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Gross morphology and Histological details of Testis and Epididymis of the Hassan Ram

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Abstract

Twelve testes were collected from local slaughter house in Hassan city of Karnataka. Six testes were used for gross study and six testes were processed for routine histological studies. The cross section of seminiferous tubules consist of stratified layers containing different stages of development of spermatogenic cells and lightly stained 14-16 single layer of sertoli cells. The interstitial tissue consisted of less number of Leydig cells, few fibrocytes and blood and lymph vessels in loose connective tissue. The rete testis was lined by simple cuboidal epithelium surrounded by loose connective tissue of mediastinum testis. The head of the epididymis is lined with thick epithelium with long branching Stereocilia and a little smooth muscle. The height of the epithelium of the body of epididymis was intermediate and had intermediate thickness of smooth muscle. The tail of epididymis was lined with thinnest epithelium of the three regions and more layers of smooth muscle compared to head and body.

Keywords: Testes, epididymis, Semeniferous tubules, Hassan sheep breed

Introduction

The testis is the site of production of the male gamets. The seminiferous tubules in the testis of any species consist of the highly specialized epithelium made up of germinal cells and supporting (Sertoli) cells. The study of the testes of Malabari goat (Harshan *et al.*, 1978) [7], Assam goats (Baishya *et al.*, 1986) [1], Beetal goats (Gupta, 1989) [6], Cashmere goats (Walkden-Brown *et al.*, 1994) [16] are available. The Hassan sheep breed as the name suggests is traditional to the Hassan district of Karnataka. The Government of Karnataka has recently proposed for Hassan sheep breed conservation and small ruminant semen station at Hassan. In this regard the present study was planned to explore basic anatomical data on the testis and epididymis.

Material and Methods

A total of twelve testes and epididymis of adult rams of Hassan Sheep Breed, Hassan District, Karnataka were collected from the local slaughter house at Hassan city to study the gross and histological details of the testis and epididymis. The determination of age was ascertained based on the eruption of teeth (Noden and de Lahunta, 1985) [14]. The morphometrical measurements of testes were recorded and the testes and epididymis samples were fixed in 10% neutral buffered formalin for 48 hours (Luna, 1968) [12]. The tissues samples were processed by routine paraffin technique and sections of 5-6 μ were stained with Haematoxylin and Eosin method, Masson trichrome, Verhoff's and Van Gieson (Luna, 1968) [12].

Results and Discussion

Gross morphology of Testes and Epididymis

The testicles of ram of the Hassan sheep breed were located in a vertical direction a little in front of the inguinal region (Fig.1&2) and are suspended in the scrotum by the spermatic cords (Fig.2). Whereas, in case of horse and dog testicles are suspended horizontally and obliquely respectively (Nickel *et al.*, 1979) [13].

Testicles are paired spherical shaped organs that produce the male germ cells. They are two in number, the right and left. They are relatively large in ram as compared to the carnivores wherein it is small (Nickel *et al.*, 1979) [13]. The testicles of the horse are usually smaller and of oval shape. The testicle of adult ram was about 13.5 ± 1.05 cm long, 5.6 ± 0.61 cm wide and 3.23 ± 0.23 cm thick (Table.1). The average scrotal circumference was about 27.33 ± 3.08 cm. The seasonal variation in the testicular size was observed in related wild species and it is absent in domestic mammals (Nickel *et al.*, 1979) [13].

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The testicles have two coverings, the tunica vaginalis and tunica albuginea. The tunica vaginalis intern have parietal and visceral layer (Fig.2), the space between two layers constitutes the cavity of the tunica vaginalis and it communicates with the peritoneal cavity through the vaginal ring. The tunica albuginea appeared thick and contains the superficial branches of the testicular arteries and veins (Fig. 2).

The epididymis was elongated, curved and closely attached along the posterior border of the testicle. It connects a testicle to a vas deferens. It consists of head, body and tail (Fig. 2).

