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Research Article

ANATOMICAL AND HISTOLOGICAL FEATURE OF TESTIS DURING PERIOD OF AFTER WEANING AND ADULTHOOD IN GUINEA PIG

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Abstract

Current work was conducted to investigate the anatomical and histological features of testis during weaning and adulthood periods. Twenty male guinea pigs involved weaning and adults were used. The testis were prepared with paraffin embedding technique and stained with H&E and Masson's trichrom stains. Anatomically, the testis after weaning were small in size had oval shaped and located outside the scrutum, the right testis was 0.40±0.02 g meanwhile the left testis 0.37±0.02 g, the length of the right testis was 3.92±0.008 g meanwhile the left testis 3.62±0.19 g and the thickness of the right testis was 3.72±0.02 g meanwhile the left testis 3.44±0.08 g. During adulthood the testes were enlarged in size had creamy colour and located in the scrotum, the weights of the right testis were 1.63±0.02 g meanwhile the left testis was 0.154±0.03 g. The length of the right testis was 14.64±1.14 g meanwhile the left testis was 14.48±1.09 g, and the thickness of the right testis was 10.11±0.95 g meanwhile the left testis 9.80±0.88 g. Histologically, the testis of weaned male was surrounded by a thin tunica albuginea. The testicular lobe had small size seminiferous tubules which lined by single layer of spermatogonial and myoid cells, the interstitium of testis was consisted of little of leydic cells. The diameter of seminiferous tubule was 45.62±4.43 µm, thickness of interstitium was 14.22±4.39 µm, and the height of germinal epithelium was129.31±0.1 µm. Atadulthood the testicular lobe had wide diameter seminiferous tubules. Seminiferous tubule was revealed active spermatogenesis which composed of very thick stratified spermatogonium epithelium. The interstitium of testis was consisted of numerous leydic cells. The germinal epithelium revealed the following six types of spermatogenic cells. The diameter of seminiferous tubules was 145.005±8.11 µm, thickness of interstitium was 22.361±1.62 µm, and the height of germinal epithelium was 52.574±2.43 µm. The statistical analysis revealed significant differences (P<0.05) between all parameters of testis in adult and after weaning.

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1. Introduction

Guinea pig is small enough to permit manipulation. It is relatively easy to care. It is an extremely docile animal and may be handled by the caretaker or experimenter with little danger of being bitten. Notable differences in physiology and behaviour, as well as in coat colour texture, and hair length exist among different guinea pig strains. It is noticeable that the development of

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sperm which is initiated in the testis is completed in the epididymis (Emma *et al*., 2020). The male reproductive system of the guinea pig is composed of two testicles, gentile way, accessory glands and penis, the testes of the guinea pig are found in scrotum. These tests are combined of exocrine and endocrine organs (Trainer, 1987). The exocrine portion is represented by the convoluted seminiferous tubules that produce spermatozoa while the endocrine portion is represented by cells of leydic and Sertoli cells, the cells are located in the septal connective tissue between the tubules of the lobule (Breazile, 1970). The testes are produce spermatozoa and testosterone hormone that important for formation of male games, embryonic differentiation and development. The ductal system and the accessory glands produce secretions and pushing the sperm outward. Secretion also provides nutrients for sperm pass through the reproductive tract. The secretions of the accessory sex glands form the seminal fluid that facilitates sperm in swim in the female reproductive tract (Mescher, 2011). The present study aimed to investigate the anatomical and histochemical features of guinea pig testes at after weaning and adult.

2. Materials and Methods

Current study was under approval of Animal Care and Use Committee at the College of Veterinary Medicine, University of Baghdad. Baghdad-Iraq. A total of twenty males guinea pig involved (10 after weaning and 10 adults) were obtained from the animal house of the national center for drug control and research. The study was done in the animal house at College of Veterinary Medicine/University of Baghdad under perfect condition.

Animal was anaesthetized by ketamine, 35 mg/kg and xylazine, 5 mg (Flecknell, 1987). The abdominal wall was incision then the testis with attached epididymis was imaged in suite to record the relation then the proper ligament of testis and ductus deferens were cut on each side, allowing removal of testes and epididymis for inspection and transaction subsequently, after obtaining the testis, the anatomical study includes weight, length, thickness organs, relationship, shape, and colour of testis. For histological preparation, the samples of testis were washed up with normal slain twice and fixed in 10 % neutral buffer formalin for 48hrs. The tissue specimens were trimmed, processed with the paraffin technique, sectioned at 5 - 6 µm, and stained with hematoxylin and eosin, combine Alcian blue (pH 2.5) - PAS stain and Masson's trichrom stain. Tissue sections were examined by Light microscopy and microphotography has been done by using Future Win Joe microscopic camera, the images have been analyzed and scored by using Fiji image analyzer system. The statistical analysis was done by using SPSS (Version-24) and data were represented by Mean±SE.

