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Studies on Postnatal Development of Testis of Guinea Pig (*Cavia porcellus*)

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Abstract

The histology and histochemistry of the testis of guinea pig of various postnatal age groups was conducted. A total of 24 guinea pigs of four different postnatal ages with six male animals each were collected from the Department of Laboratory Animal Medicine, Madhavaram Milk Colony, Chennai as per the Ethical committee approval. After collection, animals were euthanized as per CPCSEA norms and testis was dissected out and was cut into small pieces, fixed and processed for paraffin embedding. Sections of 4–5 µm thickness were cut and used for the routine and special histological and histochemical staining techniques. Testes wereencapsulated by tunica vaginalis and tunica albuginea. Septa from the capsule divided the testicular parenchyma into lobules. Each lobule consisted of seminiferous tubules which consisted of spermatogenic cells in stratified layers and sertoli cells. Pre-weaning and weaning group of guinea pigs seminiferous tubules showed wide lumen with only type 1 and type 2 spermatogonia and sertoli cells. Young and adult animals seminiferous tubules showed narrow lumen with type 1 and type 2 spermatogonia, primary spermatocytes in various stages of differentiation, secondary spermatocytes, spermatids (early and late) and sertoli cells. Sertoli cells were large oval shaped cells with lightly stained irregular shaped nucleus. Interstitial tissue contained leydig cells in all ages.Leydig cells appeared as varied in shape. In all the age groups studied, PAS activity was noticed in the capsule and basement membrane. The micrometrical parameters increased as age advanced in both right and left testis.

Keywords: Postnatal development, histology, histochemistry, testis, guinea pig

1. Introduction

Guinea pigs are large rodents and best experimental animals for physiological, pharmacological, clinical and anatomical research because of their large body weight, stout, compact body, easiness of handling and rapid adaptation in laboratory situation (Rowlands and Weir, 1974). Guinea pig (*Cavia porcellus*) is also known as cavy and was probably first introduced into Europe from South America some 400 years ago (Wagner and Manning, 1976). The usage of rodents as experimental model was well known, guinea pigs being chosen as subjects in many studies (Stan, 2015). The male reproductive components of laboratory animals were

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relatively similar in most species and included the principal organs namely the testis, spermatic ducts and accessory sex glands (Barone, 2001). The male reproductive system consists of testes, epididymis, ductus deferens, accessory sex glands and penisas stated by Stan (2015) in guinea pigs and Sharma et al. (2011) in dogs. The development of the male reproductive organs was influenced by various factors like hormone composition and androgenic stimulation in rodents (Cepeda et al., 2006). Environmental factors, time of year and mating season also influenced the morphology and function of the reproductive organs (Breedet al., 2014). The morphology of the principal organs of the reproductive system in guinea pigs was common with that of other species like cattle, sheep, swine, lagomorphs and even humans (Stan, 2015). The histology of the testis consisted of seminiferous tubules and interstitial tissue. A myoid cell layer of contractile nature was found surrounding the basement membrane of the seminiferous tubules of rodents (Maekawa et al., 1996). Rodents testis had scanty interstitial connective tissue whereas human and dog testis had distinct connective tissue (Foley, 2001). The adult seminiferous epithelium was divided into 14 stages in rats and 12 stages in mice based on the associations of spermatogenic cells in four cycles of spermatogenesis (Meistrich and Hess, 2013 and Nakata et al., 2015). The function of the testes is to produce sperm and male sex hormone testosterone (Andromaand Khasanah, 2017). The knowledge regarding histomorphological growth of male reproductive organ is necessary to understand the normal physiology, surgical anatomy and breeding aspects (Hassan et al., 2018). The histological and histochemical studies on the testis of adult guinea pigs were studied by Bansal et al. (2009). The histological and micrometrical changes in the descending testis during the postnatal development of rabbit were studied by Ahmed et al. (2012), Pathak et al. (2016), Gopi et al. (2017), Hasanin et al. (2018). Mehanna et al. (2018) studied the normal histoarchitecture of testis of adult rams, adult goats, adult male albino ratand hoary fox. Ultrastructural changes of spermatogensis during pubertal and postpubertal stages were studied by Simoes et al. (2016) in guinea pigs. Postnatal differentiation of spermatogenic cells in the testis of rams were studied by Kishore et al. (2012). Leydig cells were the testosterone producing cells and were located in the interstitial tissue between the seminiferous tubules (Diagone et al., 2012). Testicular weight is an important parameter in the reproductive evaluation of males owing to its high and positive correlation to sperm production in rats (Soliman et al., 2014). Large scrotal circumference is also associated with early puberty, more sperm, a higher percentage of morphologically normal sperm, and better reproductive performance in closely related females (Kastelic, 2014). Spermatogeneis was studied in different mammals under normal and various disease conditions which would pave the way for regeneration of sperm production (Santos et al., 2013). Literature available on the postnatal development of histology and histochemistry of testis in guinea pigs is scanty. So the present investigation is undertaken to study the postnatal histological and histochemical development of testis of guinea pigs.

