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Histo-morphological studies of testis & epididymis of *Panthera leo persica*

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Abstract

In the present investigation, the anatomical study of testes and epididymis of a 15 year old Asiatic lion revealed that the oval shaped testis were located outside the abdominal cavity within the scrotum in pair. The morphometrical profile of right testis was higher than the left one. The body of epididymis passes at dorso-lateral face of testes. Microscopically thick tunica albuginea and tunica vaginalis of testis was composed of abundant connective tissue. The testicular mass was composed of seminiferous tubules of variable sizes with various generations of spermatogenic cell. The elongated large Sertoli cells were few in number with basally located dark stained nuclei. Spermatogonium cells were seen at the basal compartment. Large active primary spermatocytes were seen with mitotic figures. The rounded spermatid cells were faintly stained. Spermatozoa were seen in the lumen attached with the free end of the Sertoli cells. Flat elongated myoid cells were seen at the basement membrane of seminiferous tubule. The epididymal ducts were lined by a pseudo stratified ciliated columnar epithelium cells with stereo cilia. Smooth muscle and abundant connective tissue fibers were seen around the epididymal duct wall. Clumps of spermatozoa were also clearly visible at the lumen of epididymal duct.

Keywords: Asiatic lion, epididymis, seminiferous tubules, spermatogenic cell, Sertoli cells, testis

Introduction

The natural habitat of Asiatic lion was western Asia and the Middle East up to northern India but currently it is restricted to the Gir National Park and in Gujarat, thus IUCN enlisted this animal under Red List because of its small population size and area of occupancy ^[1]. As hybrid lions have no conservation value, the Central Zoo Authority of India stopped breeding Indian-African crosses lions ^[2]. Now only pure native Asiatic lions are bred in India. The testis is an endocrine as well as an exocrine gland. Tubules within the epididymis secrete important substances that help the sperm survive and mature. While in the body of the epididymis, sperm are nurtured by epididymal secretions and undergo further stages of their maturation ^[3]. The anatomical details of testes and epididymis of lion has not been sufficiently investigated. Therefore, this present study was undertaken to investigate the macro and micro anatomical details of testes and epididymis.

It is worth nothing that key role in the control of germ cell development is assigned to Sertoli cells. Thus in this study another approach is made on visualization of Sertoli cells in the seminiferous tubules which help in the formation of the blood testes barrier. As sperm production and maturation depends upon the testicular physiology and epididymal physiology, histological study can provide baseline information to the researchers.

Material and Methods

The present study was carried out in the department of Veterinary Pathology, C.V.Sc & A.H., R.K. Nagar, Agartala, Tripura (W). For the present study, intact right and left testes of *Panthera leo persica* along with relevant parts were collected and preserved in 10% neutral buffered formalin solution without removing peritoneal attachment for 48 hours. The samples were collected from the department of Pathology, C.V.Sc & A.H., R.K. Nagar, Agartala, Tripura (W) which was brought for postmortem examination. Approximately 2-4 cm Samples were fixed in 10% neutral buffered formalin (NBF) solution and washed by keeping the samples under running tap water for 16 hours. Routine procedure ^[4] was followed for dehydration, clearing and paraffin embedding of tissues. Paraffin blocks were cut by rotary microtome to obtain 5 µm thick serial sections.

The sections were stained with haematoxylin and eosin as per standard procedure [4]. Statistical analysis for mean, standard errors was calculated by taking 10 readings from each slide using Statistical Package for the Social Sciences (SPSS 7.5). Photography and measurements were performed using Image J 1.34s software.

Results and Discussion

Gross Anatomy Testis and Epididymis

Both the testes are oval in shape, located outside the abdominal cavity, within the skin folds called scrotum [5]. Externally it looks slightly yellowish in color and surrounded by tunica albuginea. The morphometrical parameters like weight, length and diameter of the right testis showed higher values than the left one (Table no.1). The epididymis was a single highly coiled tube surrounded by connective tissue and blood vessels, divided into three distinct regions are referred to as the head, body and tail. The head, tail and body were attached with cranial, caudal and dorso-lateral aspect of the testes respectively.

Microscopic Anatomy

Testicular tissue and Tunica albugenia

The testes of Asiatic lion were surrounded by a thick dense fibrous capsule, the tunica albuginea with a thickness of 302.4207 ± 2.34 pixel with numerous blood vessels. Tunica vasculosa was present between the tunica albuginea and parenchyma of the testis which is presented in Fig. III. The same cellular feature was observed in domestic cat [5]. From this layer connective tissue septa extended into the testicular tissue and subdivide the testicular tissue into numerous indistinct lobules. Each lobule contained 2 – 5 nos of seminiferous tubules (Fig. IV) of variable size. These kind of similar histological findings were observed in Northern great grey kangaroo [6]. The tunica albuginea is covered by the tunica vaginalis, composed of connective tissue with fibroblasts and blood vessels. The similar findings were observed in dog and sheep [7].

