

**Morphometric and Morphological Characterization of The Donkey Testis in Relation to Age**

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**ABSTRACT**

Histomorphological changes in testicular parameters and seminiferous epithelium of different age groups male donkeys; pre-pubertal-pubertal and post-pubertal periods were observed. The present study was performed on Testes of 14 donkeys obtained from local markets from Menoufia governorate. Animals divided into four age groups (pre-pubertal-pubertal - post-pubertal and senile after the collection, gross anatomical features of the testes were examined and recorded. The present anatomical result showed that the testis of the donkey was not completely descended into the scrotum around birth. The mean weight, length and width of both left and right testicles in groups differed significantly ( $p \leq 0.05$ ) from each other. The both testicles were appeared not symmetrical, the left testes were larger than right one. All morphometrical parameters were significantly increased when age advanced. The beginning of seminiferous epithelial cycle at 1.5 years old. Marked morphometrical changes in donkey testes occurred from period of puberty until middle of sexual maturity at 6- 7-year-old after that a serious of regressive changes occurred. A marked increase in the weight and diameter of the testes was recorded at 2-6-year-old; The parenchyma of testis was dark reddish in color; it was covered by a thick tunica albuginea consisting of outer and inner fibrous layers in addition to middle vascular one. The diameter of the seminiferous tubules was greatly increased than those recorded at the early stage of the postnatal development. The present histological results emphasized an irregular arrangement of the testicular lobules of the donkey during various stages of the postnatal life. Such arrangement does not allow some lobules to have direct contact with the mediastinum testis. Numerous seminiferous tubules with different shapes and size were highly active.

**Keywords:** Donkey; Morphometry; Testis; seminiferous tubules.

**INTRODUCTION**

Donkey is a member of the Equidae family with the horse and Zebras. It is fully adapted to the variations of temperature of the arid zones of Africa and Asia (Sanni, Suleiman, 1997). There are over 40 million donkeys worldwide found in Asia, Africa and Latin America living mainly in arid and semi-arid regions and are valuable to farmers and traders, providing power and rural transport at low costs (Starkey, 1995).

The adult mammalian testis is the important organ within the male reproductive system and has two major important physiological functions: the production of spermatozoa and testosterone production (Siuan and Cheng, 2004).

The testis is a bipartite glandular organ; they produce both a cellular component (sperm) and the testosterone hormone, the testes of most mammals are located extra abdominally in the scrotum

(Gartner and Hiatt, 1987). Basic structure of testicle is highly fixed among vertebrates (Capel, 2000). Consequently, morphometric data can be used to investigate testis development (França and Russell, 1998). At birth, the mammalian testis appears as an immature organ, which take its full function only at puberty. Testicular maturity is evidenced by a number of morphological and morphometric changes (Wrobel, *et al.*, 1988).

Several anatomical studies have been carried out on the morphology and Morphometry of testes of domestic animals, (Abdel-Raouf, *et al.*, 1975) in one-humped camel, (Neves, *et al.*, 2002) in donkey and mule's testes (Collins, *et al.*, 1987) on in tree-shrew.

The donkey testis is surrounded by two capsular layers; the visceral layer of the tunica vaginalis and tunica albuginea. The tunica albuginea of stallion consists of collagen fibers and few elastic fibers which contain Smooth muscle cells; the tunica albuginea is continuous with the septulae testis, which converges towards the mediastinum testis, (Dellmann and Wrobel, 1976). A vascular layer is present in the tunica albuginea of most species (Banks, 1993). Testicular parenchyma is composed of two compartments, a seminiferous tubular compartment and an interstitial compartment (Ravindranath, *et al.*, 2003). The testicular mass is composed mostly of seminiferous tubules, The outer wall of each seminiferous tubule is a single layer of boundary tissue cells similar in some ways to smooth muscle, sometimes called myoid cells or peritubular contractile cells, and in in some species such as rodents, they are known to be contractile, though contractility hasn't been proven of all animals (Caceci, 2008).

The age-related changes in the adult testis of donkey during post-natal period studied by (Saber, 1994) and (Neves, *et al.*, 2002). (Nipken and Wrobel, 1997) divided sexual maturity in the donkey testis into two periods; a progressive post pubertal (1.5-5 years) and regressive period (5-10 years). The aim of the present work was to study the morphological and morphometric age-related changes in the testis of the male donkey during pre-pubertal and post-pubertal period. The present work to describe the different stages of sexual maturation in different ages of the donkey.

## MATERIALS AND METHODS

The present study was performed in the Department of Anatomy, Faculty of Veterinary medicine, Sadat City University, Cairo, Egypt.

### Animal

The experimental work was carried out on right and left testes of 14 donkeys were obtained from local markets in Menoufia governorate, in the Department of Anatomy and histology, Faculty of Veterinary medicine, Sadat city university, Egypt.

Testes were removed from animals, ranging in age from one month up to 25 years old in good health. Ages of animals were determined by correlating the appearance of specific dental features Animals were classified according to age into four age groups (Table1)

### Anatomical work:

The testes samples were obtained from apparently healthy animals shortly after slaughter, the testes were maintained at 34°C in sterile 0.9% saline then photographed with digital camera after that the testicular measurements (weight and diameter) recorded shortly after slaughter. The weight was recorded in gm and diameters (length x breadth) were recorded in cm by using of digital caliper. Data were statistically analyzed by one-way ANOVA. Testes were fixed in 10% neutral buffered formalin for histological work.

### Histological work:

The testes (right and left) were taken; Small pieces (1.0 cm×1.0 cm×0.5 cm) of testis tissue were fixed in 10% neutral buffered formalin solution, the tissues were transported to the laboratory then the tissue was passed through the routine histological procedure (**Bancroft and Gamble, 2002**). The samples were immersed in a fixative for 24-48 hours. The testicular tissue was thoroughly washed by running tap water to remove fixative from them then the water was removed by treatment in ascending graded of ethanol concentrations (70, 80, 90 and 100%), cleared in xylol to remove alcohol and to permit the fixed tissues to be miscible with paraffin wax. the tissue was embedded in paraffin, the testicular tissue was cut 5 µm thick ness and stained with Haematoxylin and Eosin, Crossman's trichrome stains for collagen and muscle fibers of capsule AND others special

stains as (PAS-alcian blue) technique for Mucopolysaccharides.