2. Materials and Methods

The histology and histochemistry of testis of Dunkin Hartley strain of guinea pig from postnatal age groups (Table 1) was conducted at the Department of Veterinary Anatomy, Madras Veterinary College, Chennai, Tamilnadu, India. Guinea pigs were procured from the Department of Laboratory Animal Medicine, Madhavaram Milk Colony, TANUVAS, Chennai-51 as per ethical committee approval (Lr. No. 1467/DFAB/IAEC/2018 dated 13.07.2018). After collection of the guinea pigs, they were euthanized as per the standard operating procedure by using the carbon dioxide asphyxiations as per CPCSEA norms and they were subjected for the dissection.

Table 1: Details of postnatal age groups of guinea pigs used for research work

Age groups	Preweaning 0-2 weeks	Weaning 2-8 weeks	Young 8-16 weeks	Adult 16-32 weeks	Total
No. of animals	6 (190-196 g)	6 (270-283 g)	6 (613-644 g)	6 (926-1013 g)	30

After careful dissection of the animals, both right and left testis wasremoved out from the scrotal sacs. After recording the morphology and both the testis were cut into small pieces. They were washed in the normal saline and fixed in 10% neutral buffered formalin andBouin's fluid for general and special histological studies. Then the tissues were dehydrated in the ascending grades of the alcohol cleared in xylene and embedded in paraffin (58-60°C). Sections of 4-5 µm thickness were cut and used for the routine and special histological and histochemical staining techniques. The following histological and histochemicaltechniques were applied for the study (Table 2).

Micrometrical measurements namely capsule thickness and diameter of seminiferous tubules in both the right and left testis of all postnatal age groups were recorded. Arithmetic mean and the standard error for the micrometrical data were calculated as per Snedecor and Cochran (1994).

3. Results and Discussion

Histological findings of the present study observed that the testis was composed of capsule, septa, seminiferous tubules, interstitial tissue and rete testis. Similar findings were also observed by Stan (2015) in adult guinea pigs. Histological and histochemical observations of right and left testis appeared similar in all the age groups studied. Similar findings were also recorded by Hanumant (2016) in goat.

3.1. Capsule and septa

The capsule covered the testicular parenchyma and was found

Table 2: Details of histological and histochemical techniques applied to the testis of postnatal age group of guinea pigs

Sl. No.	Histological and histochemical staining technique	Purpose	Reference
1.	Standard haemotoxylin and eosin method for paraffin sections	Routine histological observations	Bancroft and Stevens, 1996
2.	Masson's trichrome staining	Demonstration of collagen and smooth muscle fibres	Luna, 1968
3.	Picosirius red method	Demonstration of Collagen fibres	Bancroft and Stevens, 1996
4.	Weigert's method	Demonstration of elastic fibres	Humason, 1979
5.	Gomori's silver method	Demonstration of reticulum	Luna, 1968
6.	Periodic acid – Schiff (PAS)	Demonstration of glycoproteins (Neutral mucosubstances)	Luna, 1968
7.	Alcian blue pH 2.5	demonstration of acid mucopolysaccharides	Luna, 1968

in two layers namely tunica vaginalis externally and tunica albuginea internally in all the age groups studied (Figure 1 and 2). Similar results were also recorded by Horst (1972) in male rodent mole and Andromа and Khasanah (2017) in adult guinea pigs. The capsule was rich in collagen fibres with few reticular, elastic fibres and smooth muscle fibres (Figure 3 and 4). Similar observations were also recorded in naked mole rat (Onyango, 1992) and in goats (Hanumant, 2016). Fibroblast and myoid cells were also observed in the capsule. Blood vessels and nerve fibres were also seen in all the age groups of testicular capsule and more pronounced in young and adult age groups than in preweaning and weaning groups (Figure 2) which may be correlated with the sexual maturity and the functional activity of the organ. The connective tissue septa aroused from the capsule and penetrated the testicular parenchyma and was found divided it into lobes. The septa were made up of collagen and reticular fibres (Figure 5). Blood vessels were also found in the septa. The septa were

