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Study on the Histoarchitecture of the Testis of Indian Mongrel Dogs

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ABSTRACT

The present study was on study on the micro anatomical details of the in indian mongrel dogs from pre and postpubertal age groups. The microanatomical details of the testes in Indian Mongrel dogs of pre-and post-pubertal groups revealed that testis was compound tubular gland covered by tunica albuginea composed of collagen, elastic, reticular and smooth muscle fibres. A well developed septulae testis divided the gland into lobuli testis. A fibrous mediastinum testis occupied a central position along the longitudinal axis of the testis. The seminiferous tubules made the bulk of the parenchyma. Seminiferous epithelium consisted of Type A and Type B spermatogonia, primary spermatocytes, secondary spermatocytes, round spermatids, elongated spermatids and spermatozoa in different stages of development and differentiation with Sertoli cells in between. The peritubular tissue consisted of collagen, elastic fibres along with few fibroblast-like cells and myoid cells. The interstitial tissue consisted of numerous Leydig cells, few fibrocytes and blood and lymph vessels in loose connective, trabeculae, mediastinum testis,seminiferous tubules and interstitial tissue.The histological structure of right and left testis was found to be similar.

Key words: Testis,histology,dog.

INTRODUCTION

A steady increase in the pet and stray animal population has paved way for increase in the infertility cases in both canine and feline species and emerging as a major threat to public health. This created the need to study and understand the canine and feline breeding and infertility. The testis functions both as an endocrine as well as an exocrine gland. The maturation of the reproductive system depends on androgen production by interstitial cells and the generation of an optimal number of viable spermatozoa from the testes (Hafez and Hafez, 2000). At various ages, males under go certain androgenic phases, during which Leydigcells of the testis secrete testosterone that influences the development of testis and accessory reproductive structures. The characterization of puberty with the associated changes in the reproductive organs is the main criteria in the selection of breeding male (Verma and Sahni, 1997). The impact of age on male fertility has been extensively studied men. However, such information is limited in dogs. In humans, testicular volume, the number of germ cells and Sertoli cells, serum testosterone levels, daily sperm production, sperm viability, and sperm fertilizing capacity are known to decrease in oldermen (Gunes *etal*., 2016). A thorough knowledge of histological changes is essential to gain a comprehensive understanding of reproductive physiology and to form a baseline for the interpretation of various pathological conditions of the testis and duct system. In that view the present study was designed in such away to create basic documentary evidence about the histological details of testis in dogs during pre-pubertal and postpubertal stages.

MATERIALS AND METHODS

The studies on the testes of the dogs were conducted at the Department of Veterinary Anatomy, Madras Veterinary College, Chennai–600007. The testicular samples for the research work were collected from the Small Animal operation unit, Teaching Veterinary Hospital, Madras Veterinary college. The samples were collected from the apparently healthy animals admitted for the elective castration. The age of the dogs were determined based on the owner's history.

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According to the age, the animals were classified into two groups as pre-pubertal and post-pubertal animals. The animals were subjected to castration as per the standard protocol under strict aseptic conditions. The tissue samples were washed with normal saline and fixed in 10 percent neutral buffered formalin and Bouin's fixatives. The tissue samples were processed as per the standard procedures (Spencer and Bancroft, 2013). Sections of 5–6 µm thickness were used for the routine and special staining techniques namely, Haematoxylin and Eosin method (Bancroft, 2013), Masson's Trichrome method, Van Gieson's method for collagen fibres and Gomori's silver method for reticular fibers (Luna, 1968). The micrometrical observations were recorded by calculating the average of four microscopic fields from each prepared slide.

