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Gross, histology and histochemical analysis of the testis in dog (*Canis lupus familiaris*)

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Abstract
A study was conducted on the testes from 10 adult dogs. The testes were collected from the healthy dogs from Veterinary Clinical Complex, Namakkal. The testes were reddish- white in colour, oval in shape located within the scrotum. The morphometric values of the left testis were higher than the right testis. The average length, width and thickness of left was 3.73± 0.07 cm, 2.51± 0.11cm, 1.77± 0.04 cm and right testes was 3.47± 0.13 cm, 2.42± 0.08 cm, 1.72± 0.22 cm. The testis of dog was surrounded by connective tissue capsule and consisted of mainly collagen fibers, few elastic fibers, blood vessels and fibroblasts. The seminiferous tubule composed of basement membrane with seminiferous epithelium. The average diameter of seminiferous tubule was 176.659 µm. The greater part of the parenchyma was formed by seminiferous tubules. The capsule, basement membrane of seminiferous tubules, spermatogonia, spermatocytes, Sertoli cells and interstitial cells or Leydig cells were moderately positive for PAS.

Keywords: Dog, testis, gross, histology and histochemistry

Introduction
The dog has been domesticated longer than any other animal. Dogs have been using for various purposes such as crime detection, battle, guide for disabled, guarding animals like sheep/goat, detection of drugs as well explosives etc. Testes are the major male reproductive organ producing spermatozoa and testosterone [1]. The gross morphology and histology of testis are the essential base for understanding normal physiology, histopathology, endocrinology, surgical anatomy and to evaluate the breeding efficiency in dogs. Meager reports are existing on the histology of testis in dog when compared with other domestic animals. Yamauchi *et al*., obviously described the histological variations in the dog testis during sexual maturity in detail. In male dogs, breedability depends on the production of androgens by the interstitial cells of testis, which also influence the process of spermatogenesis [2, 3]. Testicular architecture disorganizes in various diseases related to the testis of male dogs, such as testicular tumors (TT) which are the most common neoplasms [4]. Others being seminomas and Leydig cell tumor [5, 6]. A complete postnatal development of testis is necessary not only to study the structural development but also to comprehend the variation of spermatogonial cells and determine the proper age of sterilization. The present study, gives basic knowledge on the histoarchitecture and histochemistry of testis in adult dogs.

Materials and Methods
Testes from ten normal, healthy adult dogs were collected immediately after castration from the Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal. The testes were washed with normal saline. The average length, width and thickness of testis were measured with vernier caliper. The tissue pieces were collected and fixed in 10% Neutral buffered formalin (NBF). The fixed tissue pieces were processed for paraffin sectioning. Paraffin sections of 3-5 µm thickness were made using Leica Rotary Microtome (RM 2145) and stained with routine haematoxylin and eosin for studying the histoarchitecture, Van Gieson's method for collagen fibers, Gomori's reticuline method for reticular fibers [7], Weigert's resorcin fuchsin for elastic fibers [8], Periodic Acid Schiff reaction with and without saliva for mucopolysaccharides and Millon's reaction for tyrosine. The histomorphology of the testis was studied with the help of Leica trinocular microscope with image analyzer (DM 1000).

Results and Discussion

Gross structure

The testes were compound tubular glands reddish-white in colour with oval shape located within the scrotum. The testes of the dog were located dorso-caudally at the perineal region and their long axis was oblique in direction. Dyce *et al*. mentioned that the long axis of testes were vertical in ruminants, horizontal in horses and dogs and tilted towards the anus in pigs and cats. The epididymis lies on the dorsolateral surface of the organ [9, 10]. Each testis was roughly oval in outline, laterally compressed and consisted of two surfaces, two borders and two extremities. Lateral surface was convex and medial surface was flattened. Anterior border was free and convex whereas the posterior border was attached with epididymis (Fig. 1a) [11-13]. Both of the testes were covered with connective tissue capsule called tunica albuginea (Fig. 1b) [10, 14]. The mediastinum testis was a fibrous extension of the tunica albuginea which extends along the long axis of the testis and spread as radiating sheets of connective tissue in testicular parenchyma (Fig. 1c&d) [15]. Mediastinum testis was white in colour, central and well developed in dogs [12].