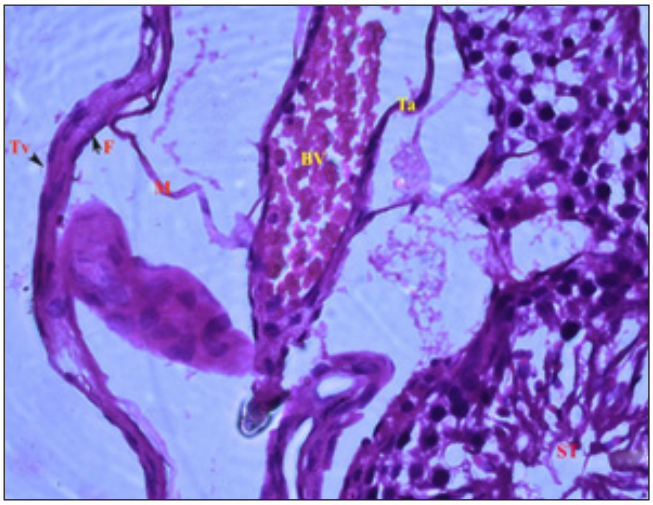

Figure 2: Photomicrograph showing the capsule with Tunica vaginalis (Tv) and Tunica albuginea (Ta) of left testis in 28 weeks old guinea pig; F: Fibroblast; M: Myoid cell; BV: Blood vessel; ST: Seminiferous tubule; H & E x 400

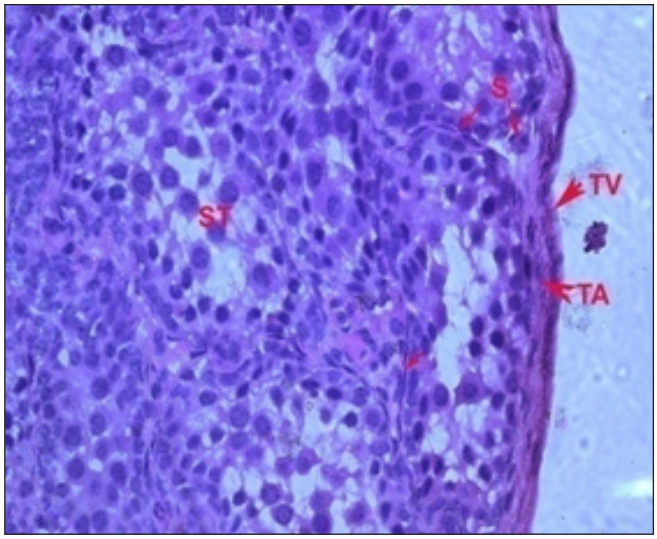

Figure 1: Photomicrograph showing capsule and septa (S, arrows) with Tunica vaginalis (TV) and Tunica albugina (TA) in right testis of two day-old guinea pig; ST: Seminiferous tubules; H & E x 100

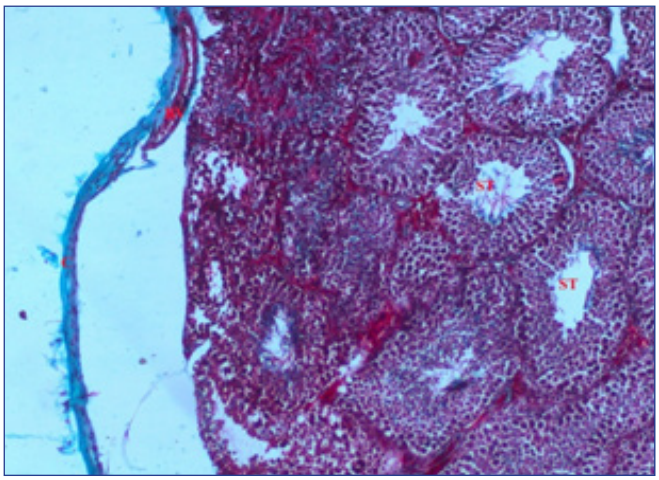

Figure 3: Photomicrograph showing the collagen fibres and smooth muscle fibres in the capsule (C) of 20 week-oldmale guinea pig; ST: Seminiferous tubules; BV: Blood vessels MTS x 100

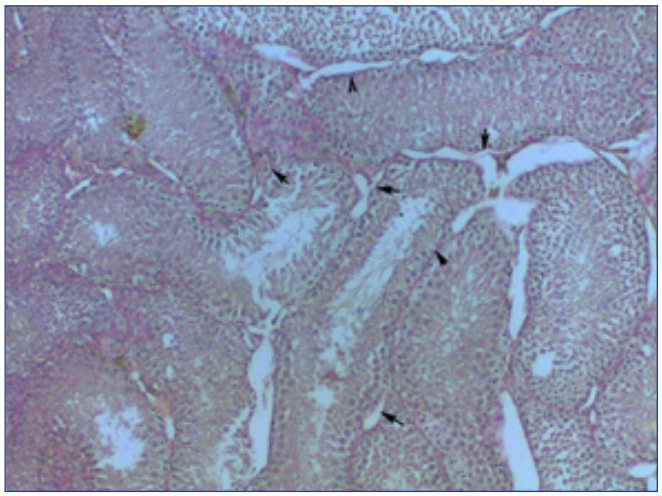

Figure 4: Photomicrograph showing the collagen fibres (arrow) in the interstitial tissue of 12 week-oldmale guinea pig; Picosirius red x100