3. Results

Morphological Results in Period of after Weaning

The testis was small in size had an oval shaped and located in the pelvic region close the inguinal ring outside the scrutum (Figure – 1 and Figure - 2). Testis showed well developed epididymus, ductus deferens and closed the sex accessary. The weight of right testis was 0.40±0.02 g meanwhile the left testis 0.37±0.02 g. The length of the right testis was 3.92±0.008 g meanwhile the left testis 3.62±0.19 g. The thickness of the right testis was 3.72±0.02 g meanwhile the left testis 3.44±0.08 g (Table - 1).

Morphological Results in Period of Adulthood

The testes were enlarged in size, had oval shape, creamy colour and located in the scrotum (Extra-abdominal position). It had cranial and caudal poles (Figure -3, 4 and 5). The weights of the right testis were 1.63±0.02 g meanwhile the left testis was 0.154±0.03 g. The length of the right testis was 14.64±1.14 g meanwhile the left testis was 14.48±1.09 g, and the thickness of the right testis was 10.11±0.95 g meanwhile the left testis 9.80±0.88 g (Table - 1).

Histological Results in Period of after Weaning

The testis of immature Guinea pig was surrounded by a thin connective tissue capsule

called Tunica albuginea which composed of inner vascular layer and outer fibrous layer. Tunica albuginea was sent numerous loose connective tissue tabeculi into parenchyma of testis to divide the organ into lobules (Figure - 6 and 7). Each testicular lobe was composed of small size seminiferous tubules. Each seminiferous tubule was composed of simple layer of spermatogonium cells rested at basement membrane and myoid cells, the interstitium of testis was consisted of loose connective tissue with little group of leydic cells (Figure - 8). The histometerical measurements of testis showed that the diameter of seminiferous tubule was 45.62±4.43 µm, thickness of interstitium was 14.22±4.39 µm, and the height of germinal epithelium was 129.31±0.1µm (Table - 2).

Histological Results in Period of Adulthood

The testis of adult Guinea pig was surrounded by a thick connective tissue capsule "tunica albuginea" which composed of inner vascular layer and outer fibrous layer. The tabeculi of tunica albuginea into parenchyma of testis was composed of thin of testicular interstitial tissue (Figure - 9, 10 and 11). Each lobe was composed of enlarged diameter seminiferous tubules. Each seminiferous tubule was revealed active spermatogenesis which composed of very thick stratified spermatogonium epithelium that rested on the basement membrane and supported by myoid cells (Figure - 10). The interstitium of testis was consisted of very thin loose connective tissue with numerous leydic cells (Figure - 10). The germinal epithelium revealed the following six types of spermatogenic cells: Spermatogonium type - A: Were small size cells which form the first simple line of cells those rested on the basement membrane. Sertoli cells: Was large cells had enlarged pale nucleus rested on the basement membrane. Spermatogonium type-B: Were the largest cells which form the seconds and third layer of cells had enlarged nuclei. Primary spermatocytes: Were slightly large cells forming the layer cells which followed spermatogonium type-B. Secondary spermatocytes: Was the smallest type of cells close the lumen. Spermatids: Was the cells types which showed differentiation of tail of spermatozoa that filled the lumen of seminiferous tubules. The histometerical measurements of testis showed that the diameter of seminiferous tubule was 145.005±8.11 µm, thickness of interstitium was 22.361±1.62 µm, and the height of germinal epithelium was 52.574±2.43 µm. The statistical analysis revealed significant differences (P<0.05) between all parameters of testis in adult and after weaning (Table - 2).

