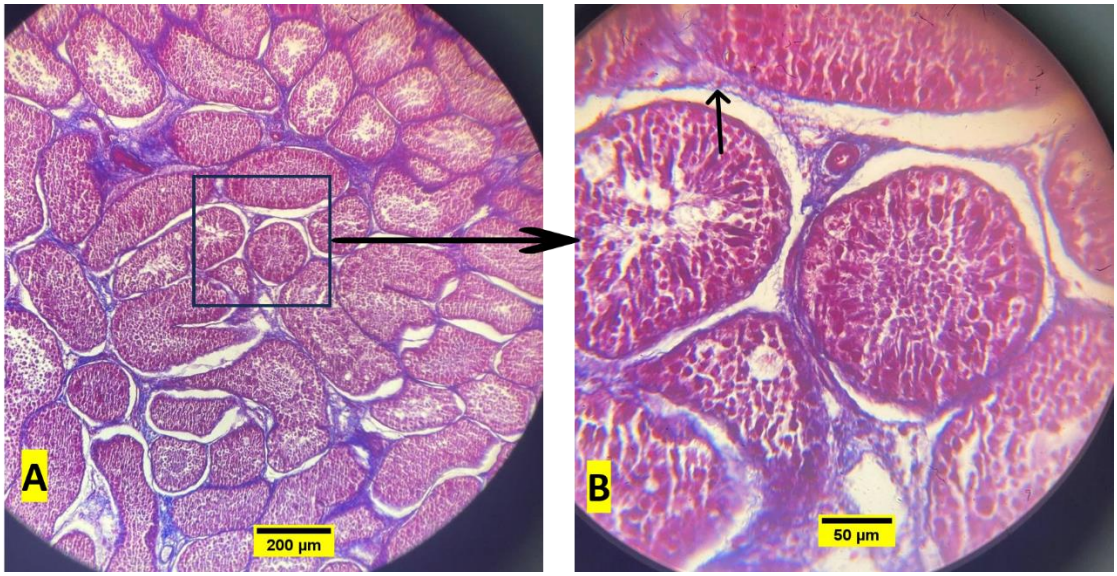

Figure 8: The histological section of testis of adult Ram showing the fibrous connective tissue within the interstitial tissue (black arrow). Masson tri-chrome stain.

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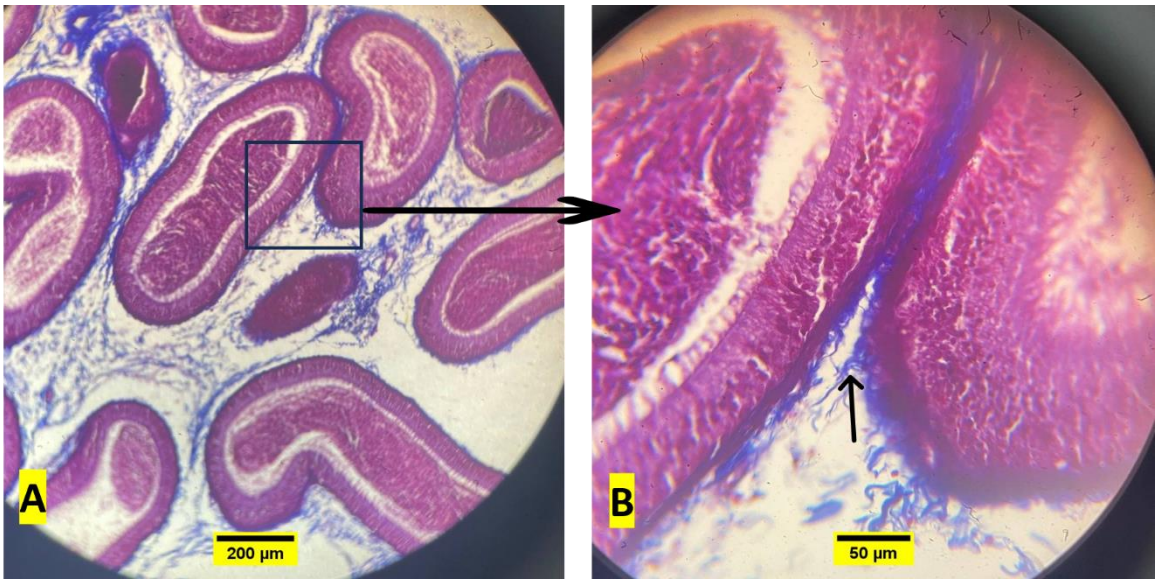

Figure 9: The histological section of head of epididymis of adult Ram showing the fibrous connective tissue within the interstitial tissue (black arrow). Masson tri-chrome stain.

IV. CONCLUSION

According to the current findings, spermatogenic cells, which comprise spermatogonia, primary and secondary spermatocytes, and spermatids that later converted into sperm, line the semiserious tubules. The current study concluded that the thickness of the epithelium of epididymis was decrease toward the tail while the thickness of the smooth muscle layer was increase toward the tail.

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