The interstitial cells (cells of leydig) between the seminiferous tubules of testis were large, polygonal in shape and often present in clumps. The nucleus was usually oval but varied in shape. Occasionally binucleate interstitial cells were observed (Fig. III & IV).

Seminiferous tubules

The testicular parenchyma was mainly composed of seminiferous tubules lined by stratified spermatogenic epithelium. Large Sertoli cells were seen at the base of the tubule. The flat myoid cells and spermatogonium cells were found at the basal compartment of seminiferous tubule.

The mean diameter of the lumen and height of the epithelium of seminiferous tubules has been mentioned in the table no. 2. Sections of these convoluted tubules appeared as a collection of round, oval or elongated structure were clearly visible (Fig. III.). The seminiferous tubules were enclosed by a basement membrane lined by stratified spermatogenic cells and supporting Sertoli cells. (Fig. IV). Spermatogenic cells were arranged in several layers from the basement membrane towards the lumen. These were spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids were observed. The clumps of spermatozoa were observed at the lumen of seminiferous tubules (Fig. IV).

Spermatogonia were seen close to the basement membrane with their dark nuclei. Primary spermatocytes were larger round shaped with distinct nucleoli and nucleolus. Primary spermatocytes were the largest cells, the spermatids appeared smaller than primary spermatocytes and lying near the lumen. The secondary spermatocytes were smaller in size than

primary spermatocytes, with nuclei and nucleoli barely visible, these, in turn, result in spermatids. The spermatogonium cells were seen in the basal compartment of the seminiferous tubule. Mitotically active cells were seen in the basal compartment of seminiferous tubule (Fig. 2B). The lumen was filled with large numbers of spermatozoa, and some of these sperms were attached to the free border of Sertoli cells. Sertoli cells appeared tall cells with dark nucleus (Fig. V). The initial stages of the spermatids were round in shape and faintly stained nuclei with the subsequent development the spermatids became elongated smaller, with dark and oval. They are mostly found in the adluminal compartment. Histologically, in the seminiferous tubules, spermatogenic cells, Sertoli cells and Leydig cells (LC) were observed which were in accordance with previous studies in other species [8, 9, 10]. Seminiferous tubules of the lion testes were well developed and were dense, compact and appeared as collection of round, oval or elongated structure, alike the findings of previous researchers in Northern great grey kangaroo and Rooster [6, 9].

Sertoli cells

Sertoli cells were few in numbers with elongated shape, extending from the basement membrane to the tubule lumen, with large oval nuclei with distinctly stained nucleoli. (Fig. V). The germ cells throughout the seminiferous epithelium, are supported and organized by the Sertoli cell, which were in support with previous findings [11]. The interstitial spaces between the seminiferous tubules were composed of abundant smooth muscle, blood vessels, connective tissues and Leydig cells appearing in polyhedral shape with different sizes, bright nuclei and nucleoli evident (Fig. 2B).

Epididymis

On histological section the single duct had the look of bunch of ducts due to hard coiling of duct that appeared circular in shape (Fig. VII). The mean height with standard error of the head, body and tail of epididymal epithelium and the mean diameter of the epididymis were measured (Table no.2).

The wall of epididymal duct was composed of all usual four layers of hollow organ; tunica mucus, tunica sub-mucosa, tunica muscularis and tunica adventitia (Fig. VIII). The epididymis was lined by pseudo-stratified columnar epithelium with stereo cilia and this similar pattern of cell structures were also found in domestic cats [12].

The caput had very thin wall smooth muscle cell layers and gradually increases its thickness towards cauda. Epithelium height in different segments was found to be highest in the corpus and lowest in the cauda (Table no. 2).

Large numbers of sperm were present within the lumen of the epididymis (Fig. VIII). Basal (nuclei are at different levels), and principal (darkly stained) cells were observed throughout the length of duct (Fig. IX). Epididymal ducts were surrounded by large number of arterioles and capillaries. The circular smooth muscle layer of epididymal duct was fairly thick surrounded by slight collagenous connective tissue (Tunica adventitia). It was characterized by lower epithelium, greater luminal diameter, greater sperm concentration within the lumen of duct, denser and shorter stereocilia, and a thicker smooth muscle wall which was similar to previous study conducted in Northern great grey kangaroo [6].

