RESEARCH ARTICLE

Histomorphology, Histochemistry and Micrometrical Study of the Testes of Tom Cat

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Abstract

The present research work was carried out on testes procured from six healthy adult non-descript male cats brought for castration/ neutering at Veterinary Clinical Complex of the College in Anand (Gujarat, India). The fibrous connective tissue capsule of testes was made up of outer tunica albugenia and inner tunica vasculosa. The tunica albugenia was thinner as compared to tunica vasculosa layer. The capsule was having collagen fibers and sparce number of elastic fibers. Mean thickness of tunica albuginea was 38.35±1.42 µm. The mediastinal testis was located centrally in the testicular parenchyma. The rete testis was lined by simple cuboidal epithelial cells, presenting prominent oval and horizontally placed nuclei in it. Each lobule of testis was oval to coiled irregular in shape. The mean diameter of seminiferous tubules was 243.37±2.52 µm, whereas the mean height of spermatogenic epithelium was 65.52±0.77 µm. There was average 17-18 Sertoli cells noticed per cross section of seminiferous tubules. The mean height of Sertoli cells was 38.35±1.42 µm. Clusters of Leydig cells were arranged in triangle, rectangle or irregular shape in the interstitial spaces. There was presence of lipid material in the Leydig cells. The mean diameter of Leydig cells was 7.4 µm. The elongated myoid cells were found at the basement membrane of the seminiferous tubules. The tunica albuginea, basement membrane of seminiferous tubules and mediastinal testis were strongly positive for PAS Alcian blue stain indicating more amount of neutral mucopolysaccharides. The seminiferous tubular epithelial cells and interstitial cells were moderately positive for neutral mucopolysaccharides.

Key words: Cat, Histochemistry, Histomorphology, Micrometry, Testis.

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Introduction

Felines are the apex predators in the food chain. They were referred to be critical species and indicators of ecosystem health (Mehanna *et al*., 2016) as their extinction could have negative effects on all trophic levels of the food chain. Being the greatest predators in tropical foods and carnivores by nature, cats have a significant influence over other species. The domestic cat is having scientific name of *Felis catus (F. cattus)*, but also having identification as *Felis domesticus (F. domestica)* with change in taxonomy in 1996. Cat *(Felis catus)* is a sole domesticated member of the family Felidae belonging to the order carnivora under the class Mammalia (Hudson and Hamilton, 2010).

The feline testis shares a similar anatomy with the majority of domestic mammals. Felines have typically oblong testes positioned obliquely inside the scrotum. It performs endocrine and exocrine functions simultaneously as it produces cellular portion of semen (sperms) and sex hormones (Hudson and Hamilton, 2010). The knowledge of complete structure of testes will help to comprehend the variation of spermatogenic cells and also to determine the proper age of sterilization.

Compared to other domestic animals, there are less reports on the histology of testes in cats. There is a dearth of information about the anatomy and histomorphology of the testes of the domestic Tom cats of India. This study was aimed to compare normal and abnormal testes and to provide baseline information regarding the reproductive histology of testes of household cats in India.

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Materials and Methods

The study was conducted on testes collected from six healthy non-descript adult male cats, which were brought for castration/neutering at Veterinary Clinical Complex, College

of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat (India). Prior to the collection of the testes, health status, normal body structure, age and body weight of each cat was recorded.

All the specimens were preserved in 10% neutral buffer formalin for at least 48 h for histomorphological studies. After fixation, the tissue pieces of 0.5 cm^3 were processed for paraffin sectioning method. Paraffin sections of 6-8 µm thickness were obtained on rotary microtome and stained with different staining solutions, *viz.*, H & E stain for routine histomorphological study, Van Gieson's stain for differentiating the connective tissue and muscles, Weighert's stain for observing the elastic fibers in glandstroma, Alcian blue PAS stain for studying the presence of mucopolysaccharides and Masson's Trichome method for studying connective tissue fibers to visualize the microanatomy of the organ. After preparation of histological stained sections, the micrometrical measurements were taken with the help of graduated 10X eyepiece under 10X and 40X objective lenses.