**RESULTS**

**Testicular measurements**

In the present investigation the Testicles were varied in size and weight, they were commonly unequal; the left testes were larger than the right, slowly increasing in mean testicular weight and diameter in group I (<1years old); in group II (1.5 to 5 years old) the mean weight and diameter of

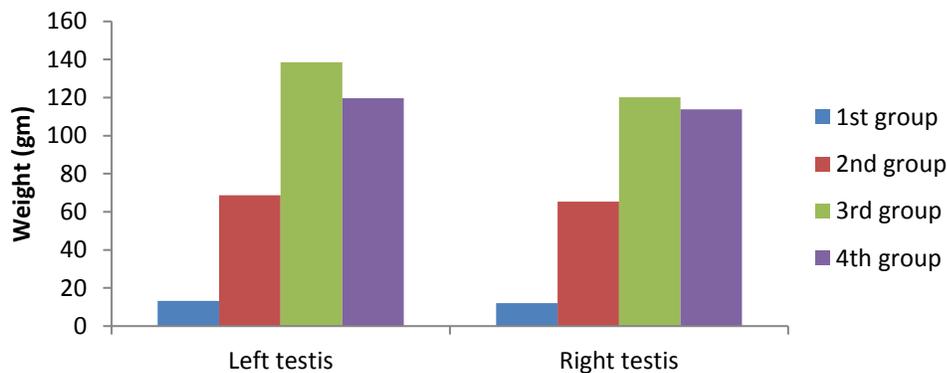
right and left testes were progressed about 3-4-fold, The mean weight and diameter in group III recorded about 6fold. Data illustrated in **Table 2**. Indifferent ages of donkeys had a significant effect on weight of testis either left or right. The most progressive and regressive changes were been obviously in left testes than right one. Weight of testis and diameter of donkey had a significant increased with age advancing in group I-II and Group III then undergo Regression in group IV summarized in and **Figure (1-2)**.

**Table 1:** Animals classified into four groups:

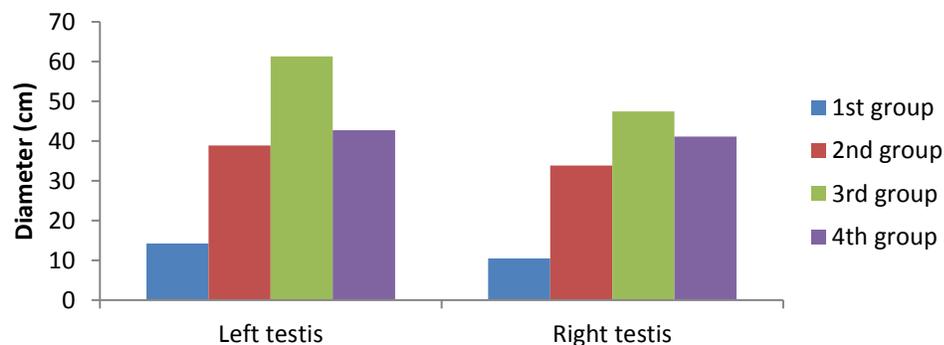
Groups	Age limits	Number of animals
G I (prepubertal)	less than 1.5 Y	4
G II (pubertal)	(2--6) Y	4
G III (post pubertal)	(7-10) Y	3
G IV (aged or senile)	more than 10Y	3

**Table (2): Overall effect of different age of donkeys on weight and diameter of testis (left and right) (Means± SE)**

Items	Group			
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>
Weight of left testis (g)	13.29±1.08 <sup>d</sup>	68.66±1.42 <sup>c</sup>	138.51±5.12 <sup>a</sup>	119.58±2.07 <sup>b</sup>
Weight of right testis (g)	12.13±1.87 <sup>d</sup>	65.33±1.79 <sup>c</sup>	120.16±4.12 <sup>a</sup>	113.75±4.36 <sup>b</sup>
Diameter of left testis (cm <sup>2</sup> )	14.27±2.04 <sup>c</sup>	38.91±2.32 <sup>b</sup>	61.33±5.28 <sup>a</sup>	42.75±1.97 <sup>b</sup>
Diameter of right testis (cm <sup>2</sup> )	10.49±1.07 <sup>c</sup>	33.85±2.12 <sup>b</sup>	47.45±3.22 <sup>a</sup>	41.14±1.35 <sup>b</sup>