Table 1: Measurement of testes

Parameters	Right testis	Left testis
Weight	44.372gm	41.837gm
Length	5.60 cm	5.20 cm
Diameter	13.00 cm	12.70 cm

Table 2: Histological parameters of seminiferous tubules and Epididymal duct

Parameters	Seminiferous tubules (Mean ±SE)	Epididymal duct (Mean ±SE)		
		Head	Body	Tail
Epithelial height (pixel)	21.75 ± 0.53	106.72±1.33	122.53±5.12	55.57±3.47
Lumen diameter (pixel)	148.39 ± 7.60	274.43 ± 18.75		



Fig 1: A photomicrograph of a testes showing right (R) & left (L) testes, vas deferens (VD), & tail of epididymis (TE) & spermatic cord (SC)

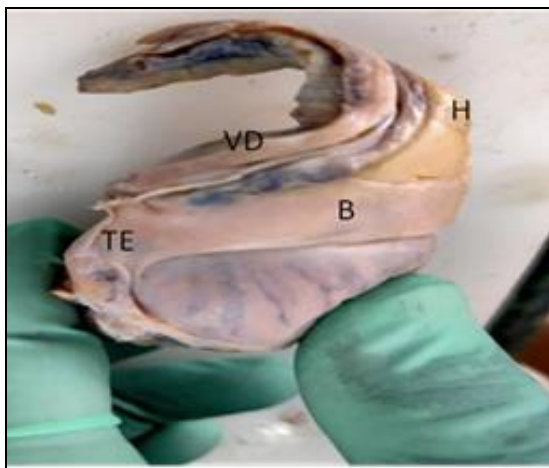


Fig 2: A photomicrograph of testis showing epididymis: head (H), Body (B), Tail (TE) and vas deferens (VD)

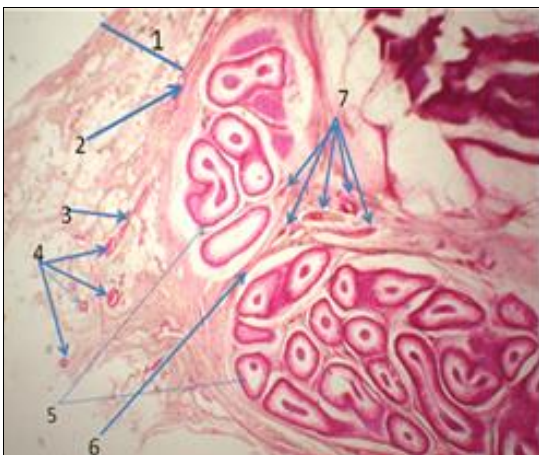


Fig 3: A photomicrograph of a section of testis showing TA, vascular layer (2), Veins(3), Artery (4), STs (5), CTs septa (6) & LCs(7).H & EX100

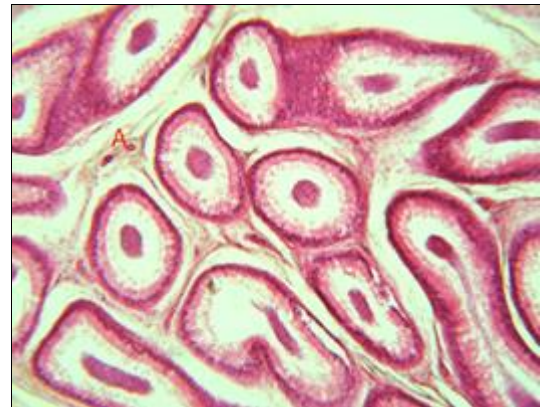


Fig 4: A photomicrograph of a section of testis showing many ST & lumen contained clumps of spermatozoa & LC (A). H & EX 200

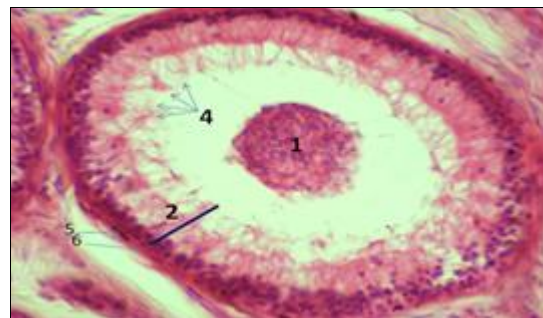


Fig 5: A photomicrograph of a section of testis showing several spermatogenic cells layers (2), spermatozoa attached with the free border of sertoli cells (4), myoid cell (5), basement membrane (6), clumps of spermatozoa (1) at the centre of lumen of ST.H & EX400