Results and Discussion

The testis was a compound tubular gland comprised of a glandular parenchyma covered by a fibrous connective tissue capsule (Fig.1).

Fig.1: Photomicrograph of cross section of cat testis showing testicular parenchyma (P), connective tissue capsule (C) and Mediastinal testis (Mt) (H & E stain, X10).

Capsule

The fibrous connective tissue capsule of testes was made up of outer tunica albugenia and inner tunica vasculosa. The tunica albugenia was thinner as compared to tunica vasculosa layer in the capsule (Fig. 2a). The capsule was having collagen fibers and sparce number of elastic fibers (Fig. 2 b,c,d). Mean thickness of tunica albuginea was 38.35±1.42 µm.

The present findings were similar to those described by Aughey and Frye (2001), Wrobel and Bergmann (2006), Bacha and Bacha (2012) and Liebich (2019) in domestic animals; Diagone *et al.* (2012) and Singh (2018) in domestic cats; Hudson and Hamilton (2010) in feline; Mehanna *et al.* (2016) in Pampas cats; Mehanna *et al.* (2018) in Hoary fox *Lycalopex vetulus*; Paul *et al.* (2020) in lion and by Kumar (2018), Singh (2018), Bhagyalakshmi *et al.* (2020) and Deka *et al.* (2021) in dogs.

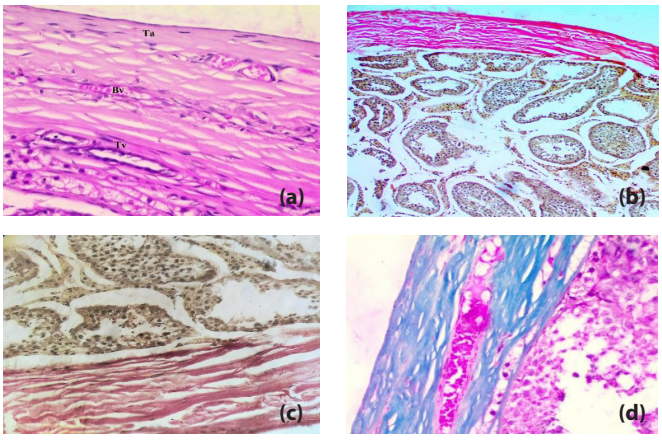

Fig. 2: Photomicrographs of section of cat testes showing (a) Capsule including tunica albuginea (Ta) and Tunica Vasculosa (Tv) with numerous blood vessels (Bv) (H& E stain, X400); (b) Pink colored collagen fibers in capsule (Van Gieson's Stain, X100); (c) Blackcolored elastic fibers in pink colored capsule (Weigert's Stain, X400), (d) Blue colored collagen fibers in capsule (Masson's Trichrome Stain, X400).

Seminiferous Tubules

Each lobule of testis was oval to coiled irregular in shape. These multilayered tubules were having basement membrane at the most peripheral part, which was made up of connective tissue. The elongated myoid cells were found at the basement membrane of the seminiferous tubules (Fig. 3a).The presence of collagen and elastic fibers was also there in basement membrane of seminiferous tubules (Fig. 3d). The mean diameter of seminiferous tubules was 243.37±2.52 µm, whereas the mean height of spermatogenic epithelium of seminiferous tubules was 65.52±0.77 µm.