**Figure (1): Overall effect of different age of donkeys on weight of testis (gm) (left and right) (Means ± SE)**

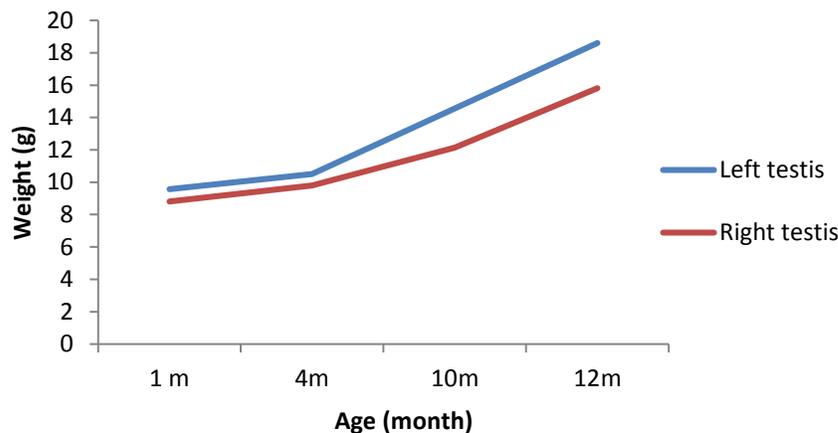


**Figure (2): Overall effect of different age of donkeys on diameter (cm) of testis (left and right) (Means ± SE).**

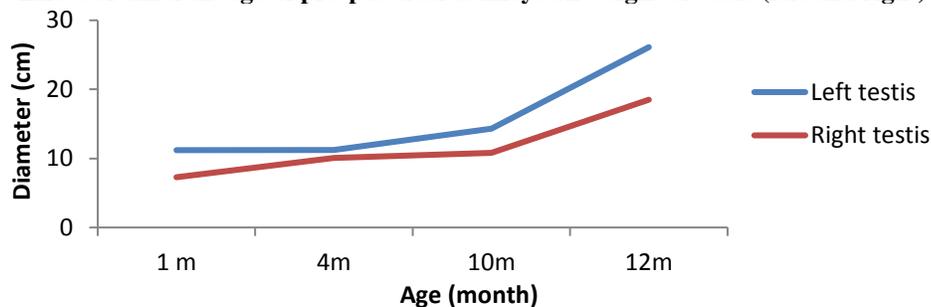
The obtained result illustrated that weight of left testis and right testis were significant increased with age in pre-pubertal donkeys from 1m, 4m, 10m to 12m **Figure (3)**. the weight of left testes showed a two stage of development first increase from (1-4m) then(4-12m) but the weight of right testes showed three stages of development from(1-4m) -(4-10m) (10-12m). As well as left testis and right testis of 12 m donkey showed the highest diameter than other ages as indicated in **Figure (4)**.

**Table (2): Effect of different age of pre-pubertal donkeys on weight and diameter of testis (left and right) (Means± SE)**

Items	Age of first group			
	1 m	4m	10m	12m
Weight of left testis (g)	9.57±0.01 <sup>d</sup>	10.51±0.06 <sup>c</sup>	14.56±0.07 <sup>b</sup>	18.60±0.01 <sup>a</sup>
Weight of right testis (g)	8.81±0.22 <sup>d</sup>	9.80±0.20 <sup>c</sup>	12.13±0.11 <sup>b</sup>	15.81±0.22 <sup>a</sup>
Diameter of left testis (cm <sup>2</sup> )	11.20±0.01 <sup>c</sup>	11.22±0.02 <sup>c</sup>	14.30±0.02 <sup>b</sup>	26.11±0.12 <sup>a</sup>
Diameter of right testis (cm <sup>2</sup> )	7.28±0.11 <sup>d</sup>	10.08±0.12 <sup>c</sup>	10.82±0.17 <sup>b</sup>	18.50±0.12 <sup>a</sup>



**Figure (3): Effect of different age of pre-pubertal donkeys on weight of testis (left and right) (Means± SE)**



**Figure (4): Effect of different age of pre-pubertal donkeys on diameter of testis (left and right)**

The result showed that the Different ages of pubertal donkeys had a significant effect on weight and diameters of left and right testis as summarizes in **Figure (5)** and **Figure (6)**. Weight of testes of GII had positive relationship with age. This group showed continuous progressive changes. Weight of left of 5 years old donkeys were higher than weight of left and right testis of 3years and 2 years old donkeys. As well as left and right testis of 2 years and 3years old donkeys showed significant lower diameter than left and right testis of 5 years old donkeys. **Left and right) (Means ± SE)**.

**Table (3): Effect of different age of pubertal donkeys on weight and diameter of testis (left and right) (Means ± SE)**

Items	Age of second group		
	2y	3y	5y
Weight of left testis (g)	42.51±0.12 <sup>c</sup>	61.11±0.11 <sup>b</sup>	121.20±0.23 <sup>a</sup>
Weight of right testis (g)	33.75±0.34 <sup>b</sup>	55.61±0.24 <sup>b</sup>	106.70±0.21 <sup>a</sup>
Diameter of left testis (cm <sup>2</sup> )	29.30±0.45 <sup>b</sup>	29.35±0.43 <sup>b</sup>	49.58±0.40 <sup>a</sup>
Diameter of right testis (cm <sup>2</sup> )	26.69±0.88 <sup>b</sup>	26.71±0.90 <sup>b</sup>	43.91±0.86 <sup>a</sup>

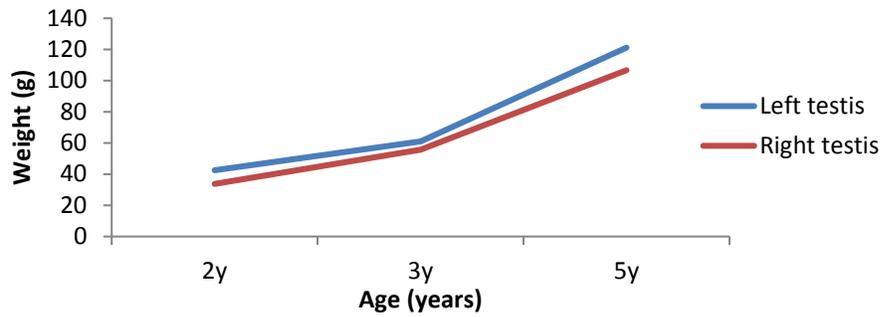


Figure (5): Effect of different age of pubertal (progressive) donkeys on weight of testis (left and right) (Means ± SE)

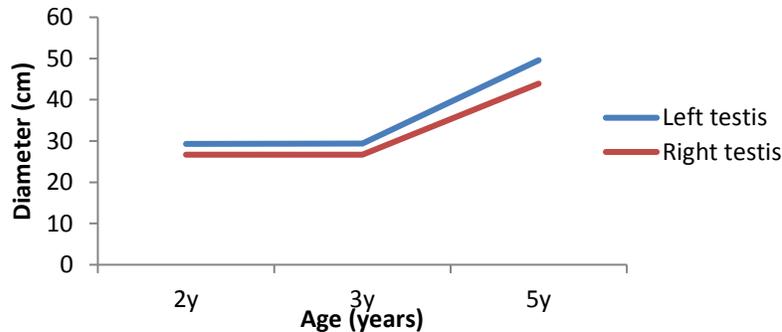


Figure (6): Effect of different age of pubertal (progressive) donkeys on diameter of testis (left and right) (Means ± SE)  
 The obtained Data illustrated that weight and diameter of testis either left or right still increase till donkeys reach to 7 years old and after that regression of testis weight and diameter occurred from 10 years old. At 6 years old, weight and diameter equal for left testis and right testis. Additionally, weight of testis at 7 years were significantly higher than others at 10 years for left and right testis. Besides, diameter of testis of donkeys at 7 years increased significantly than 10 years either left or right testis. Data Represented in Figure (7) and Figure (8).