Table - 1: Weight, Length and Thickness of the Right and Left Testes at after Weaning and Adult Guinea pig

Group	Right testis			Left testis		
	Weight/g Mean±SE	Length/ mm Mean±SE	Thickness/mm Mean±SE	Weight/g Mean±SE	Length/mm Mean±SE	Thickness/mm Mean±SE
After weaning	0.40±0.02a	3.92±0.008a	3.72±0.14a	0.37±0.02b	3.62±0.19b	3.44±0.08b
Adult	1.63±0.02 a	14.64±1.14a	10.11±0.95a	1.54±0.03a	14.48±1.09a	9.80±0.88a
LSD	0.0825*	2.3923*	1.9815*	1.17*	10.86*	6.36*

(*) is denoted significant differences at (P0.05) between the after weaning and adults. Similar small litters (aa) is denoted no significant differences at (P0.05) between the right and left tubes of seminal vesicle. Different small litters (ab) are denoted significant differences at (P0.05) between the right and left tubes of seminal vesicle.

Table - 2: Diameter of Seminiferous Tubules, Height of Germinal Epithelium and Thickness of Testicular Interstitium of Testis in after Weaning and Adult Guinea Pig

	Diameter of seminiferous tubules/µm	Height of germinal epithelium/µm	Thickness of Interstitium/µm
After Weaning	45.62±4.43	12.31±0.19	14.22±4.39
Adult	145.05±8.11	52.57±2.43	22.361±1.62
LSD	99.43*	40.26*	8.35*

(*) Represents significant differences (P<0.05)

Figure - 1: Dissected guinea pig (after weaning) shows; Pelvis position of left testis (Lt) & right testis (Rt), Right ductus deferens (Rd), Left ductus deferens (Ld), Seminal vesicle (S), Coagulating gland (C,) Epididymus(e)

Figure - 2: Reproductive organ of guinea pig (after weaning) shows Right testes (Rt), Left testis (Lt), Epididymis (e), Left ductus deferens (Ld), Right ductus deferens (Rd), Prostate (P), Coagulating gland (C) &Seminal vesicle (s)

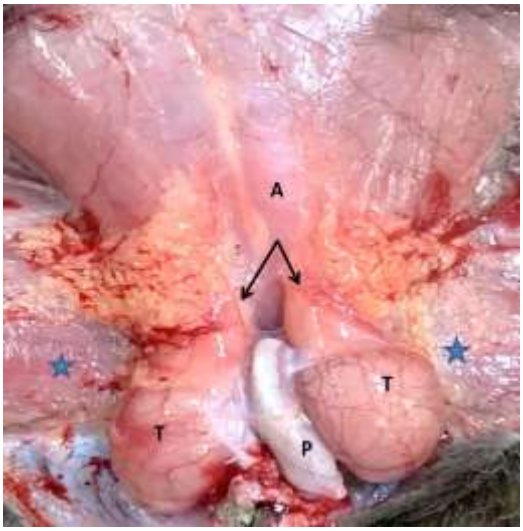

Figure - 3: Abdomen of adult guinea pig involved inguinal region shows testis (T), Penis (P), Scrutum(Asterisks), Abdominal wall (A) andInguinal canal (arrows)

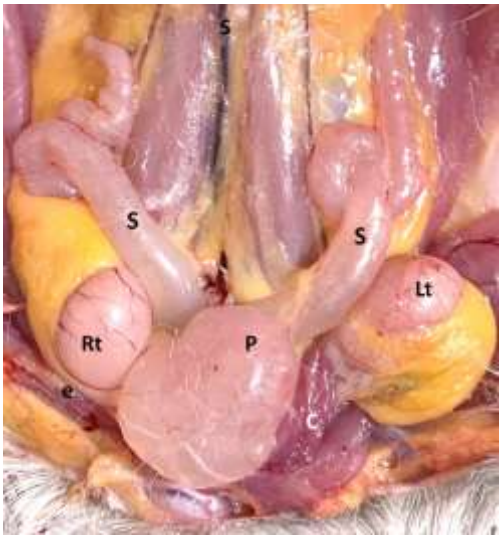

Figure - 4: Dissected abdominal muscles of Adult Guinea pig shows; Pelvis position of left testis (Lt), Right testis (Rt), Right ductus deferens (Rd), Left ductus deferens (Ld), Seminal vesicle (S), Coagulating gland (C) &Epididymus(e)