The average length, width and thickness of left testis was 3.73± 0.07 cm, 2.51± 0.11cm, 1.77± 0.04 cm whereas the right testis was 3.47± 0.13 cm, 2.42± 0.08 cm, 1.72± 0.22 cm (Table.1). The left testis was observed to be larger and longer than right testis, which is in accordance with the findings of Seema Sikarwar *et al.*, in pig (Fig. 2**)** and Hamid Karimi *et al.*, in ghezel rams [14, 16].

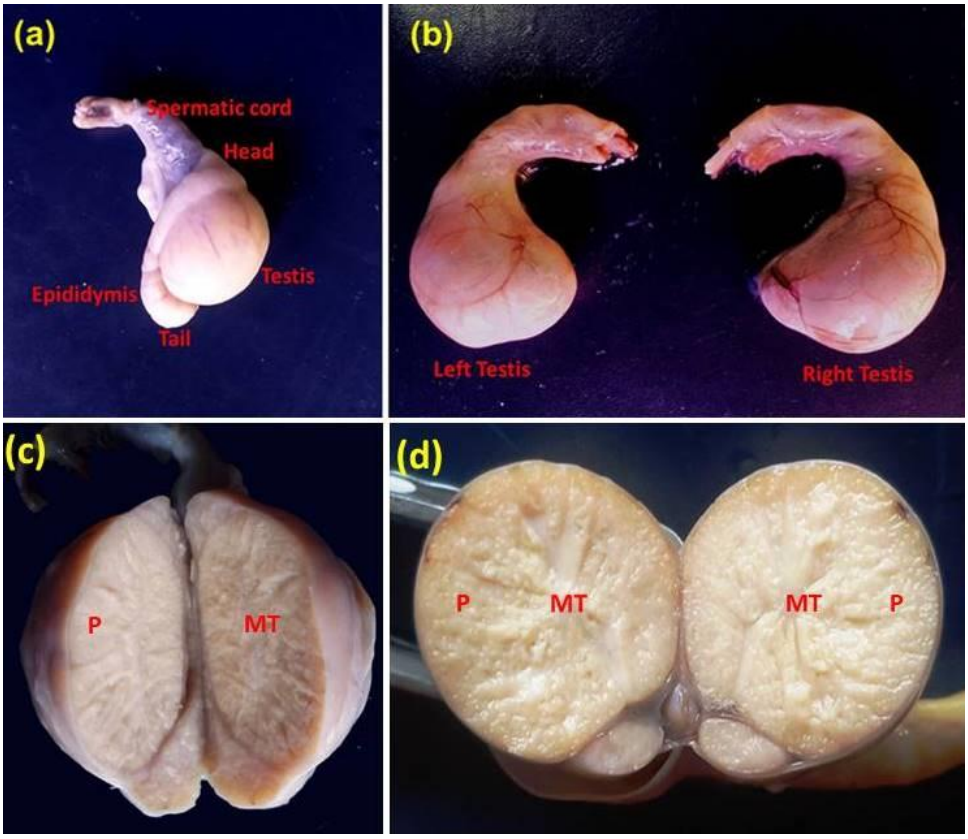

Fig 1: Gross photograph of testis in adult dog showing (a) Spermatic cord, Epididymis and Testis, (b) Left and right testis, (c&d) Vertical and horizontal bisected testis showing mediastinum testis (MT) and parenchyma (P).

Table 1: Biometrical values of the testes of dog.