Table (4): Effect of different age of regressive donkeys on weight and diameter of testis (left and right) (Means ± SE)

Items	Age of third group		
	6y	7y	10y
Weight of left testis (g)	125.55±0.32 <sup>c</sup>	155.80±0.030 <sup>a</sup>	135.20±0.30 <sup>b</sup>
Weight of right testis (g)	105.50±0.06 <sup>c</sup>	124.10±0.06 <sup>a</sup>	113.60±0.01 <sup>b</sup>
Diameter of left testis (cm <sup>2</sup> )	58.59±0.12 <sup>b</sup>	72.20±0.16 <sup>a</sup>	44.40±0.11 <sup>c</sup>
Diameter of right testis (cm <sup>2</sup> )	47.20±0.65 <sup>b</sup>	58.55±0.35 <sup>a</sup>	39.05±0.30 <sup>c</sup>

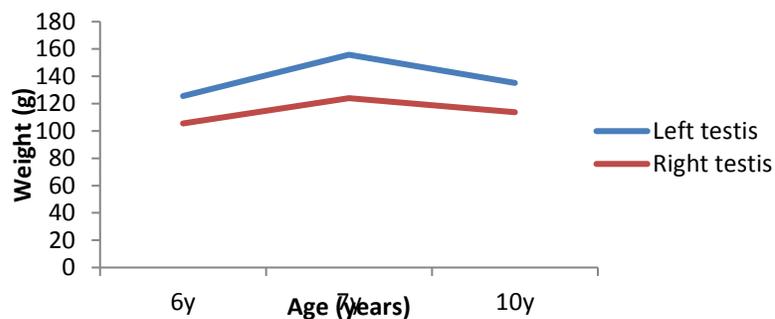
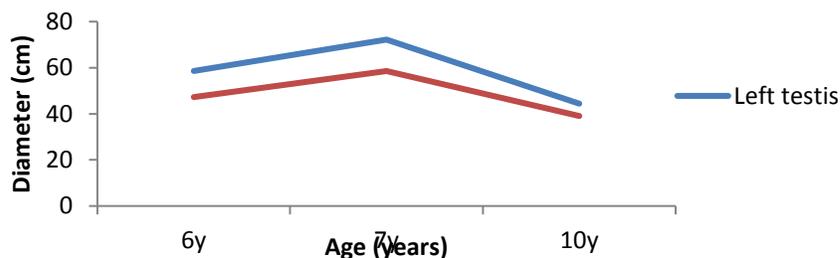


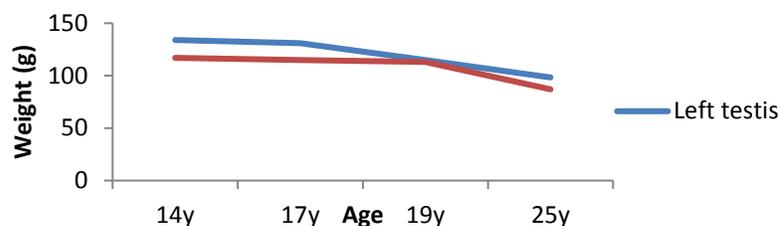
Figure (7): Effect of different age of post pubertal (regressive) donkeys on weight of testis (left and right) (Means ±SE)



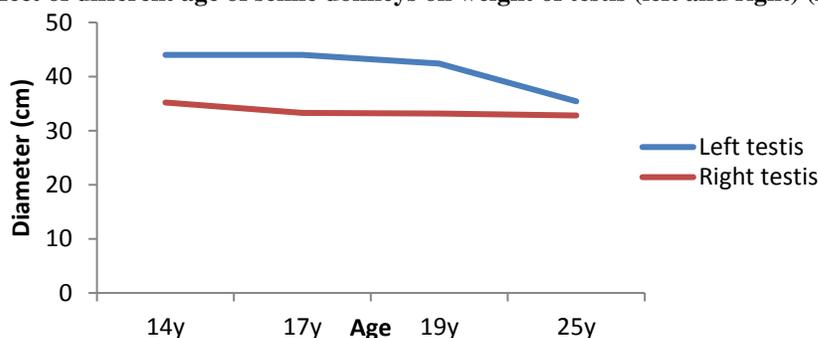
**Figure (8):** Effect of different age of post-pubertal donkeys on diameter of testis (left and right) (Means ±SE)  
 Senile donkeys showed significant decrease of weight of left testis and right testis from 14 years, 17 years, and 19 years till 25 years old as shown in **Figure (9)**. Moreover, **Figure (10)** illustrated that diameter of left testis and right testis equal for 14y, 17y, 19y and 25years old.