RESULTS AND DISCUSSION

Tunica albuginea

In the present study, in Indian Mongrel dog the tunica albuginea was a dense irregular with a connective tissue, composed mainly of collagen, (Fig.1) few elastic and reticular fibres (Fig.2). Few fibroblasts were observed between the collagen fibre bundles and few smooth muscle fibres were also noticed. A similar observation was made by Sharma *et al*. (2011) in dogs, Bacha and Bacha (2000) in boar. However, Islam *et al*. (2002) reported that tunica albuginea in the Black Bengal goat did not contain any smooth muscles fibres, where as, Trautmann and Fiebiger (1957) reported that the tunica albuginea was predominantly muscular in horses. Smooth muscle cells are the contractile units responsible for spontaneous contractions of testicular capsule which may play an important role in non-motile spermatozoa out of the testis (George *et al*., 1978). The tunica albuginea was thinner in post-pubertal animals which may be attributed to an increase in parenchymatous testicular tissue with advancing age (Table 1). The tunica vasculosa was abutting the parenchyma in dogs, where as it was located in the middle of the tunica albuginea in horses and boars (Trautmann and Fiebiger,1957).

Septulae testis

In the present study, the septulae testis extended from the capsule and divided the testicular parenchyma into a number of lobuli testis (Fig.3). These findings concur with the observations of Archana *et al.* (2008) in goats, who described the septula testis as connective tissue strands extending from the lateral, anterior and posterior sides of the mediastinum testis, joining the tunica albuginea at the periphery and dividing the testicular parenchyma into various small lobuli testis. In this study, the trabeculae were composed of thick strands of collagen, elastic and reticular fibres in which blood and lymph vessels were observed. These trabeculae varied in thickness and converge centrally. Thickness of septulae testis was found to be more in post-pubertal group when compared to pre-pubertal group.This agrees with the findings of Banks (1993) in domestic animals. Bacha and Bacha (2000) opined that the septulae testis were thinner in ruminants and thicker in carnivores, stallions and boars.

Mediastinum testis

In the present study, the mediastinum testis in dogs occupied a central positional on the longitudinal axis of the testis (Fig.4). Similar observations have been reported in other domestic animals, except in horses where the mediastinum testis is absent (Getty, 1977). In goats, the mediastinal testes lay as an axial connective tissue strand in the central parenchyma of the testis, extending dorso-ventrally from the cranial extremity towards the caudal extremity for about $3/4^{th}$ of the length of the testicular parenchyma (Archana *et al.*, 2008). In this study, the mediastinum testis was composed mainly of collagen fibres and lodged the rete testis. In dogs, the mediastinum testis consisted of tubular and intertubular components. The intertubular components was composed of dense fibrous tissue with a few elastic fibres, reticular fibres and smooth muscle cells (Fig. 5), as reported by Girish *et al*. (2001) in dogs and Eurell and Frappier (2006) in boars, ruminants and dogs. Additionally, Archana

et al. (2008) found that in goats the mediastinum testis was loose and gelatinous in neonatal kids but became whiter and denser in pubertal and post pubertal goats.

Lobuli testes

The lobuli testes were wedge-shaped portion soft testicular parenchyma. Each lobuli contained convoluted seminiferous tubules (Fig.6). Similar observations were reported by Sharma *et al.* (2011) in dogs and Eurell and Frappier (2006) in domestic animals.

Seminiferous tubules

In the present study, the wall of the seminiferous tubules consisted of an outer lamina propria and an inner seminiferous epithelium. The lamina propria was composed predominantly of collagen fibers with a few elastic fibres (Fig.7). This finding is in accordance with the observations of Trautmann and Fiebiger (1957) in domestic animals. The inner seminiferous epithelium consisted of two different types of cell populations i.e., non-proliferating Sertoli cells and highly proliferating spermatogenic cells at different stages of development (Fig.8). This observation agrees with the reports of Banks (1993) and Dellmann and Brown (1986) in domestic animals. The diameter of the seminiferous tubules and height of the seminiferous epithelium in the post-pubertal group were greater when compared to the pre-pubertal group (Table 1). Similar observations were recorded by Franca *et al,* (2000) in domestic cats, White *et al.* (2005) in Grizzly bears and Nishimura *et al*. (2000) in male Tokara (Japanese native) goats. In the pre-pubertal group myoid cells (Fig.9) were numerous and smaller in size. In contrast, in the post-pubertal group, myoid cells became lesser in number but larger in size and were arranged in a single layer external to the seminiferous tubules (Fig.10). Similar findings were reported by Zade (2007) in dogs and Copenhaver *et al.*(1978) in lower animals.

