



SPERMATOGENESIS AND SPERMIOGENESIS STUDY IN THE TESTES OF ADULT DONKEY (*EQUUS ASINUS*)

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ABSTRACT

The present study was designed to investigate the spermatogenesis and spermiogenesis in the testes of the adult male donkey (*Equus asinus*). The testis of adult male donkey lies within the scrotum. Each testis consists of two surfaces, two borders, and two extremities. The epididymis was firmly attached to the dorsolateral surface of the testis. The testis was covered by a capsule housing a vascular bed. The capsular septae divided the testis into lobules. The lobular testis was composed of more than one of the seminiferous tubules. Multilayered germ cells were noted in the seminiferous tubules of the testis in the adult donkey. The first layer included three types of spermatogonia: type-A-Spermatogonia, the B-spermatogonia, and intermediate type. The next holds six types of primary spermatocytes known as Preleptotene, Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis. The last type of primary spermatocyte produces the secondary spermatocyte. Secondary spermatocytes were converted into spermatids. The final layer contained seventeenth stages of spermatids. These metamorphosis of spermatids yield the spermatozoa which possess heads, mid pieces, and tails.

KEYWORDS: Donkey, Testes, Spermatogenesis, Spermiogenesis

INTRODUCTION

Spermatogenesis and Spermiogenesis comprise the process of transformation of spermatogonial cells into spermatozoa over an extended period of time within the testicular seminiferous tubule^[1,2]. This process was made up of different stages which based on the arrangement of spermatids within the germinal epithelium, the shape of the spermatid nucleus, the nuclear chromatin, and the morphology of developing spermatids^[3,4,5]. Medical researchers were found some variation in the stages of the seminiferous epithelium in the testes of some vertebrates^[6,7,8]. A search of spermatogenesis and spermiogenesis in donkey (*Equus asinus*) were somehow devoted or remains poorly investigated^[7]. The aim of this study was organized to build up some histological remarks and to search the spermatogenesis and Spermiogenesis stages in the testes of donkeys so as to establish a norm for future physiological and pathological works in this tissue.

MATERIALS & METHODS

Six adult male donkeys were obtained from different locations in Baghdad city, Iraq. These animals were housed under conventional conditions in the animal stable at the College of Veterinary Medicine, University of Baghdad, and maintained in a pathogen-free environment. All animals were fed with enough green diet and supplied with enough water for more than two weeks prior to orchiectomy^[9]. Orchiectomy was done for all these animals, some anatomical remarks were recorded. Each testis was cut into pieces and dipped into 10% formalin. The specimens were washed with water several times in order to remove a large proportion of

fixative. Dehydration was achieved by passing the specimens through ascending grades of ethanol. Clearing these fixated specimens by passing through xylene. Specimens were immersed in a mixture of xylene and paraffin wax at 54-60°C. These specimens were transferred to melted paraffin for two to three hours. Tissue block was then placed in a small container of fresh melted paraffin until solidification occurs which encasing the tissue blocks. Trimming and sectioned with microtome by five μm thick. The obtained histological sections were stained with hematoxyline and eosin according to Luna^[10].

RESULTS & DISCUSSIONS

The adult male donkey possesses a pair of testes that lie in a pouch of skin or scrotum. The scrotum was black in color and oily to touch, and commonly asymmetrical, since one testicle more often, the left, appeared the larger and placed a little further caudal. The presence of scattered short fine hairs was noted. The testes were marked centrally by a longitudinal raphe which continued cranially on the prepuce and caudally on the perineum. The testes were ovoid in form but compressed from side to side. Each testis showed two surfaces, two borders, and two extremities. The medial surface appeared convex but somewhat flattened by contact with the scrotal septum. The lateral surface was also convex. The free border positioned ventrally and appears somewhat convex. The epididymal border was in the dorsal area (Fig.1). The long axis of the testis was a proximally longitudinal. Equine testicle in case of drawn up or has not completed its descent into the scrotum, the long axis was remained almost vertical. The result