**Table (5):** Effect of different age of senile donkeys on weight and diameter of testis (left and right) (Means ± SE)

Items	Age of fourth group			
	14y	17y	19y	25y
Weight of left testis (g)	134.00±0.38 <sup>a</sup>	131.00±0.30 <sup>b</sup>	115.00±0.31 <sup>c</sup>	98.32±0.27 <sup>d</sup>
Weight of right testis (g)	115.70±0.41 <sup>a</sup>	113.00±0.41 <sup>b</sup>	113.00±0.45 <sup>b</sup>	80.11±0.34 <sup>c</sup>
Diameter of left testis (cm <sup>2</sup> )	44.00±0.06 <sup>a</sup>	44.40±0.02 <sup>a</sup>	42.38±0.02 <sup>b</sup>	35.42±0.05 <sup>c</sup>
Diameter of right testis (cm <sup>2</sup> )	35.20±0.78 <sup>a</sup>	33.27±0.65 <sup>b</sup>	33.15±0.43 <sup>b</sup>	32.80±0.54 <sup>b</sup>



**Figure (9):** Effect of different age of senile donkeys on weight of testis (left and right) (Means ± SE)



**Figure (10):** Effect of different age of senile donkeys on diameter of testis (left and right) (Means ± SE)

**Testicular morphology-Gross Anatomical study**

The right and left testes were appeared unequal, the left testes larger than right one. They were ovoid in shape but compressed from side to side, the long axis of the testes was horizontal in position. the greater part of testes was covered by a serous mesothelium membrane (tunica vaginalis); beneath it a tunica albuginea which contained collagen and few elastic fibers **Fig11 (A-B)**.

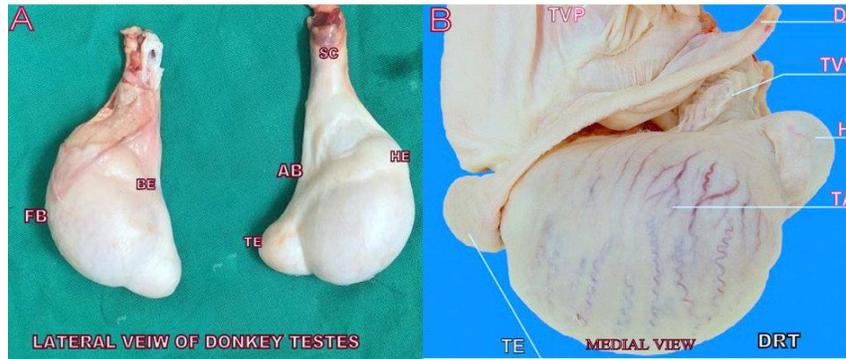


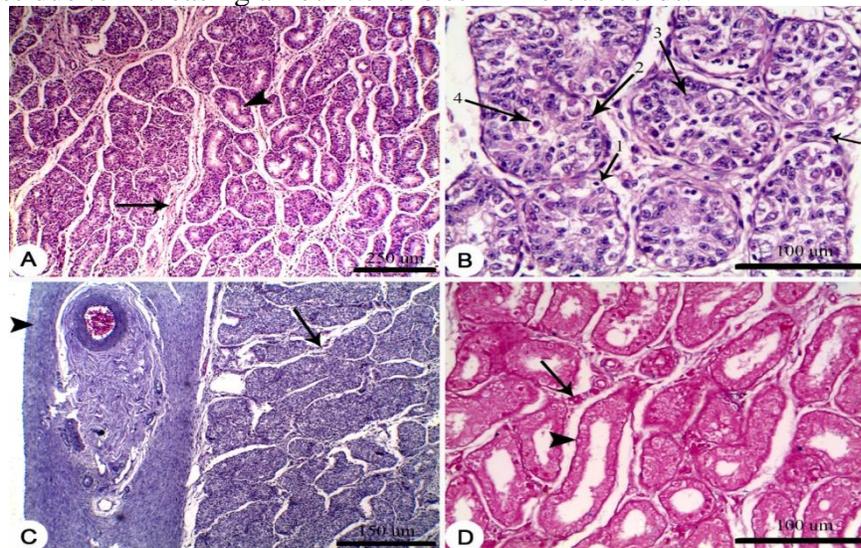
Figure 11.A Photograph of Lateral view of donkey Epididymal Head (EH), Epididymal Body (EB), Epididymal tail (ET), AB (attached border), FB (free border). Figure 11.B Photograph of Medial view of donkey (DRT) Right testes TA (Tunica albuginea, ductus deferens (DD), TVP (tunica vaginalis paritalis) and TVV (tunica vaginalis visceralis)

### Microscopic study

Histologically, the testes of donkey were covered by a tunica albuginea consisting of outer and inner fibrous layer in addition to middle vascular one. The seminiferous tubules were the major part of testicular parenchyma; in the seminiferous tubules of the donkey testes showed normally formed germinal epithelium, which contained spermatogenic cells and Sertoli cells as well as interstitial Leydig cells were observed. The seminiferous tubules had an irregular form more relatively coiled, the space between it was filled with interstitial tissue, where various size blood vessels and capillaries were observed.

### Group I (Pre pubertal animals)

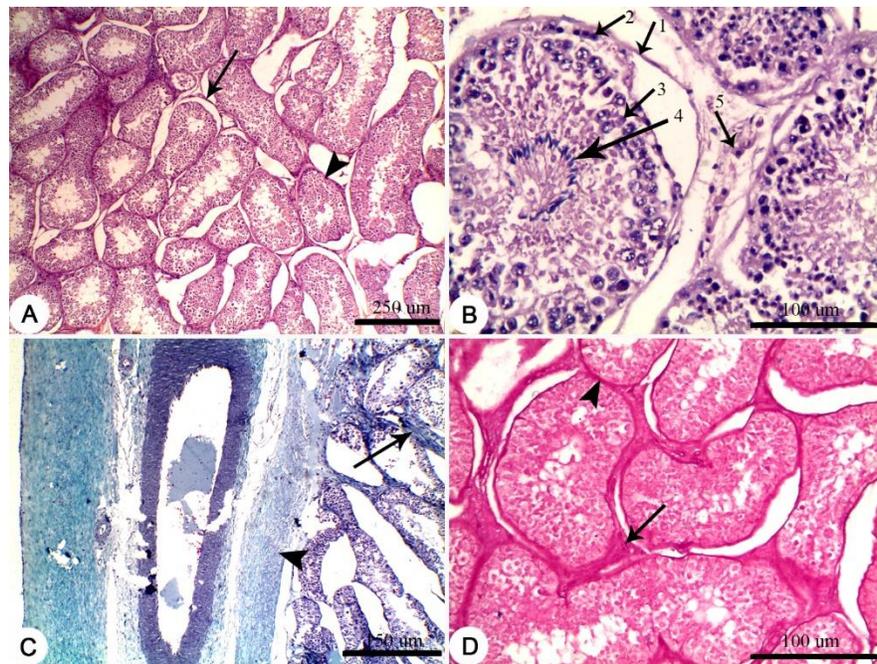
The present study emphasized that the testes was surrounded by a thick tunica albuginea which covered by one layer of tunica vaginalis. The testicular capsule showed no smooth muscle fibers at early stages of age **Fig12c**, Most of testicular parenchyma of consisted of large numbers of a solid irregular seminiferous cord surrounded by a thick connective tissue septa with no lumina the germ cells were oval or spherical with light stained cytoplasm; These cords separated by a large amount of interstitial tissue which decreased by the time due to lamination and convolution of seminiferous cords shortly before puberty the round to elongated spermatid were observed **Fig ( a, b)**. Leydig cells were variable in shape and size mostly they were polyhedral cells, in PAS-AB-stained section few Leydig cell stained positive **Fig12d**. Shortly before onset of puberty (at1.5 Y); Marked increasing in seminiferous tubule diameter in addition to the tunica albuginea thickness due to increasing amount of the seminiferous cords.