Seminiferous epithelium

The seminiferous epithelium was stratified in nature and consisted of spermatogenic cells and Sertoli cells. The spermatogenic cells at different phases of development were located between the sertoli cells.This observation agrees with the findings of Zade (2007) in dogs, Gofur *et al.*(2008) in bulls and Abdul *et al.*(2011) in goats.

Spermatogenic cell

Spermatogenic cells consisted of sperm atogonia, primary spermatocytes, secondary spermatocytes, round spermatids, elongated spermatids and spermatozoa at different stages of development and differentiation. The sperma togonia were small and rounded in shape with a darkly stained, centrally placed rounded nucleus. These cells were arranged in a single layer adjacent to the basement membrane. Two types of Spermatogonia were identified based on nuclear morphology–Type A and Type B. Of these, Type A served as reserve cells and type B cells undergo mitotic division to form primary spermatocytes. This observation is in agreement with the findings of Zade (2007) in dogs. The primary spermatocytes were located above the spermatogonia and were large cells with spherical nuclei. The secondary spermatocytes were smaller than the primary spermatocytes but larger than the round spermatids. They were rounded in shape, with centrally placed spherical nuclei showing small dot-like chromatin clumps. The nuclear membrane was distinct with indistinct nucleolus. The cytoplasm was scanty and eosinophilic (Fig.11). The early spermatids were spherical and occurred in bundles towards the lumen of the seminiferous tubules, while late spermatids were elongated with an oval head and along faint tail. Similar observations were made by Zade (2007) in dogs. There was significant difference observed in the height of the seminiferous epithelium beween the pre and post pubertal age groups (Table 1).

Sertoli Cells

In the present study, Sertoli cells were tall and triangular with a broad base resting on the basement membrane. The nucleus was basal portion of the cells. The centrally placed nucleolus was large and intensely stained (Fig.8) this is in accordance with the findings of Paul *et al.* (2020) in Asiatic lion.

Interstitial tissue

The interstitial tissue occupied the space between the adjacent seminiferous tubules and consisted of numerous Leydig cells, a few fibrocytes and blood and lymph vessels embedded in loose connective tissue. Fawcett *et al.*(1973) explained the organization of the interstitial tissue in the mammalian species, stating that Leydig cells and interstitial tissue were minimal in laboratory animals. Clusters of Leydig cells were widely scattered in loose connective tissue drained by lymphatic vessel in ram, bull, elephant and monkey, where as these cells were a dominant feature with minimal interstitial tissue in boar, zebra, and opossum. In the present study, Leydig cells occurredin groups as reported by Trautmann and Fiebiger (1957). Leydig cells were polyhedral in shape, with spherical nuclei positioned eccentrically. This is in line with the findings of Banks (1993) in domestic animals.

Table -1 Statistical analysis of micrometrical observation of various parameters of the right and left testis of pre- and post-pubertal indian mongrel dog

Sr. No.	Parameter	Groups	Mean ± SE		RESULT
			Right	Left	
1.	Thickness of testicular capsule (µm)	Prepubertal	151.56 ± 45.52	151.03 ± 45.50	S
		Post pubertal	136.10 ± 23.94	135.31 ± 24.65	S
2.	Diameter of the seminiferous tubules (µm)	Prepubertal	40.50 ± 10.17	40.41 ± 10.16	S
		Post pubertal	102.07 ± 17.90	102.27 ± 17.74	S
3.	Height of geminal epithelium	Prepubertal	18.47 ± 5.45	18.08 ± 5.55	S
		Post pubertal	29.12 ± 6.75	28.32 ± 6.81	S
4.	Number of sertoli cells /mm^2	Prepubertal	36.52 ± 9.74	36.54 ± 10.15	S
		Post pubertal	16.88 ± 2.39	16.82 ± 2.37	S