Parameters	No. of animals	Left testis	Range	Right testis	Range
Length (cm)	10	3.73±0.07	3.34-4.67	3.47±0.13	2.57-4.49
Width (cm)	10	2.51±0.11	2.23-2.83	2.42±0.08	2.22-2.64
Thickness (cm)	10	1.77±0.04	1.35-2.13	1.72±0.22	1.34-1.94

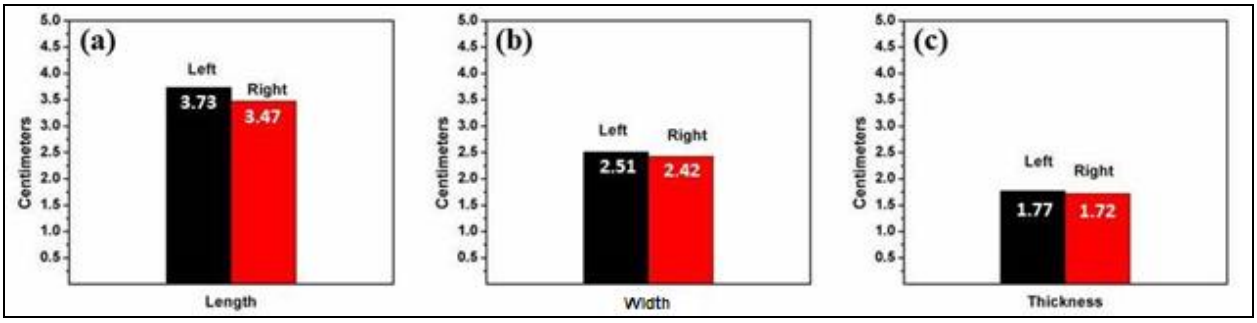

Fig 2: showing the comparison of length (a), width (b) and thickness (c) of left and right testes of dog.

Histology

The testis of dog was surrounded by a dense irregular connective tissue capsule, termed as tunica albugenia which composed of mainly collagen (Fig. 3a&d), few elastic and reticular fibers along with few blood vessels and fibroblasts. The septula testis (trabeculae) were connective tissue strands which divided the testicular parenchyma into different number of testicular lobules, each lobule consisted one to four seminiferous tubules. The septula testis composed of collagen fibers. The collagen fibers were noticed at capsule and around basement membrane of the seminiferous tubule by Van Gieson's stain (Fig. 3a) and Masson's trichrome stain (Fig.3d). Elastic fibers were primarily noticed in the blood vessels of tunica vasculosa by Weigert's stain (Fig. 3c) [17, 18]. The reticular fibers were observed at capsule and around basement membrane of the seminiferous tubule by Gomori's reticulin stain (Fig. 3b). The interstitial tissue was consisted of delicate loose connective tissue fibers and blood vessels surrounding the seminiferous tubule [19].

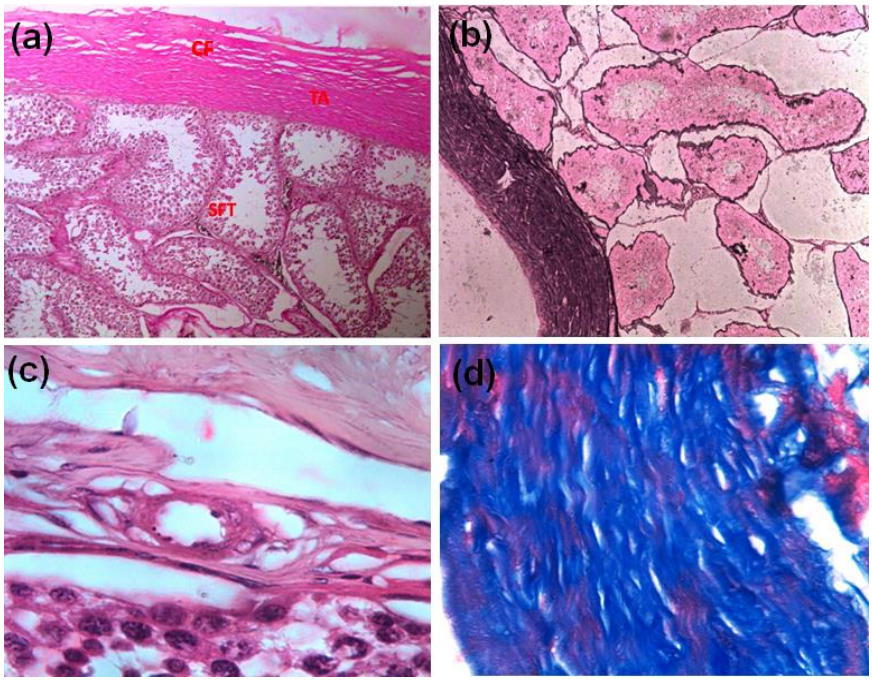

Fig 3: Photomicrograph of testis in adult dog showing different fibers with various stains (a) Van Geison's (×400), (b) Gomori's reticulin (×400) (c) Weigert's (×1000) (d) Masson's trichrome (×1000) SFT - Seminiferous tubules, TA-Tunica albugenia and CF- Collagen fibers