Under the basement membrane, the spermatogonia cells were observed which were large in size with oval nucleus. Amongst the spermatogonia cells, there was presence of Sertoli cells. Above the spermatogonia cells, towards the lumen of the tubule, there were primary spermatocytes, which were larger than spermatogonia cells with more prominent nucleolus in the nucleus. The nucleus of primary spermatocytes was darker as compared to other cells of seminiferous epithelium. The secondary spermatocytes were located above the primary spermatocytes towards the lumen of tubule. These cells were smaller than primary spermatocytes and spermatogonia cells, whereas larger than spermatids. The spermatids were round cells located in the luminal part of seminiferous tubules with faintly stained nucleus. Elongated spermatids under the metamorphosis were also evident at several places near Sertoli cells in the lumen (Fig. 3b,c). There was average 17-18 Sertoli cells noticed per cross section of seminiferous tubules. The mean height of Sertoli cells was 38.35±1.42 µm.

The present results were completely at par with the description mentioned in domestic animals by Aughey and Frye (2001), Wrobel and Bergmann (2006) and Liebich

(2019), and in dog, cat, fox and panther by others (Kumar, 2018; Mehanna *et al.*, 2018; Singh, 2018; Bhagyalakshmi *et al.*, 2020; Paul *et al.,* 2020; Deka *et al.,* 2021). However, Wrobel and Bergmann (2006) mentioned that the nucleus of sertoli cell was deeply infolded and had a big nucleolus. No such infoldings were found in the nucleus of the Sertoli cells in the present study.

The present findings were in contrast with Egger and Witter (2009) in testis of the dog *(Canis lupus familiaris)* wherein 2-3 layers of spindle-shaped contractile cells were concentrically organised in the lamina propria of the seminiferous tubules. No such findings were obtained in the present study. The present findings were at par with Bacha and Bacha (2012) in domestic animals, except cleft like infoldings in the nucleus of Sertoli cells were mentioned by them.

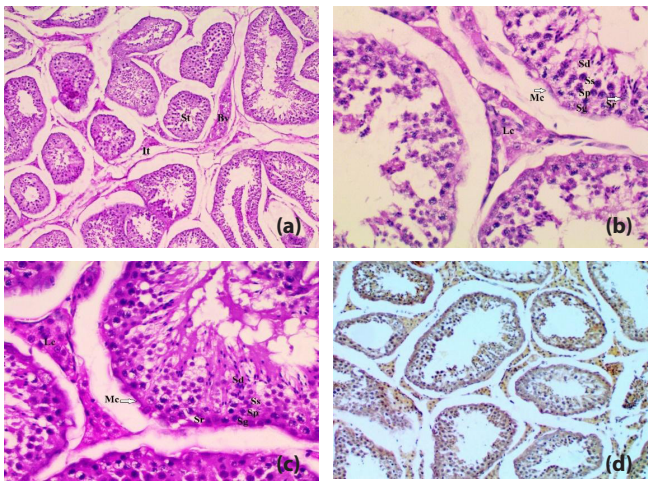

Fig. 3: Photomicrograph of section of cat testis showing (a) Seminiferous tubules (St) lined by spermatogenic cells, blood vessel (Bv) and the interstitial tissue (It) amongst the tubules (H& E stain, X100); (b) & (c) Seminiferous tubules lined by spermatogenic cells *viz.*; Spermatogonia (Sg), Primary Spermatocyte (Sp), Secondary Spermatocyte (Ss), Spermatids (Sd), Sertoli cell (Sr). Leydig cells (Lc) in the interstitial spaces and Myoid cells (Mc) (arrow) in the basement membrane of seminiferous tubule (H& E stain, X400); (d) parenchyma presenting yellow colored lining epithelial cells with black nuclei (Van Gieson's Stain, 100X).