** = t <0.01, * = t <0.05 and t >0.05 = Non-significant (NS)

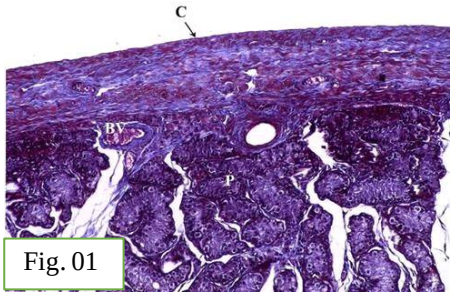
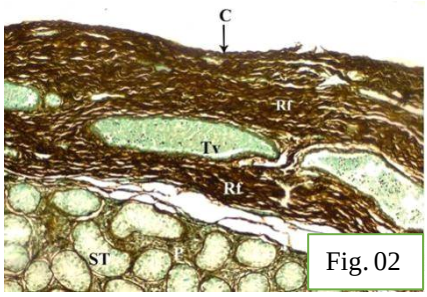


Fig. 01



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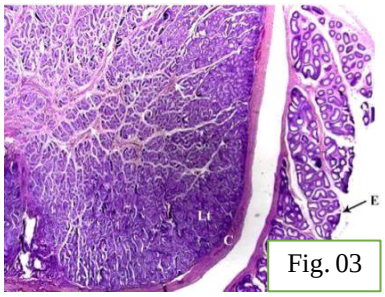


Fig. 03

Fig.1 Photomicrograph of testis from pre-pubertal dog showing the capsule (C), parenchyma (P) and Blood vessel (BV), **Fig.2** Photomicrograph of testis from pre-pubertal dog showing the reticular fibers (Rf) in the capsule (C) and parenchyma (P). Seminiferous tubule (ST), Tunica vasculosa (Tv)**.Fig.3** Photomicrograph of testis from a pre-pubertal dog showing septulae testis (arrows), capsule (C), epididymis (E), lobuli testis (Lt) and blood vessel (BV).

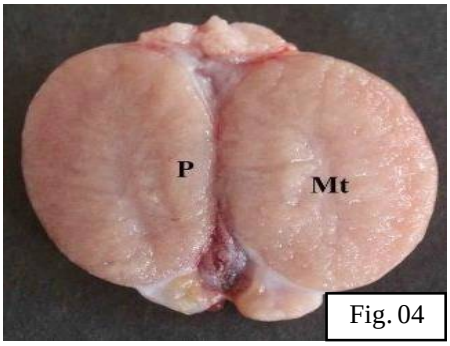
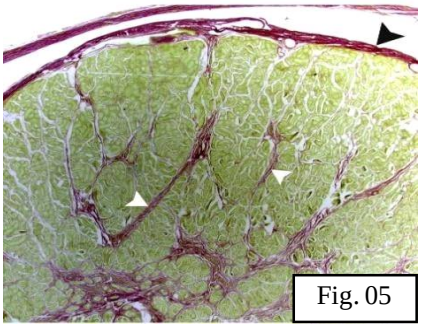


Fig. 04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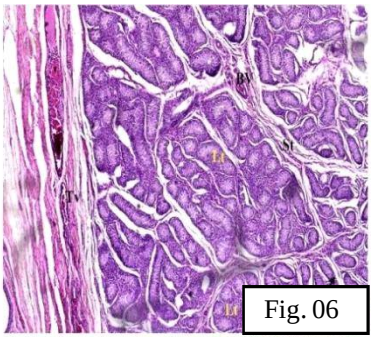

Fig. 06

Fig.4 Photogrph of post-pubertal testis of dog (c.s) showing P-Parenchyma Mt-Mediastinum testis;**Fig.5** Photomicrograph of pre pubertal dog testis showing the distribution of collagen fibres (arrow heads) in the capsule and parenchyma,Van Gieson's Method X 12.5; **Fig.6** Photomicrograph of testis from pre pubertal dog showing the lobuli testis (Lt), St- Septulae testis, BV-Blood Vessel, Tv-Tunica vasculosa, H& E X 50.