**Figure 12. Testes, Donkey, one-year-old.** **A)** showing immature seminiferous tubules (ST) with average diameter 80 µm (arrowhead), and marked interstitial tissues (arrow). HE stains. **B)** showing the normal histological structure of immature seminiferous tubules which contain smooth muscle wall (1), spermatogonia cells (2), Sertoli cells (3), Few Spermatids (4), and interstitial Leydig cells (5). HE stain. **C)** showing collagen fibers in tunica albuginea (arrowhead) surrounding the testis with average thickness 260 µm, and minimal amount of collagen fibers in the interstitium (arrow). Trichrome stain. **D)** showing positive staining for interstitial cells (arrow) and smooth muscle wall (arrowhead). PAS and Alcian blue staining.

**Group II (pubertal animals)**

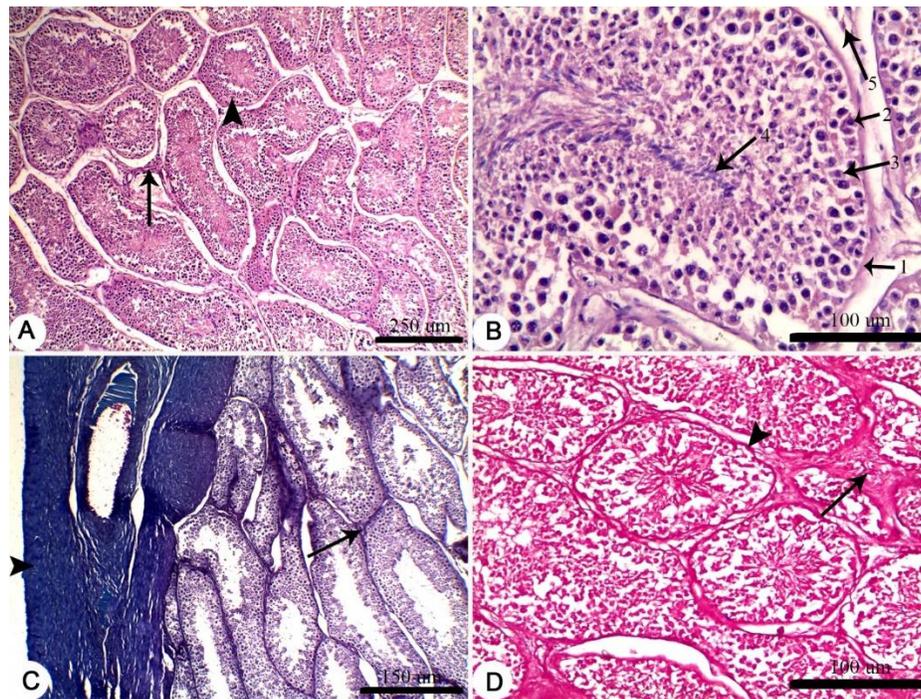
Complete spermatogenic cycle observed in all seminiferous tubules (spermatogonia-primary –secondary-spermatid and sperm); they were lied on basement membrane marked increased in testicular parenchymal and tubular diameter with epithelial thickness in addition to Decreasing of interstitial tissue amount. The testicular parenchyma was composed of islets of highly tortious irregular seminiferous tubules, some tubules had an irregular high-density lumen, the highest value of seminiferous tubule diameter were observed. **Fig13 (a, b)**. The tunica albuginea maintained its previous structure of outer and inner fibrous layers in addition to the middle vascular one, each layer was composed of collagen and few elastic tissues, and the vascular one was composed of variable sized arterial, venous and lymphatic vessels several connective septa originated from tunica albuginea. Presence of bundles of smooth muscles in outer fibrous layer of capsule for sperm transport. **Fig13c.**In PAS-AB section no change as compared with the previous stage **Fig13 d**



**Figure 13. Testes, Donkey, three-years-old.** **A**) showing mature seminiferous tubules (ST) with average diameter 215 μm (arrowhead), and slight interstitial tissues (arrow). HE stain. **B**) showing the mature seminiferous tubules which contain smooth muscle wall (1), spermatogonia cells (2), Sertoli cells (3), well developed spermatids (4), and interstitial Leydig cells (5). HE stain. **C**) showing collagen fibers in tunica albuginea (arrowhead) surrounding the testis with average thickness 410 μm, and collagen fibers in the interstitium (arrow). Trichrome stain. **D**) showing strong positive staining for interstitial cells (arrow) and smooth muscle wall (arrowhead). PAS and Alcian blue staining.