Histology of the Testes and Epididymis

The parenchymas of testes were composed of tunica albuginea, trabeculae, mediastinum testis, seminiferous tubules and interstitial tissue.

Tunica albuginea was the outermost covering of the testis. It contain thick connective tissue capsule consisting of more of collagen and less of elastic fibers and myofibroblast internal to which was vascular layer of loose connective tissue called the tunica vasculosa (Fig.3). The connective tissue extends inwards into the testicular parenchyma to form the interstitial connective tissue which divided the testicular parenchyma into lobules, this surround, binds and support seminiferous tubules. These observations are in agreement with Gofur, (2008) [5], Eurell and Frappier, (2006) in domestic animals, in boar (Ohanian *et al.*, 1979); in bull (Ahmed, 2005 [1] and Gofur *et al.*, 2008) [5]; in deer (Moonjit and Adcharatt, 2007), in horse (Shukla *et al.*, 2013), in domestic pig by Reddy *et al.* (2016) [15].

The space present between the seminiferous tubules, consisted of less number of Leydig cells, few fibrocytes and blood capillaries in loose connective tissue (Fig.4), whereas, numerous leydig cells were found in testes of goat (Bhosale, 2016) [3]. Leydig cells constitute approximately 1% of the entire testicular volume in adult ram, approximately 5% in bulls and 20-30% in boars (Eurell and Frappier, 2013) [4] and abundant leydig cells were observed in domestic pig by Reddy *et al.* (2016) [15].

The mediastinum testis consisted of the connective tissue cords, which extended through the long axis of the testis. The mediastinum testis was composed of collagen and elastic fibers and contained labyrinthine, which communicated with rete testis (Fig.5). Similar observations were recorded in domestic goat by Kakade and Singh (1990) [8]. The rete testis was continuous with the ductuli efferentes. Similar observations were recorded by Eurell and Frappier (2006) in domestic animals.

The rete testes were lined by simple cuboidal epithelium surrounded by loose connective tissue of mediastenum testis (Fig.5). Whereas, Kishore *et al.* (2006) [9] reported that rete testis was lined by simple cuboidal to simple squamous epithelium in Madras Red ram.

The seminiferous tubules comprised of major portion of testicular parenchyma. It consisted of myofibroblast cells between the basement membrane and lamia propria. The cross section of tubules consisted of stratified layers containing different stages of developing spermatogenic epithelial cells and lightly stained 14-16 sertoli cells in a single layer (Fig.6). Lightly stained sertoli cells (Sustanticular/nurturing cells) were elongated and irregularly outlined in shape, the broad base of the cells rest on the basement membrane and narrow

end of the cells extends toward the lumen. The nuclei of these cells were oval to pear in shape and located at the broad basal portion of the cells with prominent nucleolus (Fig.7). Similar findings were recorded by Eurell and Frappier, (2006) in domestic animals. Sertoli cells were elongated with ellipsoidal shaped nuclei in ram (Kishore *et al.* 2011) [10]. Whereas, Johnson *et al.* (2008) [11] reported that the sertoli cells number was not stable in adult horse. Bashir *et al.* (2012) [2] noticed that sertoli cells were irregularly columnar extended into the lumen and the nucleus was large, lightly stained and irregular in shape with a distinct nucleolus. In the present study no difference was noted in sertoli cells of right and left testicle.

Seminiferous tubules consisted of spermatogonia (germinal cells), primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. The spermatogonia were the most immature spermatogenic germ cells. They were small and rounded in shape with dark stained rounded nuclei at the center of the cells. These cells were situated adjacent to the basement membrane in single layer (Fig. 6). The primary spermatocytes were found above the spermatogonia which were the largest cells within the lumen having dark spherical nuclei. The elongated spermatids presented oval nucleus and differentiated spermatozoa were noticed adjoining the lumen (Fig.6). Similar findings were recorded by Banks (1993) and Eurell and Frappier, (2006) in domestic animals and in pig by Reddy *et al.* (2016) [15].