Interstitial Tissue

There was presence of interstitial tissue between the seminiferous tubules. It contained loose connective tissue with fibroblasts, cells of Leydig (Interstitial cells) and numerous blood vessels. The Leydig cells were varying in shape and having spherical nucleus. The cytoplasm of Leydig cells was vacuolated giving the cells a foamy cytoplasm, which suggested the presence of some lipid material in it. These cells were having 7.4 µm diameter (Table 1). Clusters of Leydig cells were arranged in triangle, rectangle or irregular shape in the interstitial tissue. The presence of collagen fibers was noticed in the interstitial tissue when stained with Masson's trichrome stain and Van Gieson's stain (Fig. 3b,c). The present results were in accordance with Aughey and Frye (2001), Wrobel and Bergmann (2006), Bacha and Bacha (2012) and Liebich (2019) in domestic animals, Diagone *et al.* (2012) in domestic cats, Mehanna *et al.* (2016) in Pampas cats, Kumar (2018) and Bhagyalakshmi *et al.* (2020) in dog, Mehanna *et al.* (2018) in Hoary fox *Lycalopex vetulus* and Paul *et al.* (2020) in *Panthera leopersica.*

Mediastinal Testis

The mediastinal testis was located centrally in the testicular parenchyma. The rete testis was lined by simple cuboidal epithelial cells presenting prominent oval and horizontally placed nuclei in it (Fig. 4a, b). The collagen and elastic fibers were also noticed in the mediastinal testis (Fig. 4c,d).

The present findings were almost in accordance with findings reported in the cat by Viotto *et al.* (1993); in domestic animals by Aughey and Frye (2001); in dog by Girish *et al.* (2001) and Egger and Witter (2009). Wrobel and Bergmann (2006) described simple squamous to columnar epithelium lines the rete testis in domestic animals. Under which, the elastic fibers and contractile cells could be found in domestic animals. In present study the only epithelial cells found were simple cuboidal type. No contractile cells were noticed.

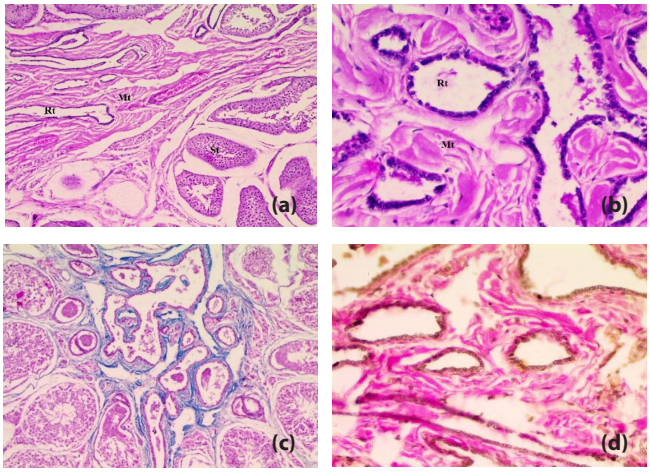

Fig. 4: Photomicrograph of section of cat testis showing (a) Mediastinal testis (Mt) including rete testis (Rt) with numerous blood vessels and loose connective tissue, Seminiferous tubules (St) and Interstitial tissue(H & E stain, X100); (b) Mediastinal testis (Mt) showedrete testis (Rt) lined by simple cuboidal cells(H& E stain, X400); (c) Mediastinal testis presenting blue collagen fibers in between pink loose connective tissue (Masson's trichrome Stain, 100X); (d) Mediastinal testis showed pink collagen fibers in loose connective tissue (Van Gieson's Stain, 400X).

Micrometry

The present findings on micrometry presented in Table 1 were almost at par with earlier reports in domestic animals (Wrobel and Bergmann, 2006), in adult jaguar (*Panthera onca*) (Costa *et al.,* 2008), in adult crab-eating fox (*Cerdo cyonthous*, Linnaeus, 1766) (Caldeira *et al.,* 2010) and in adult feral tom cats (Boher *et al.*, 2015). The present observations in cats were relatively higher than those of dogs reported by Kumar (2018) and Bhagyalakshmi *et al.* (2020). The present findings regarding diameter of seminiferous tubules were nearly at

Table 1: Micrometrical observation of cat testes (n=12)