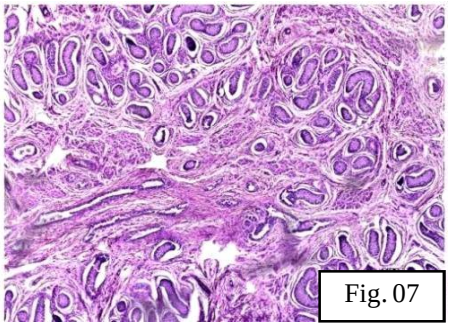
Fig. 07

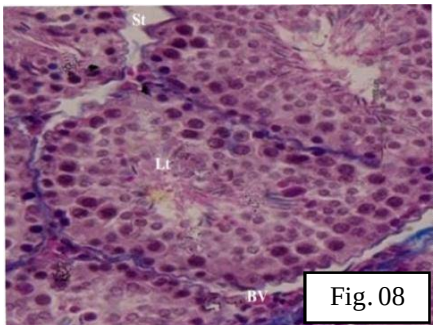

Fig. 08

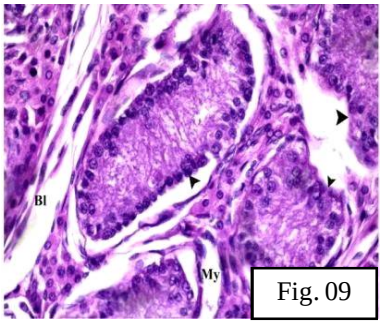

Fig. 09

Fig.7 Photomicrograph of testis from pre-pubertal dog showing the distribution of seminiferous tubules (ST) in testicular parenchyma H& E X 50;**Fig.8** Photomicrograph of testis from post- pubertal dog showing the cellular population in the seminiferous tubules Lt- Lobuli testis (Lt), St- Septulae testis, BV-Blood Vessel,H& E X 50; **Fig.9** Photomicrograph of testis from pre pubertal showing the seminiferous tubules Bl-Basal lamina, Arrow heads-Cellular layer My-Myoid cell, H& E X 400

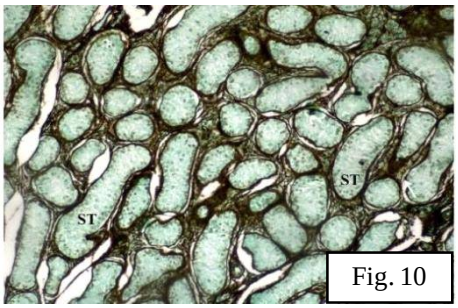

Fig. 10

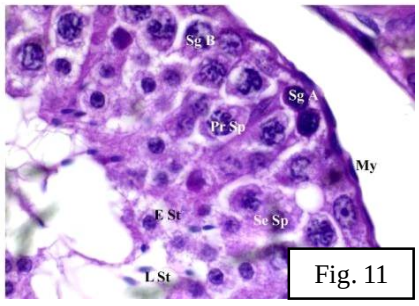

Fig. 11

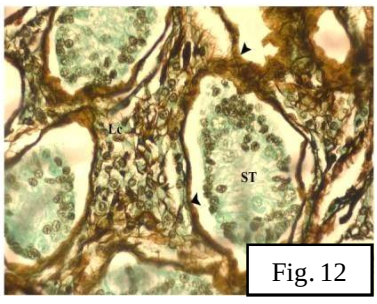

Fig. 12

Fig.10 Photomicrograph of testis from post pubertal dog showing the distribution of reticular fibres in the parenchyma, ST-seminiferous tubules Gomori's method X 100; **Fig11** Photomicrograph of testis from pre-pubertal dog showing the seminiferous epithelium My-Myoid cell, SgA-Type-A Spermatogonia, SgB-Type-B Spermatogonia, Pr Sp-Primary Spermatocyte, Se Sp-Secondary Spermatocyte ,E St-Early spermatid L St-Late spermatid, H& E X 1000;**Fig.12** Photomicrograph of testis from pre pubertal dog interstitial tissue Le-Leydig cells, ST- Seminiferous Arrow heads – Peritubular connective tissue Gomori's method X
400

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