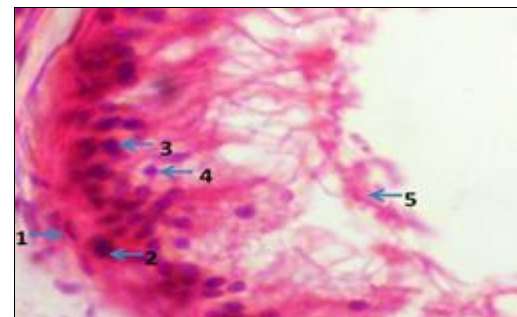


Fig 6: A photomicrograph of seminiferous tubules showing spermatogonia (1), sertoli cell (2), primary spermatocyte (3), secondary spermatocytes (4), spermatid (5) & basement membraneH & E, 400X

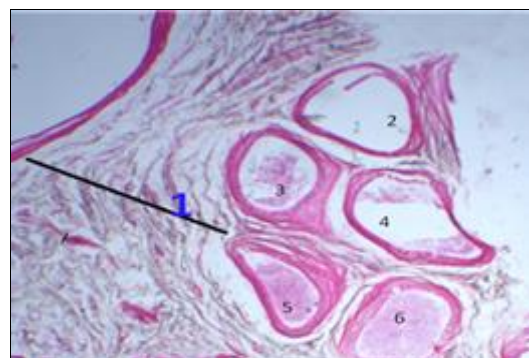


Fig 7: A photomicrograph of a section of epididymis showing epididymal duct and lumen contained spermatozoa (2,3,4,4) was surrounded by CT Coat (1) with numerous blood vessels. H & E X100.

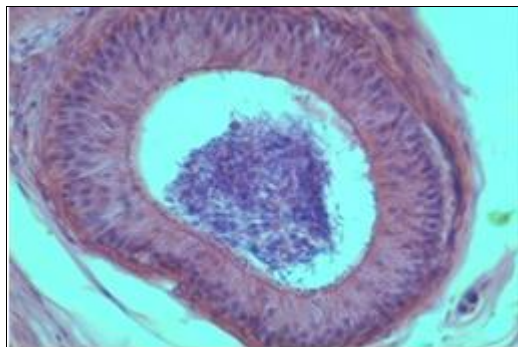


Fig 8: A photomicrograph of a section of caput of epididymis showing columnar cell, short stereocilia & clumps of spermatozoa. H & E X400.

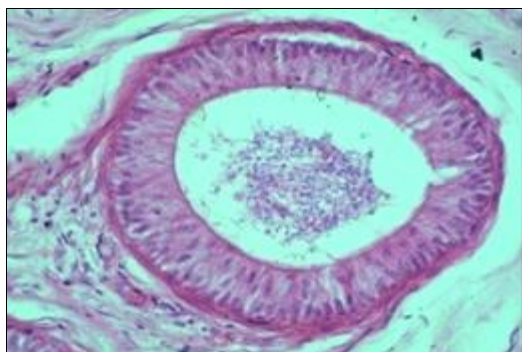


Fig 9: A photomicrograph of a section of caput of epididymis showing columnar cell, stereocilia & clumps of spermatozoa. H & E

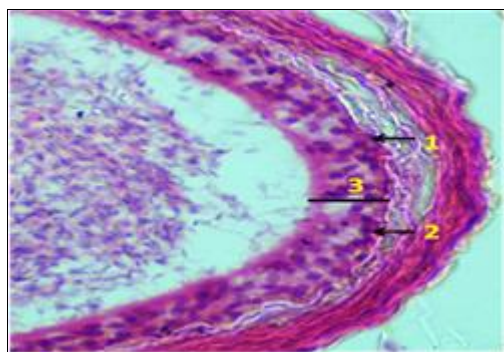


Fig 10: A photomicrograph of a section of epididymis showing basal (1) & intermediate cells (2) of epithelium & height of epithelium (3). H & EX400

Conclusion

In general, the histology of testis and epididymis of the Asiatic lion was found to be similar to other mammalian species in their general organization with few exceptions like the tunica albuginea in dogs and sheep are having a thin layer of dense connective tissue whereas in case of *Panthera leo persica*, it was found fairly thick. The seminiferous tubules with well developed stratified epithelium and irregular shaped Sertoli cells were observed. Both uninucleated and lesser amount of binucleated Leydig cells were also found in between the seminiferous tubules. The epididymal ducts showed pseudo-stratified columnar epithelium with stereocilia, present on a basement membrane. The condition of the seminiferous tubules, epididymis and the fine structure of the testicular interstitial cells indicated that this 15 year old animal was reproductively active. The reproductive history of the lion supports the above observations.

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