The Leydig cells were polyhedral in shape having large, spherical nuclei with accentric nucleoli (Fig.4). Similar observations were recorded by Bashir *et al.* (2012) [2] in Bakerwali goat, Banks, (1993) in domestic animals, Eurell and Frappier, (2006) in domestic animals. Whereas in boar, testicular interstitium contained abundant Leydig cells arranged in large clusters between seminiferous tubules and located in close vicinity to the arteriole and capillaries (Reddy *et al.*, 2016) [15]. In seasonal breeding male *viz*, camel leydig cell volume and number may change during year (Eurell and Frappier., 2013) [4].

The ductuli efferentes showed simple ciliated columnar cells with vesicles of endocytosis at apical cytoplasm, indicative of involvement of resorption and digestion of ductular fluid. The findings were in accordance with Eurell and Frappier, (2013) [4] in domestic animals.

The head of the epididymis receives spermatozoa via the efferent ducts from the testis. The Head, body and tail of the epididymis was lined with pseudostratified columnar epithelium with stereocilia. The height of lining epithelia of head was more with long branching stereocilia with little amount of loose convective tissue and circular smooth muscle fibers surrounding it (Fig.8). There was presence of multivesicular bodies in the apical cytoplasm indicating most of the fluid that leaving testis is reabsorbed in the proximal part of head of epididymis. The height of the epithelial cells of the body was intermediate with reduced size of the stereocilia and increased smooth muscle layers as compared to the head (Fig.9). The height of the epithelial cells of the tail was less compared to the head and body and more layers of smooth muscle surrounding epithelium were found as compared to the head and the body (Fig.10). In seasonal breeder such as camel, the height of epididymal epithelium and the innervations density of the muscular layer display characteristic changes over the year (Eurell and Frappier., 2013) [4].

The testes of the ram of Hassan sheep breed are paired and

placed in a vertical direction, a little in front of the inguinal region. The epididymis is elongated, curved and closely attached along the posterior border of the testicle. The seminiferous tubules were lined with stratified layers containing different stages of development of spermatogenic cells and lightly stained 14-16 single layer of sertoli cells. The Leydig cells were less in number as compared to the other species indicating less quantity of semen in comparison to other domestic animals. The head, body and tail of the epididymis have more, intermediate and thinnest layer of epithelium and in reverse order of smooth muscle respectively.

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Table 1: The testicle of adult ram was about 13.5 ± 1.05 cm long, 5.6 ± 0.61 cm wide and 3.23 ± 0.23 cm thick

SI No	Length of the scrotum (Cms)	Width (Cms)	Thickness (Cms)	Circumference of the scrotum (Cms)
1	14.0	6.5	3.5	25
2	15.0	5.0	3.25	27
3	14.0	6.0	3.0	26
4	13.0	5.5	3.0	32
5	12.0	5.0	3.5	24
6	13.0	6.0	3.15	30
Mean \pm SE	13.50 ± 1.05	5.67 ± 0.61	3.23 ± 0.23	27.33 ± 3.08



Fig 2: Photograph showing head (ED), body (EB), tail (ET) of the epididymis, superficial blood vessels (Arrow), visceral layer (V) of tunica Vaginalis and Spermatic cord (C)



Fig 3: Photomicrograph showing seminiferous tubules (S) with tunica albuginea (T), Tunica vasculosa (V). The head of epididymis (EH) surrounded by collagen fibers (Arrows). Van Gieson x10



Fig 1: Photograph showing right testes (RT), left testes (LT) and Epididymis (ET) enclosed in the scrotum