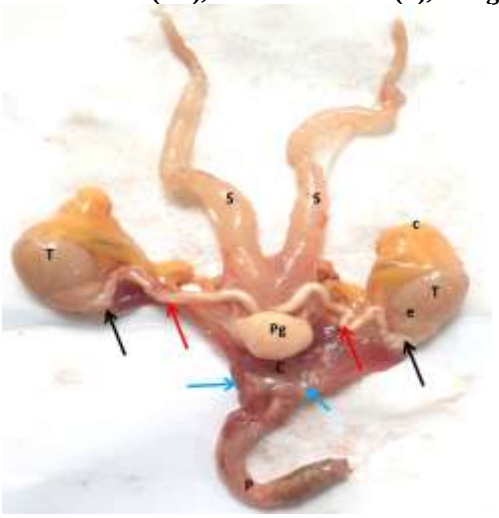

Figure - 5: Reproductive organs of adult guinea pig shows testis (T), Cranial pole of testis (C), Caudal pole of testis (e), Ductus deferens (Red arrows), Seminal vesicle (S), Coagulating gland (C), Tail of epididymis (black arrows) andProstate (Pg)

Figure - 6: Histological section of testis-guinea pig (after weaning) shows: Thintunica albugina (black arrow), Thin trabeculi of connective tissue (red arrows), Numerous small size seminiferous tubules (asterisks) andBlood vessel (blue arrow). H&E stain 40x.

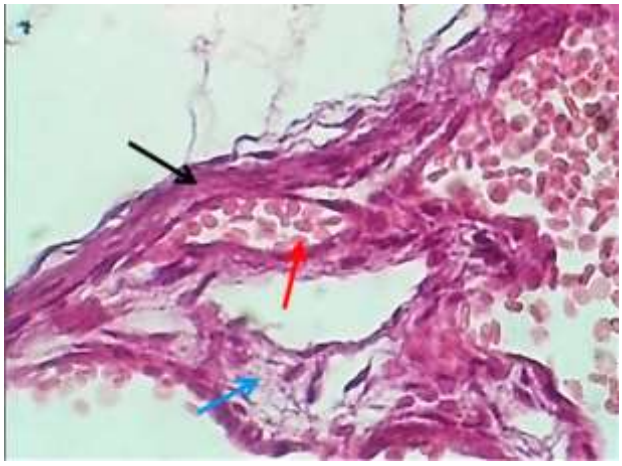

Figure - 7: Histological section of tunica albugina of testis-guinea pig (after weaning) shows: Fibrous layer (black arrow), Vascular layer (red arrow) thin trabeculi of connective tissue (blue arrows). H&E stain 400x.

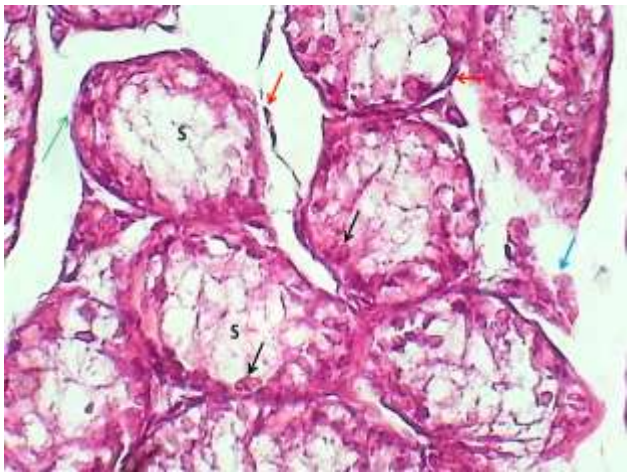

Figure - 8: Histological section of testis-guinea pig (after weaning) shows: thin tunica albugina (black arrow), myoid cells (red arrows), leydic cells (blue arrow) numerous small size seminiferous tubules (S), spermatogonium type-A, (black arrows), basement membrane (green arrow). H&E stain 400x.

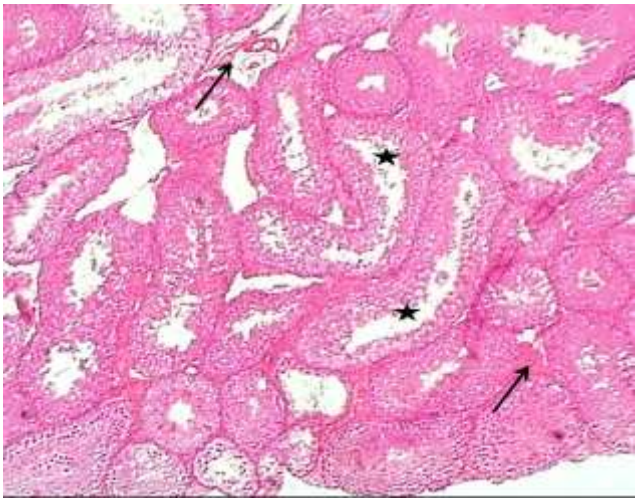

Figure - 9: Histological section of testis of adult guinea pig shows: thin testicular interstitium (black arrows), numerous enlarged in size seminiferous tubules (asterisks) revealed marked spermatogenesis. H&E stain 40x.