**Group III (Regressive)**

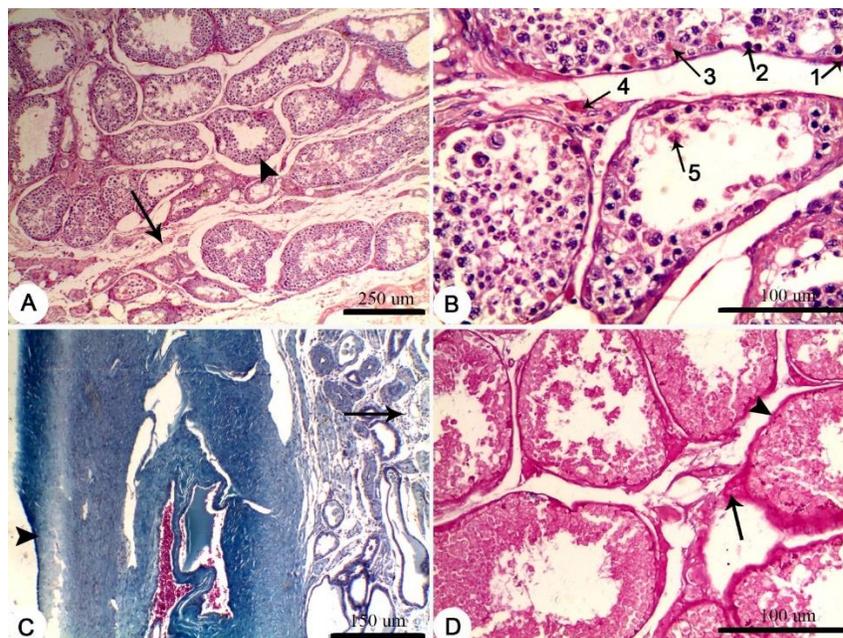
It already began at 7 years old. The arrangement of seminiferous tubules had a previous picture. The diameter of seminiferous tubule was decreased with marked Increasing in lamina propria thickness with age advancing due to declination of seminiferous epithelial efficiency in addition to decreasing of young secondary spermatocyte and decreasing of spermatogenic precursor proliferation were observed. At the age of 10, the basal lamina is thicker, displays irregular projections into germ and Sertoli cells **Fig14 (a, b)**. The thick layer of tunica albuginea with a bundle of smooth muscles layer was observed **Fig14c** In PAS-AB-stained section a positive reaction of interstitial and basement membrane were appeared **Fig14d**.



**Figure 14. Testes, Donkey, seven-years-old.** **A)** showing mature seminiferous tubules (ST) with average diameter 235  $\mu\text{m}$  (arrowhead), and slight interstitial tissues (arrow). HE stain. **B)** showing the mature seminiferous tubules which contain smooth muscle wall (1), spermatogonia cells (2), Sertoli cells (3), well developed spermatids (4), and interstitial Leydig cells (5). HE stain. **C)** showing collagen fibers in tunica albuginea (arrowhead) surrounding the testis with average thickness 400  $\mu\text{m}$ , and collagen fibers in the interstitium (arrow). Trichrome stain. **D)** showing strong positive staining for interstitial cells (arrow) and smooth muscle wall (arrowhead). PAS and Alcian blue staining.

**Group IV (senium)**

The seminiferous cords were appeared corrugated due to shrinkage of outer lining membrane with decreeing in tubular diameter in addition to A rising volume of intertubular tissue is also observed in the ageing . **Fig 15(a,b)**,the thickset tunica albuginea as comparing with the previous age groups .**Fig 15c**. In PAS-AB-stained section highly positive reaction of interstitial and basement membrane were appeared **Fig15d**.



**Figure 4. Testes, Donkey, twenty-five-years-old.** **A)** showing atrophied seminiferous tubules (ST) with average diameter 148  $\mu\text{m}$  (arrowhead), and marked interstitial tissues (arrow). HE stain. **B)** showing the atrophied seminiferous tubules which contain thick smooth muscle wall (1), a few spermatogonia cells (2), hyaline degenerated Sertoli cells (3), hyaline degenerated interstitial Leydig cells (4), and degenerated spermatocyte (5). HE stain. **C)** showing collagen fibers in tunica albuginea (arrowhead) surrounding the testis with average thickness 390  $\mu\text{m}$ , and collagen fibers in the interstitium (arrow). Trichrome

stain. **D)** showing strong positive staining for interstitial cells (arrow) and thick smooth muscle wall (arrowhead). PAS and Alcian blue staining.

## DISCUSSION

Previous studies have illustrated that a great difference in time of descending of testes in different species. The current observation revealed that the testes of donkey not completely descend in scrotum at 1week contrarily with Abdel-Hafeez (2005) who recorded that the testes of donkey descend in scrotum at 1week after birth, in addition to Bergin, *et al.*(1970) who gave a range in the testicular descent in the horse from about 30 days before birth to 10 days after birth . The testes of the donkey were smaller and lighter, weighed about half those of the stallion similar result obtained by Jacob (1978). In the over all, the significant difference was noticed in the weight and diameter of the testes of donkeys between four age groups with advancing ages. The testis of the donkey was used as a model to study age-related changes in the period between birth and senium. The postnatal testicular development in the donkey was divided into four phases: pre pubertal (birth to 1.5 year) - pubertal (2-5) - post pubertal (6-10) and senile (more than 10y) from the same respect, the postnatal testicular development in the bovine was divided into four phases: infantile, proliferation, pre pubertal and pubertal phase (Abdel-Raouf, 1960; Sinowatz *et al.*, 1983). During these phases, the testicular cells undergo gradual developmental changes to attain the pubertal form at about 40 weeks of age.

The higher numerical value of the left donkey testis than the right one similar to the reports of Getty (1975), who reported that the both testes of donkey are appear unequal. from current result in agreement with el-Jack (1980) in one-humped camel, who reported that the left testicle is larger than the right one due an enlarged pampiniform plexus on the left side similar to the result obtained from Otet *al.* (1982) in sheep; Nasir *et al.* (2014) in goat, who reported that the left testis was 10% larger than right one.