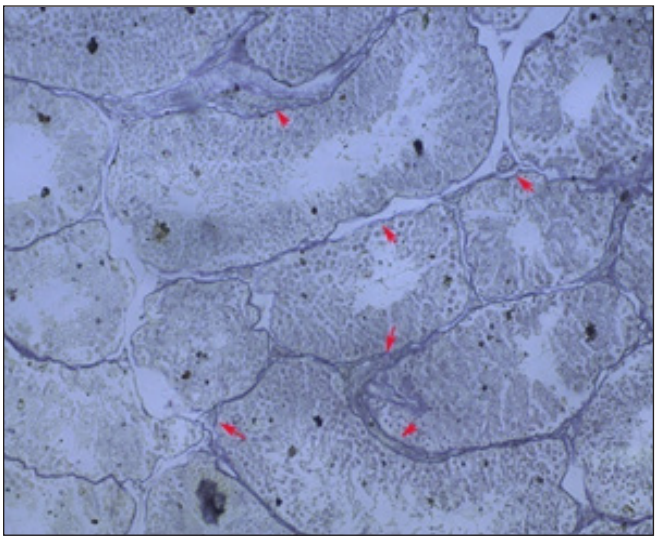

Figure 5: Photomicrograph showing the reticular fibres (arrow) in the basement membrane and peritubular connective tissue of 10 week-oldmale guinea pig; Weigert's x100

found thin near the capsule and were loose and thick in the rete testis. The above findings were in line with the findings of Hanumant (2016) in goats.

3.1.1. Rete testis

Rete testis was found from weaning to adult age groups of present study within the teticular parenchyma in longitudinal fashion (Figure 6) and was not evident in preweaning groups. Seminiferous tubules approaching near the mediastinum testis formed the tubuli testis. One tubulitesis was connected with other tubuli testis and formed rete testis (Figure 7). Rete testis was lined by simple cuboidal epithelium with basal lamina (Figure 8). Similar findings were observed in male rodent mole by Horst (1972). But Androma and Khasanah (2017) found the lining epithelium of rete testis as simple squamous epithelium which was contrast to our present observation.

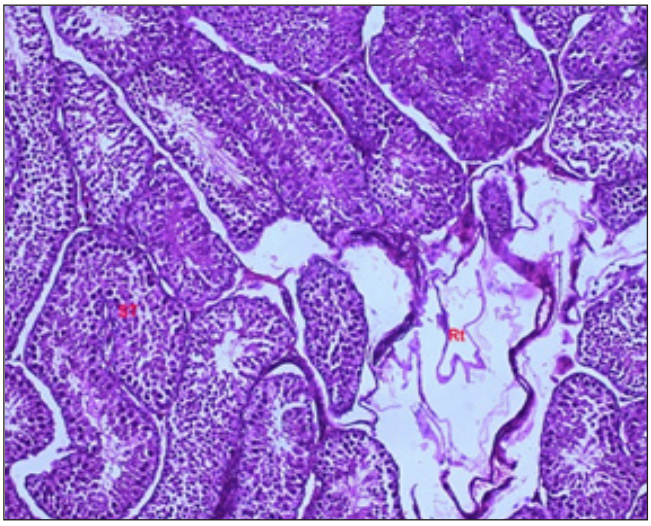

Figure 6: Photomicrograph showing rete testis (Rt) of right testis which was surrounded by seminiferous tubules (ST) in 10 week-oldmale guinea pig. H & E x 100