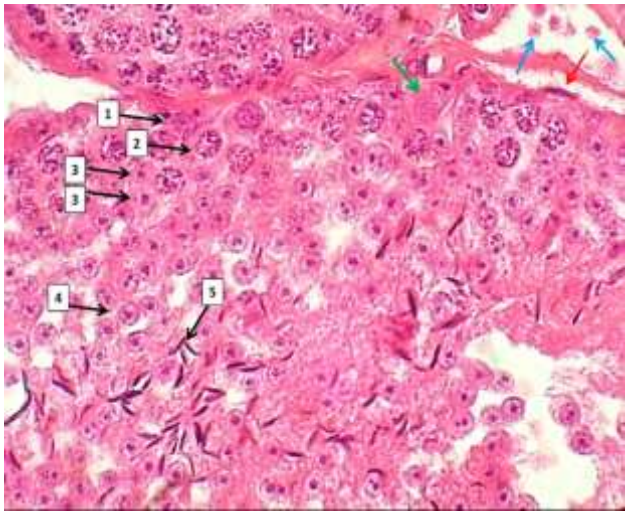

Figure - 10: Histological section of seminiferous tubules-guinea pig (adult) shows: myoid cell (red arrow), leydic cells (blue arrows), sertoli cells (green arrow), spermatogonium type-A (1), type-B (2), primary spermatocytes (3), secondary spermatocytes (4) & spermatids (5). H&E stain 400x.

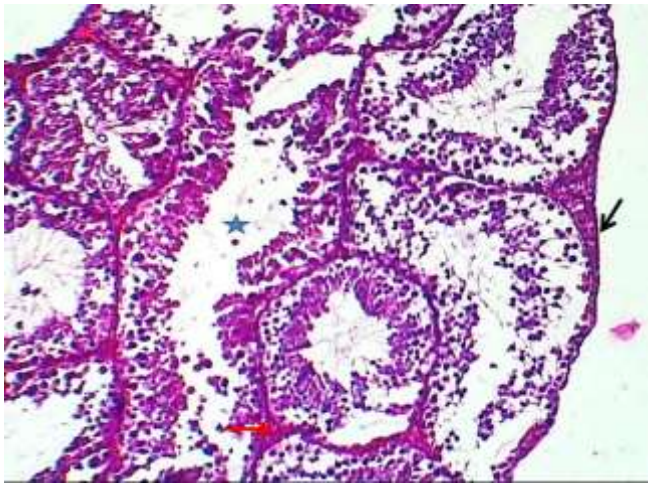

Figure - 11: Histological section of seminiferous tubules-guinea pig (adult) shows: tunica albuginea (black arrow), interstitial tissue (red arrows) & seminiferous tubules (asterisk). H&E stain 100x.

4. Discussion

In general, the results of shape and position of testis during after weaning are accord with results of Yamomoto (2015), Vanderlip (2013), Barone (2001), the study agree with fact that the inguinal canal is persist open during this periods as that seen in rabbit and other small rodents (Quesenberry and Carpenter 2012; Thematt *et al*., 2009; Merchant *et al*., 1993; McCracken *et al*., 2008), this result is disagree with that seen in small carnivores by Dawood and Abood (2019) who recorded that the testis is situated about halfway between the inguinal region and the anus and the long axis of the testis is oblique and is directed dorsally and caudally. However, the testis is descend into the scrotum through the inguinal canals near the end of the fetal development, this migration is occurs under the stimulation of testosterone (Nishino *et al*., 2004; Pelletier, 2002; Amory and Bremner, 2001).This suggest that the level of the testosterone is low level during period of after weaning. The scrotum is to keep the temperature of the testes lower than that of the rest of the body and moving the testicles closer to the abdomen when the ambient temperature is cold, this is done by using contraction and relaxation of the cremaster muscle in the abdomen and the dartos fascia (muscular tissue under the skin in the scrotum (Kastelic *et al*., 1997; Arimura *et al*.,