Furthermore, the mean testicular weight increased slowly during early ages of Group I After that; shortly before puberty, testicular measurement recorded progressive increasing. The current observation showed that significant increase in weight of both testes with increase ages of pre-pubertal donkeys from 1m, 4m, and 10 m to 12m respectively; Amann and Curtis

(1981) discussed the testicular diameter increasing during post-natal development due to increasing in parenchymal mass occupied by the seminiferous tubules. The current observation revealed that there was a relatively slow testicular parenchymal growth from 1 month to 4 months of age. There was a pronounced increase in the testicular weight and diameter at one year. From the current observation we can notice that the testicular diameter recorded higher values compared with the weight in mature animals due to the spaces occupied by a lumen inside seminiferous tubules, which weight nothing. Berndtson (1986) who gave similar results in the donkey-that a period of increasing tubular diameter is immediately followed by a period of decrease during sexual maturity. In contrarily with Abdel-Rouf, *et al.* (1975) who reported that testicular weight, dimension and volume of camel testes are stable in aged animals.

### **Histological changes of the testes**

The current result indicated that The donkey testis can be divided into two compartments, a seminiferous tubular compartment and an interstitial; the seminiferous epithelium lied on the a cellular basement membrane and contains two types of cells, Sertoli cells and germ cells in agreement with Mai HM (2014)

The present observations revealed that the testis of the donkey was covered by a tunica albuginea consisting of an outer and inner fibrous layer with middle vascular one this in agreement with Naden et al (1990). Tunica albuginea had a striking property due to presence of bundles of smooth muscles in its outer fibrous layer for sperm transport in agreement with (Dellmann and Worbel 1983) reported that smooth muscles bundles are seen in outer fibrous layer of stallion tunica albuginea. Contrarily with Chacon-Arellano and Wooley who denied the role of this muscles in sperm transport in testes of horse, pig and sheep.

The present study showed an irregular arrangement of the testicular lobules of the donkey during various stages of the postnatal life. So, some lobules failed to have a direct contact with the mediastinum testis. Unlike, the case in other domestic animals such as ruminants, pigs and dogs where the mediastinum occupied a central position (Wrobel and

Dellmann, 1993), the testicular lobules of donkey testes had an irregular arrangement in all age groups; such arrangement in agreement with Moustafa *et al.* (2015). On the other hand, Getty., (1975) who reported that there was no distinct mediastinum in equine testes. The present investigation revealed that the epithelial lining of tubules including germ cell and supporting cell showed a certain morphological differentiation during post-natal development in all stages. They decreased in numbers with age advancing this result has been observed in human from newborn to adulthood

The Sertoli cells located on the outermost portion of the tubule, right up against the basement membrane with an oval nucleus, the current study revealed that the population of Sertoli cell was constant at age advancing because they didn't normally divide and proliferate, the similar result discussed by (Johnson *et al.*, 2008).

Our results correspond with our previous observations. The interstitial tissue was, this tissue composed of vascular tissue and the Leydig cells were dispersed between the seminiferous tubules in agreement with (Leeson and Forman, 1981).

The current result revealed that the Spermatogenic cells were basally located at the periphery of the tubule near basal lamina and can be identified by their densely stained, round nuclei. At first; the spermatogenic cells were located near the base of the "basement membrane" and as they developed into later stages, the spermatocytes were moved up toward center. By the time they were located close to the tubule lumen; they have completed their meiotic division and become Spermatids, haploid cells undergoing the final morphological transformation into sperm similar to (Getty, 1975).

The current study showed that

The parenchyma of the testes in group I; it was composed of a solid irregular cord with one to two layers of germinal epithelium resting on one layer of mesothelium cells; shortly before puberty it was composed of time numerous densely packed irregular and laminated seminiferous tubules with interstitial cells in between which are in accordance with the findings of Treuting and Dintzis (2012).

At 1 year old; The testicular capsule showed no smooth muscle fibers at early stages of the postnatal development, most of testicular parenchyma of consisted of a solid irregular seminiferous cord surrounded by a thick tunica albuginea with no lumina.; These cords separated by a large amount of interstitial tissue which decreased by the time due to they were laminated. The amount of interstitial tissue was increased in prepubertal and senile groups but decreased in pubertal and post pubertal group in agreement with Nipken and Worbel (1997) who was demonstrated the age-related increase of tubular convolution with a decreasing of interstitial space shortly before puberty. Leydig cells were variable in shape and size mostly they were polyhedral cells. Shortly before onset of puberty Marked increase in testicular weight and diameter in addition to the tunica albuginea thickness (at 1.5 Years old) the amount of seminiferous cords increased in agreement with Abd-Elhafeez, (2017). at 2-6 years old; Complete spermatogenic cycle observed in all seminiferous tubules; marked increased in testicular parenchymal and tubular diameter with epithelial thickness .The diameter of seminiferous tubule was decreased with marked Increase in lamina propria thickness with age advancing. At the age of 10, the basal lamina is thicker, displays irregular projections into germ and Sertoli cells. These changes of the basal lamina are in accordance with previous results in man (Johnson *et al.*, 1986) The seminiferous cords in testicular parenchymal were appeared corrugated due to shrinkage of outer lining membrane with the decrease in tubular diameter A rising volume of intratubular tissue is also observed in the ageing.

## CONCLUSION

We can conclude that the study has shown the significant differences in terms of Morphometry, gross morphology and histology of right and left testes of donkey at different age groups especially, weight and dimensions of the testes differ from age to age. The testes of the donkey grow relatively slowly until approximately 1y of age and then a rapid phase of growth occurs until puberty, at 1.5y of age.

During the early postnatal phase of slower growth of the testis. The pre-spermatogonia and some spermatogonia are established, adult

Leydig cells appear and undifferentiated Sertolicele are produced. The rapid testicular growth, after 1.5 years of age, consists of marked increases in the diameter and length of the seminiferous tubules, dramatic proliferation and differentiation of germ cells, with mature spermatozoa occurring between 2-5 years of age. The adult Leydig cell and Sertoli cells population is largely in place. The progressive changes extend from puberty at 3 years old until 10 years the productive capacity will be decrease.

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