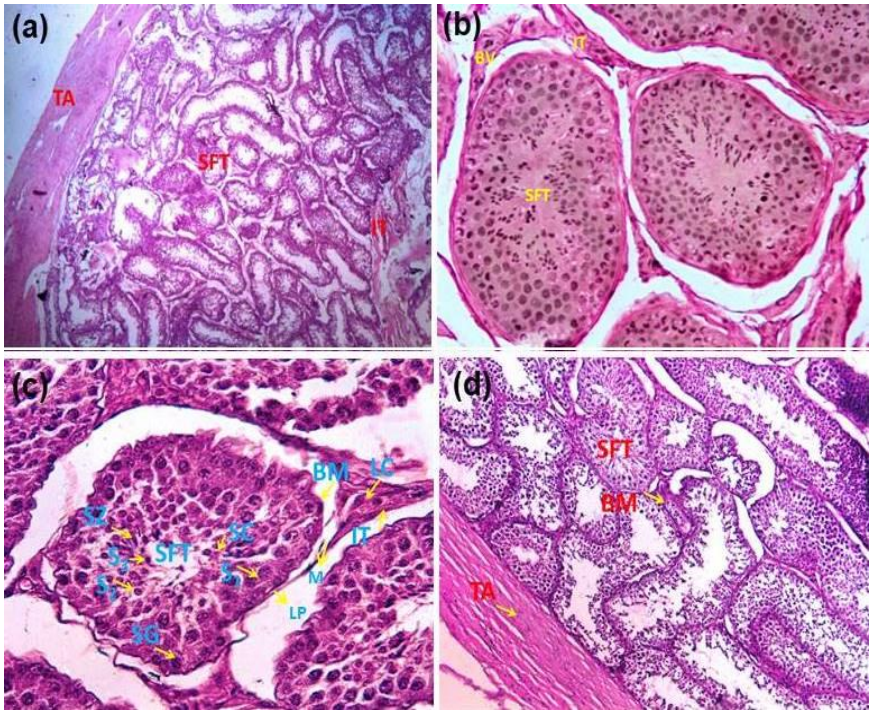

Fig 4: Photomicrograph of testis in adult dog showing (a) Tunica albugenia (TA), seminiferous tubules (SFT). H&E X40 (b) Seminiferous tubules (SFT), Blood vessel (BV) and Interstitial tissue (IT) Van Gieson's X400 (c) Seminiferous tubules (SFT), Basement Membrane (BM), Sertoli Cells (SC), Spermatozoa (SZ), Primary Spermatocytes (S_1), Secondary Spermatocytes (S_2), Spermatid (S_3), Spermaogonia (SG), Leydig cell (LC), Myoid cells (M) and Interstitial Tissue (IT) H&E X1000 (d) Seminiferous tubules (SFT), Basement membrane (BM) and Tunica Albugenia (TA) PAS X100