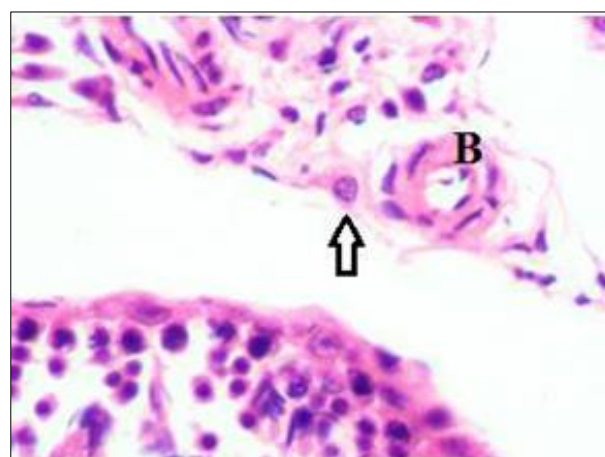


Fig 4: Interstitium showing the presence of leydig cell (Open arrow) and blood vessel (B). x40

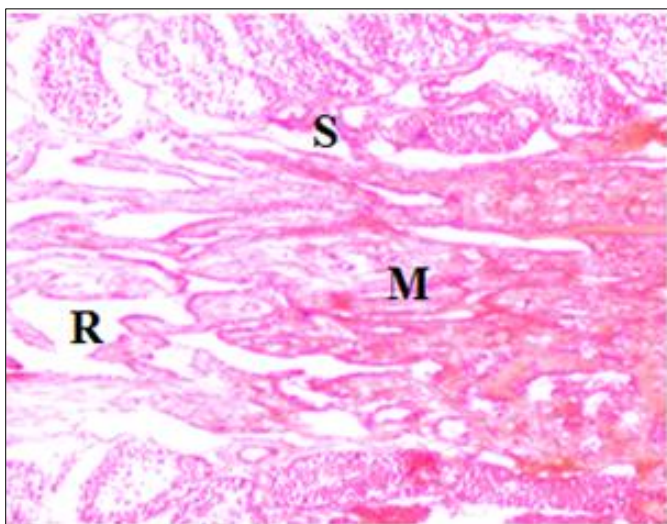


Fig 5: Photograph showing mediastinum testes (M) and rete testes (T), straight part of the seminiferous tubule. (S) H&E. x 4

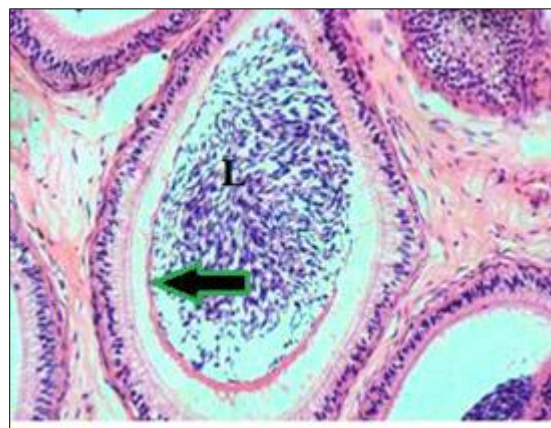


Fig 8: Epididymis head pseudostratified columnar epithelium with stereocilia (Arrow) H&E. x40

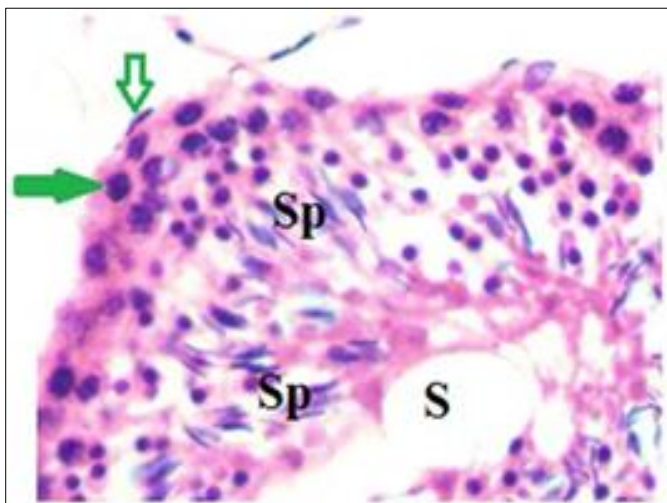


Fig 6: Seminiferous tubule (S) comprises stratified epithelium containing spermatogonia cells (solid arrow), myoblast (Open arrow), developing spermatid and spermatozoa (Sp) H&E. x 40