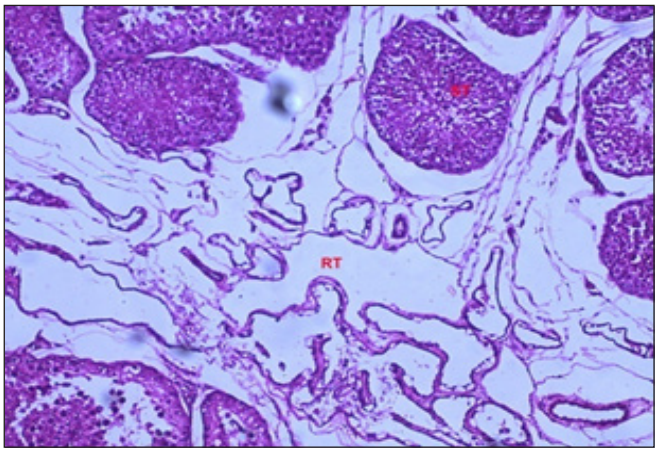

Figure 7: Photomicrograph showing rete testis (RT) of left testis which was surrounded by seminiferous tubules (ST) in 24 week-oldmale guinea pig. H & E x 100

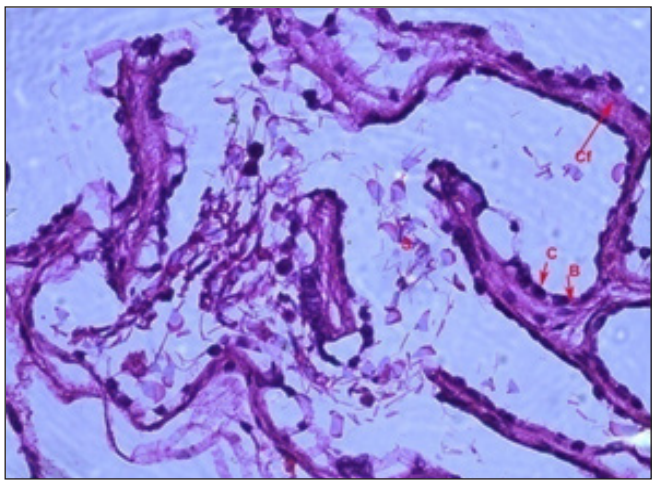

Figure 8: Photomicrograph showing rete testis (Rt) of left testis in 24 week-oldmale guinea pig; C: Cuboidal cell; B: Basement membrane; Cf: Collagen fibres; S: Spermatozoa; H & E x400

The height of the cuboidal epithelium was found increased as age advanced in the present study. The epithelium was found surrounded by connective tissue fibres especially collagen fibres and smooth muscle fibres. The lumen was observed small in weaning and young age group of animals and was wide in adult groups of animals. Hanumant (2016) found reticular fibres in pre pubertal goats, elastic, reticular and collagen fibres in pubertal goats and mostly collagen in post pubertal goats. But in the present study, preweaning and weaning groups of animals showed mostly reticular fibres surrounding the lining epithelium, young animals showed collagen and reticular fibres and adult animals showed only collagen fibres. Rete testis was continued as efferent ductules.

3.2. Testicular parenchyma

Testicular parenchyma in all the age groups of present study consisted of seminiferous tubule, interstitial tissueand rete testis. Each seminiferous tubule in all the age groups was surrounded by peritubular connective tissue which was a thin layer of connective tissue surrounding the basement membrane of the tubules (Figure 9). The peritubular connective tissue consisted of collagen and reticular fibres with fibroblast and myoid cells. Similar results regarding the presence of myoid cells were also found by Onyango (1992) in naked mole rats. The proportion of the fibres and cells were more pronounced in young and adult age groups than in preweaning and weaning groups of animals.

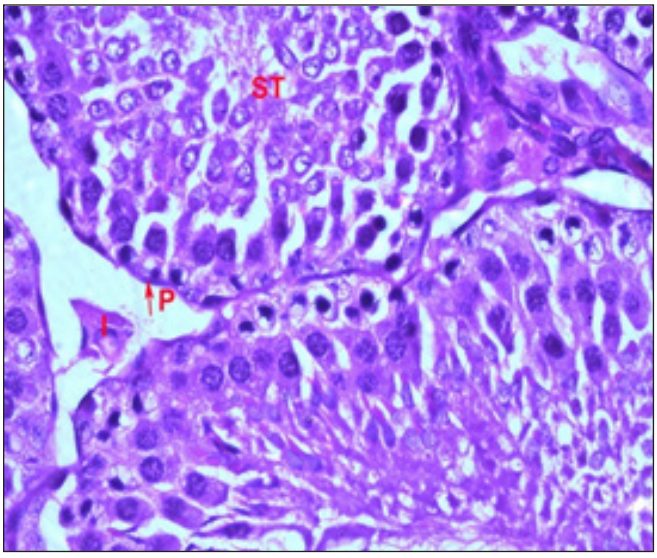

Figure 9: Photomicrograph showing peritubular connective tissue (P) surrounding the Seminiferous tubule of left testis (LT) in 12 week-oldmale guinea pig; I: Interstitial tissue H & E x 400