Sr. No.	Parameters	Range	Mean ± SE	SD	CV %
1	Seminiferous tubular diameter (µm)	164.34 - 335.82	243.37±2.52	33.86	1146.60
2	Seminiferous epithelial height (µm)	46.44 - 100.03	65.52±0.77	10.36	107.40
3	Tunica albugenia thickness (µm)	142.90 - 271.51	211.49±5.87	32.15	1033.70
4	Sertoli cell height (µm)	22.20 - 55.50	38.35±1.42	8.16	66.58

par with Liebich (2019) in domestic animals, but variations were found in the thickness of tunica albuginea (1-2 mm) and height of Sertoli cells (70-80 µm) in domestic animals, which were found lower in the present work. It might be due to differences of species and age. The present results are in contrary to Yogesh (2021) with testes of dog as the mean thickness of tunica albuginea was 725.95±4.47 µm and mean height of germinal epithelium layer was 164.56 ±0.88 µm, which might be due to differences of age and species.

Histochemistry

The tunica albuginea, basement membrane of seminiferous tubules and mediastinal testis were strongly positive for PAS Alcian blue stain indicating more amount of neutral mucopolysaccharides. The seminiferous tubular epithelial cells and interstitial cells were moderately positive for neutral mucopolysaccharides (Fig. 5). The present findings were almost at par with the report of Gopikrishna *et al.* (2017) in adult ram, Liebich (2019) in horse and cat, and Bhagyalakshmi *et al.* (2020) in dog *(Canis lupus familiaris)*.

The present findings were in contrary with Gofur *et al.* (2008) as they have reported that spermatogonia, primary spermatocytes and secondary spermatocytes reacted negatively in testes of bull for neutral mucopolysaccharides.

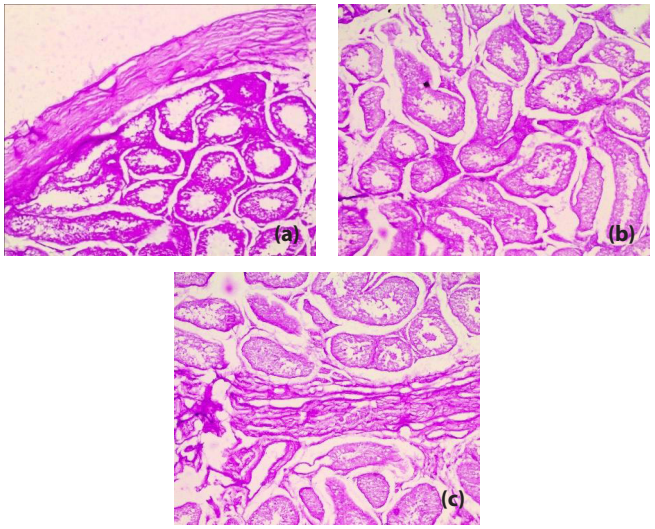

Fig.5: Photomicrograph of section of cat testis presenting (a)capsule and basement membrane of seminiferous tubules showing strong positive reaction for neutral mucopolysaccharides; (b) seminiferous tubular epithelium and interstitial tissue showing moderately positive reaction for neutral mucopolysaccharides; (c) strong positive reaction for neutral mucopolysaccharides (PAS Alcian blue stain, 100X).

Conclusions

The tunica albugenia was thinner as compared to tunica vasculosa layer in the testicular capsule of Tom cat, and comprised of mainly collagen fibers. The Leydig cells were varying in shape and having spherical nucleus. The foamy appearance due to vacuolation was suggestive of the presence of lipid materials in the cytoplasm of Leydig cells. The mediastinal testis was located centrally in the testicular parenchyma. The simple cuboidal epithelial cells of rete testis presented prominent oval and horizontally placed nuclei. There was an average 17-18 number of Sertoli cells per cross section of seminiferous tubules with the elongated myoid cells at the basement membrane. The tunica albuginea, basement membrane of seminiferous tubules and mediastinal testis were strongly positive, whereas the tubular epithelial cells and interstitial cells were moderately positive for neutral mucopolysaccharides.

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