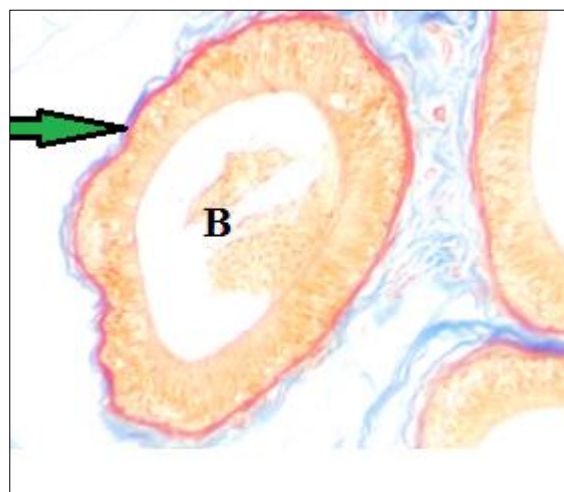


Fig 9: Body of the epididymis (B) showing 2-3 layers of smooth muscle (Arrow) Masson's trichrome x10

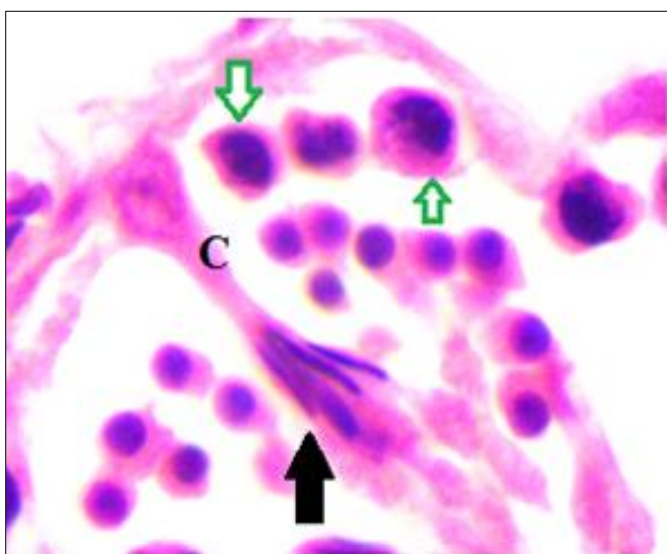


Fig 7: Seminiferous tubule comprises stratified epithelium containing sertoli cells (C), primary spermatocyte (Open arrows) and developing spermatid and spermatozoa (Solid arrow) H&E. x100

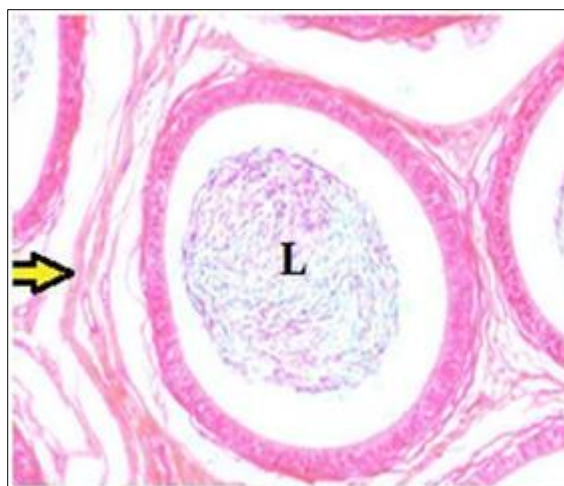


Fig 10: Tail of Epididymis pseudostratified columnar epithelium (L) with thick muscular layer (Arrow) H&E. x10

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