3.2.1. Seminiferous tubules

Seminiferous tubules in the present study were of various sizes and shapes. Seminiferous epithelium was rested on the basement which was surrounded by peritubular connective tissue. Seminiferous epithelium in young and adult group consisted of multilayered germinal epithelium and sertoli cells. Similar observations were also recorded by Onyango (1992) in naked mole rat, Hanumant (2016) in goats and Andromaand Khasanah (2017) in adult guinea pigs. Preweaning and weaning group of guinea pigs seminiferous tubules showed wide lumen with only type 1 and type 2 spermatogonia and sertoli cells (Figure 10). In this group, the germinal epithelium showed only 2-4 layers. Type 1 cells were appeared cuboidal in shape with oval nucleus. Type 2 cells were round shaped with vesicular nucleus and prominent nucleoli. Young and adult animals seminiferous tubules showed narrow lumen with type 1 and type 2 spermatogonia, primary spermatocytes in various stages of differentiation namely leptotene, zygotene, pachytene and diplotene, secondary spermatocytes, spermatids (early and late) and sertoli cells (Figure 11). The spermatocytes were differentiated by their nuclear-

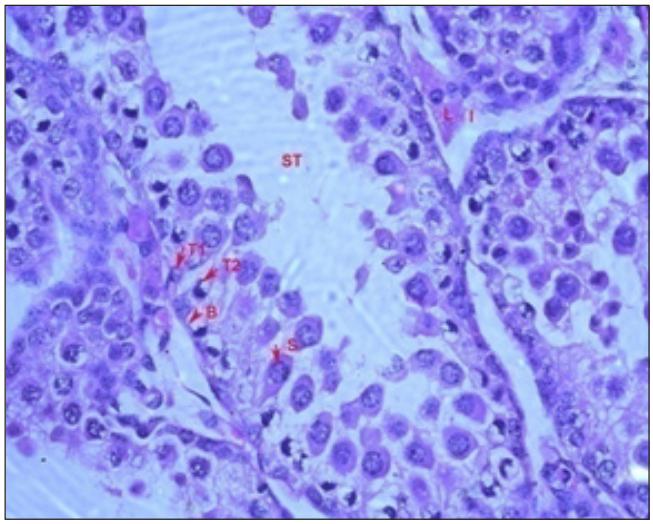

Figure 10: Photomicrograph showing Seminiferous tubule (ST) of left testis in 10 day-oldmale guinea pig; T_1: Type A Spermatogonia; T_2: Type B Spermatogonia; B: Basement membrane; S: Sertoli cell; L: Leydig cells; I: Interstitial tissue H & E x 400

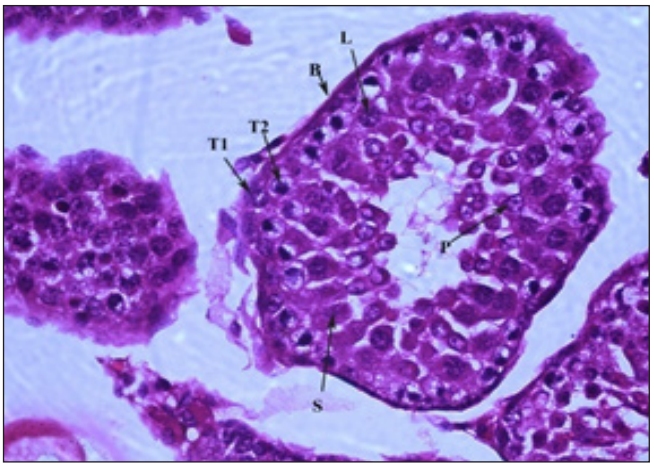

Figure 11: Photomicrograph showing Seminiferous tubules of right testis (RT) in 4 week-old guinea pig; T_1: Type A Spermatogonia; T_2: Type B Spermatogonia; B: Basement membrane; L: Leptotene stage; P: Pachytene stage; S: Sertoli cell H & E x 400

cytoplasmic ratio, shape and size of the cell and the nucleus and the the granularity of cytoplasm in H & E staining. Similar findings were noticed in adult Bakerwali goat by Bashir et al. (2012). In this group, the germinal epithelium was found with 7–8 layers. Type 1 and Type II spermatogonia are immature germ cells. They appeared as small, round shaped with dark round nucleus centrally. Type 1 cells were found near the basement membrane whereas type 2 cells found medial to type 1 cells. Primary spermatozoa were large cells with spherical nucleus and found medial to type 2 cells in various stages of differentiation. Leptotene cells were identified with chromatin dispersed nuclei. Zygotene cells were found with large crescent shaped nuclei and were vacuolated. Pachytene cells were found with patchy appearance of chromatin in nucleus.Diplotene stage was identified with disintegrated nuclear membrane.Secondary spermatocytes were found assmallspherical cells with light cytoplasm and were not found mostly in all the seminiferous tubules as they were transformed to spermatid immediately. Early spermatids were round shaped whereas late spermatids were elongated in shape. In the lumen, spermatozoa were found (Figure 12). Similar findings were also observed by Onyango (1992) in naked mole rat and Hanumant (2016) in goats.