1976). However, the results showed that the of weight, length and thickness of the right testis were significantly exceeded that of the left once, such result dissimilar to that mentioned by Anderson *et al*. (2002), this result is beyond species variations. The result showed that at the adulthood the testes are located within scrutum, because they have a dual function as the production of the sperm, synthesis and secretion of male sex hormones, so during the period of reproductive it is safe to be in the scrotum to protect spermatozoa from high of body temperature (Schmahl and Capel, 2003; Anderson *et al*., 2002; Kastelic *et al*., 1997; Merchant *et al*., 1993; Arimura *et al*., 1976). The testis had transient in position as seen in other laboratory animals at adulthood as in mouse the testes are located outside the abdomen in the inguinal (groin) region housed in sac of skin called the Scrotum (Thematt *et al*., 2009) while in other animals like small carnivores (Dawood and Abood, 2019) and large animals they persist within scrutum. The results revealed significant differences between all parameters of adult and those at after weaning such differences were associated with functional status of both testicles under the effects of gonadotropic hormones produced by the anterior pituitary. Luteinizing Hormone (LH) results in testosterone release. The presence of both Testosterone and Follicle-Stimulating Hormone (FSH) is needed to support spermatogenesis (Stamatiades and Kaiser, 2018; Yonkers and Simoni, 2018 Barbieri, 2014; Arimura *et al*., 1976). Testis weight of rat was 3.3 g (Collins, 1978), these weight in comparison with guinea pig testis of the present study was significant variation and even in European hedgehog 3.93 g was significant at ($P < 0.5$), also highly significant in comparison with chinchillas 4.3 g. The length of the testis in the chinchilla 26.2 mm (Cepeda, 2006). The weight of testis of the right side in mice was 0.0178 mg/kg, and the left was 0.534, these data were significant in comparison with results recorded in the guinea pig of the present study On the other hand, other factors that related with increase the weight length and thickness of testis is the testosterone which produced by leydic cells, it were mostly store within the seminiferous tubules in order to maintain spermatogenesis and other function related with stimulation of accessary sex gland (Elbing *et al*., 2000; Bremner *et al*., 1994; Sanborn *et al*., 1975). The testosterone is controlled by LH male that acts upon the Leydig cells of the testis and is regulated by GNRH (Pitteloud *et al*., 2008; Maghuin *et al*., 1995).

Histologically, the immature testis was as description by Nines *et al*. (2017) when reported that at 2 weeks of weaning of guinea pig, no signs of the presence of Sertoli cells, but indicated at 8 weeks and form different kinds of germ cells. Also Amory and Bremner (2001) was reported that the testis is divide the testis into two major compartments: tubular (seminiferous tubules) and interstitial tissue. The germinal epithelium showed only spermatogonium type-A and sertoli cells at the basement membrane as that recorded by Al-Khuzae (2007), Al-Aboudi (2009), Martin (1973), Bustos (1970). Bremner *et al*. (1994) revealed that androgen assists in the development of male sex glands, particularly spermatogenic cells and acting for Sertoli cells receptors, as well as the LH and FSH from the pituitary gland control for good development of testicular SNTs *via* secretion of testosterone by the leydig cells. Thus, step of development in the guinea pig needs few weeks, so this fact is in agree with data of (Neelam *et al*., 2009).

The histological structure of testis during period of adulthood were similar to results of Fukuda *et al*. (2001) and Trainer (1987). These results reflect the well development and differentiation which seen at the adult stage. This development influenced by the production of an endocrine hormone (testosterone) (Liza *et al*., 2017). The testis had a thick tunica albuginea and in comparison, after weaning appeared thinner to that of an adult. This description was in agreement with Al-Khuzae (2007). The result revealed numerous myoid cells at the basement membrane of seminiferous tubules which are localized cell with loose connective tissue proper as dominance of fibroblast (Elzbieta *et al*., 1999). The statistical analysis revealed significant differences between all parameters of testis in adult and after weaning

that related with active spermatogenesis within the germinal epithelium under the influences of sex hormones (Barbieri, 2014; Stamatiades and Kaiser, 2018). The upward description for testicular tissue was a similar description to that reported by Ahura *et al*. (2001) in the rat. The present result showed significant variation with that of after weaning of the testis, so the present study of adult guinea pig testis was in agreement with the Adebayo *et al*. (2009) for his description for the testicular tissue of the rat.

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