The testicular parenchyma in adult dog consisted numerous highly convoluted seminiferous tubules lined by a stratified epithelium which showed two distinct populations of cells *i.e.*, spermatogenic cells and non spermatogenic cells [20]. Various shapes of seminiferous tubules were observed such as circular, oval, curved, elongated and straight tubules (Fig. 4a). The average diameter of seminiferous tubules was 176.659µm. The myoid cells with flattened shape were also observed on the external part of the basement membrane. Each seminiferous tubule of the testis displayed basement membrane, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, Sertoli cells and spermatozoa (Fig. 4c) [18, 21]. The seminiferous tubule showed small round with darkly stained nucleus cells called spermatogonia near basement membrane. The spermatogonia divided into primary and secondary spermatocytes. In the present study, both primary and secondary spermatocytes were observed in which primary spermatocytes were spherical in shape with biggest darkly stained nucleus whereas the secondary spermatocytes have spherical nucleus with scattered chromatin and smaller than the primary spermatocytes. The secondary spermatocytes form spermatids. The spermatids were spherical with pale nuclei which appeared near lumen of the seminiferous tubule. The clumps of elongated spermatozoa were observed in the lumen of the seminiferous tubule. The Sertoli cells were tall columnar cells present between spermatogenic cells which were arranged radially from basement membrane to lumen of seminiferous tubule (Fig. 4c). The interstitial tissue composed of interstitials spaces and interstitial endocrine cells of testis namely Leydig cells, blood vessels and nerve fibers which were also reported in pig and in ram (Fig. 4b) [21, 22]. Leydig cells were polygonal cells present in between seminiferous tubules arranged in the form of clusters and consisted of granular cytoplasm with spherical nucleus. These findings are in agreement with Dhyana *et al.*, in pig, and Gofur *et al*., in bull [22, 17]. The walls of the convoluted seminiferous tubule consisted of thin basement membrane which was supported by reticular fibers (Fig. 4d) [17, 23].

Histochemistry

The periodic acid Schiff (PAS) staining method was used to demonstrate the glycogen in various components of the testis. The capsule, spermatogonia, primary and secondary spermatocytes, Sertoli cells and interstitial cells or Leydig cells were moderately positive for PAS which indicates the presence of mucopolysaccharides (Fig. 4d). The basement membrane of seminiferous tubules was strongly positive for PAS which indicates the presence of mucopolysaccharides. These observations are in agreement with Gopikrishna *et al*., in adult ram [24].

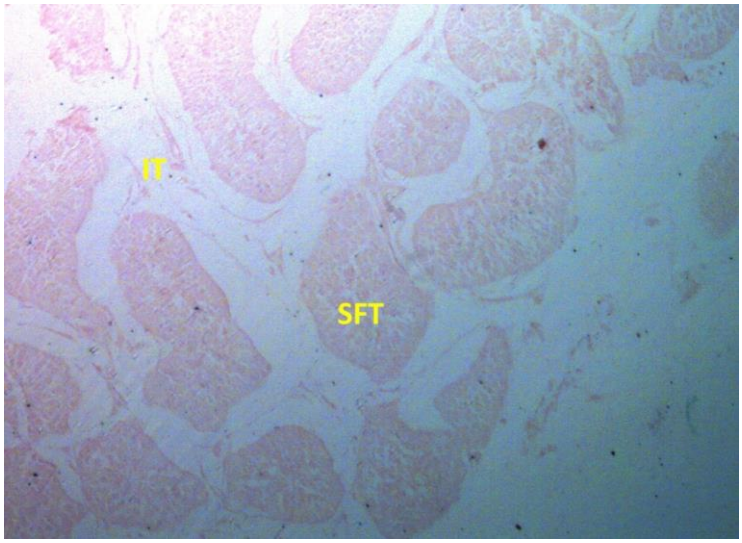

Fig 5: Photomicrograph of testis in dog showing seminiferous tubules (SFT), and interstitial tissue (IT) Millon's reaction X100

The capsule, basement membrane of seminiferous tubules, spermatogonia, primary and secondary spermatocytes, spermatids, Sertoli cells and Leydig cells were moderately positive for tyrosine (Fig. 5) and these findings are in agreement with the reports of Rao, *et al*., in the testis of domestic duck [25].

Conclusion

The testes of dog were compound tubular glands reddish-white in colour with oval shape located within the scrotum. The left testis was observed to be larger and longer than right testis. The testis was surrounded by a dense irregular connective tissue capsule, termed as tunica albugenia which composed of mainly collagen, few elastic and reticular fibers along with few blood vessels and fibroblasts. The testicular parenchyma in adult dog consisted numerous highly convoluted seminiferous tubules lined by a stratified epithelium. The average diameter of seminiferous tubules was 176.659µm. The capsule, spermatogonia, primary and secondary spermatocytes, Sertoli cells and interstitial cells or Leydig cells were moderately positive for PAS.

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