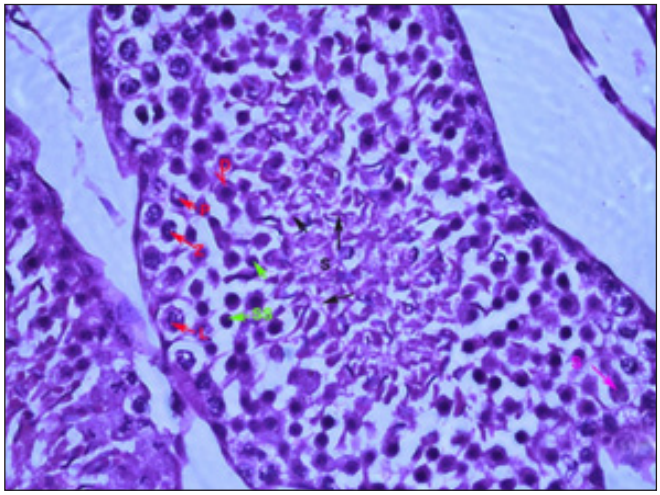

Figure 12: Photomicrograph showing Seminiferous tubule (ST) of left testis in 12 week-old guinea pig; S: Sertoli cell; L: Leptotene; P: Pachytene; Z: Zygotene; D: Diplotene stages of primary spermatocytes; SS: Secondary Spermatocytes; S: Sperms H & E x 400

3.2.2. Sertoli cells

In all the age groups of present study, sertoli cells were appreciated within the seminiferous tubules. Sertoli cells were large oval shaped cells with lightly stained irregular shaped nucleus (Figure 10, 12). But Onyango (1992) in naked mole rat observed sertoli cells as tall, irregular columnar cells and Hanumant (2016) in goat observed it as tall and triangular shaped. This may be due to species difference. In all age groups, sertoli cells were found perpendicular to the lumen and extended from basement membrane to the lumen. Similar results were also found by Onyango (1992) in naked mole rat and Hanumant (2016) in goats. The nucleus of the cell was found in the basal or middle part. It had irregular nuclear membrane with fine chromatin materials. Nucleolus was lightly stained. In contrast to this Onyango (1992) in naked mole rat and Hanumant (2016) in goats found prominent nucleolus. The cytoplasm of sertoli cells were found acidophilic. Cytoplasmic processes of sertoli cells in young and adult group of animals were in contact with the germinal cells or spermatid.

3.2.3. Interstitial tissue

Interstitial tissue was loose connective tissue with collagen and reticular fibres. It was found between the seminiferous tubules in all the age groups studied. But it was wider in preweaning and weaning group as compare to young and adult group of animals. Interstitial tissue contained leydig cells in all ages. In addition to leydig cells, it also contained fibroblast, mast cells, myoid cells and plasma cells (Figure 13). Blood vessels of various sized were also found in the interstitial tissue. Similar observations were also recorded by Onyango (1992) in naked mole rats and Hanumant (2016) in adult goats. The blood vessels were few in preweaning groups

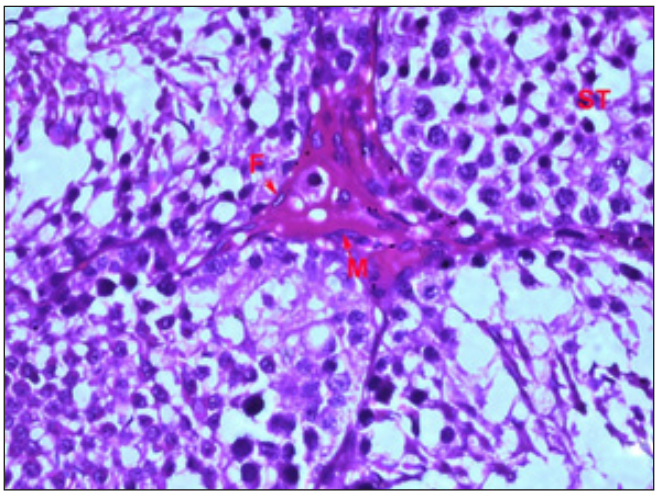

Figure 13: Photomicrograph showing interstitial tissue of right testis which was surrounded by Seminiferous tubules (ST) in 12 week-old guinea pig; F: Fibroblast; M: Myoid cell. H & E x 400

and the proportion of blood vessels were found increasing from weaning to adult group of animals which states the functional activity of the gland. Leydig cells were associated with blood vessels in all age groups studied. Leydig cells were found singly and also in groups of 2-3 cells. They appeared as varied shaped but frequently found as triangular, oval and polyhedral shaped. But Onyango (1992) in naked mole rats and Hanumant (2016) in adult goats found cluster of polyhedral shaped leydig cells which may be due to species difference. The basophilic nucleus of leydig cells were found as flattened or rounded with unclear nucleolus. The cytoplasm appeared as acidophilic and homogenous in all ages. Leydig cells were large

sized cells in preweaning and weaning groups and were small in size in young and adult groups which proves the functional adaptation of the cells.

3.3. PAS Staining

Neutral mucopolysaccharides were detected by using PAS staining reaction. In all the age groups studied, PAS activity was noticed in the capsule and basement membrane (Figure 14). Similar results were also recorded by Hanumant (2016) in adult goats. But the intensity of the reaction was mild in preweaning groups and found increasing from preweaning to adult age groups as age advanced. Sertoli cells of young and adult groups showed moderate reaction. Only in young and adult groups, PAS reaction was found in the spermatozoa (Figure 14) and was not noticed in preweaning and weaning groups as sperms were not observed in these age groups. PAS activity was not observed in spermatogonia and spermatocytes. Similar findings were also observedby Kannan et al. (2008) in Japanese quail and Hanumant (2016) in adult goats.

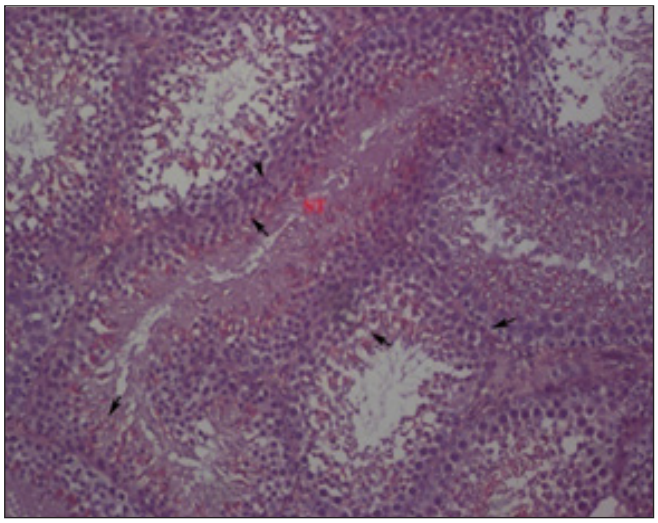

Figure 14: Photomicrograph showing the PAS positive reaction in the sperm head region (arrow) within the seminiferous tubules (ST) and basement membrane (arrow) in the left testis of 12 week-old guinea pig. PAS x 400

3.4. Micrometry

In the present study, the micrometrical observations namely capsule thickness and diameter of seminiferous tubules of right and left testis of various postnatal age groups were recorded in Table 3. The micrometrical parameters increased as age advanced in both right and left testis. The capsule thickness was more in right testis when compared to left study in all age groups. The diameter of the seminiferous tubules was found more in right testes than in left. Hanumant (2016) in goats found different values for the diameter of seminiferous tubules. He found that diameter of the seminiferous tubules of the left testis was more than the right testis which was in contrast to our present observation. This may be due to age and species differences.

Table 3: Postnatal micrometrical parameters of testis in guinea pig

Age groups	Capsule thickness		Diameter of seminiferous tubules	
	Right	Left	Right	Left
0-2 weeks	15.8±0.01	15.2±0.02	184.8±0.1	176.2±0.01
2-8 weeks	17.3±0.02	16.54±0.03	310.5±0.04	305.8±0.02
8-16 weeks	20.29±0.11	19.48±0.01	330.8±0.05	325.4±0.11
16-32 weeks	25.2±0.13	23.9±0.11	246.5±0.11	252.9±0.05
F value	49.52**	47.28**	174.65**	162.87**

*: Significant difference among groups ($p \leq 0.05$); (Mean ±SE) in µm

4. Conclusion

The histology of postnatal development of testis of guinea pigs showed many similarities with that of rodents and other mammals. In this study, it was concluded that the even though guinea pigs attained sexual maturity from 4th week onwards but the testis started functioning for sperm production in young and adult age groups. Butsperm production was not observed anatomically in preweaning and weaning age groups of guinea pigs which is unique in this study.

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