revealed that the epididymis adhered to the attached border of the testicle and over laps the lateral surface (Fig.2). The head of the epididymis from an enlargement at the cranial part. The caudal, slightly enlarged end form the tail and the intermediate narrow part of the body of the epididymis which was closely attached to from a pocket beneath the epididymis. The testicle of the donkey was richly supplied with blood by testicular artery. This description is in agreement with^[11]. Histologically, each testis of the donkey was covered by a capsule of dense irregular connective tissue housed a vascular bed that feeds and drains the tissue (Fig. 3). Some researchers^[12] declared that this vascular bed or larger can be deep in some animals, including boar and stallion and sometimes was referred to separately as the tunica vasculosa. The mediastinum testis, the actually a thickened portion of the testicular capsule, converge inward but does not exist completely. Similar structure has been mentioned in the horse^[13]. Some authors^[14, 15] noted that the connective tissue capsule converge posteriorly in feline and rodent species and centrally in canine, porcine, and ruminant species to form mediastinum testis. The microscopically examination showed that the parenchyma of the testis contained trabecular and interlobular septa which form a network and showed no special condensation in any part of the testis. In correlation with this, absence of a rete testis formed by the anastomosis of the seminiferous tubules in the mediastinum. The septa cause the testicular stroma to divide into lobules. Each lobule possed the seminiferous tubules which supported by loose intralobular connective tissue (Fig.4). The tubules were very tortuous; they unite with other tubules to form straight tubules. The later unite with adjacent tubules and converge toward the cranial part of the attached border of the testis to form larger efferent ducts, which pierced the capsule and enter the head of the epididymis. One to four looping seminiferous tubules were eventually the site of spermatogenesis (Fig.5). Each seminiferous tubule was lined by the seminiferous epithelium. These epithelium was composed of two cell types; Sertoli cells that extends from the basement membrane to the tubular lumen. The general picture of Sertoli cell; showed a broad base containing oval to irregular nucleus. The apical portion of Sertoli cell was highly irregular (Fig. 6). The lateral sides of adjacent Sertoli cells form numerous infoldings. Often, the Sertoli surface was deeply indented by old spermatid. Here, the Sertoli cells were joined with each other by tight junction. This is in agreement with^[12] whom they also added that the tight junctions constitute the blood-testis barrier, that provide a microenvironment in the ad luminal compartment within the complicated processes of meiosis and Spermiogenesis occur without major outside disturbance. In addition the work of^[16] assumed that Sertoli cells have had nutritive, protective, and supportive functions for the spermatogenic cells. Sertoli cells can phagocytize regressive spermatozoa and detached residual bodies of spermatids. Beside,^[13] noted [that Sertoli cells release the spermatozoa into the lumen of the seminiferous tubules,

mediate the action of gonadotropic hormones on the germ cells, produce an androgen-binding protein, and secrete the intra tubular fluid. This study showed that the germinal stratified epithelium of the seminiferous tubules in the testis of the donkey was attached to a thin wall of connective containing myofibroblastic cells which lie next to the germinal epithelium whereas the fibrocytes lie more peripherally (Fig. 5 and 7) . This coincides with^[17, 18]. The view of microscopic study in this research revealed that the spermatogenic lineage in the testis of the donkey began with spermatogonia. The spermatogonia showed type A-spermatogonia, type B-spermatogonia, and I-spermatogonia or intermediate spermatogonia (Fig. 4 and 5). A-spermatogonia aggregated singly or in clusters. They were rounded cells possesses oval nuclei. Their nuclei showed dispersed chromatin was but some of chromatin was in the form of flakes. B- spermatogonia have large oval to round nuclei and their chromatin was in the form of crusts. The general picture of the I-spermatogonia or intermediate spermatogonia was in between the main outlines described for A and B spermatogonia. Their nuclear chromatin was dispersed in the form of small granules and a few flakes of chromatin were often seen located in the center of their nuclei. A study on different types of spermatogonia in boar, monkey and buffalo had led to further classification into A₀, A₁, A₂, intermediate, B₀, B₁, and B₂^[9,8]. B- spermatogonia in this research revealed mitotic activity to give rise to primary spermatocytes which larger cell and more circular (Fig. 6). There were six step of primary spermatocytes; Preleptotene, Leptotene, Zygotene, Pachytene, Diplotene, and Diakinesis. The final step affects the nuclear membrane which disintegrates and completely disappeared. In this case, the primary spermatocyte generated two secondary spermatocyte (Fig. 6). The nucleus of secondary spermatocyte had clumps of heterochromatin blocks along the inner facet of the nuclear envelope. The meiotic division led to form the spermatid. The spermatids pass through metamorphosis. The spermatids can be classified in this study according to their nuclear chromatin pattern, size, and shape into seventeenth stages. In the first stage, the nuclear chromatin appeared granular associated with some flakes of chromatin. Their nuclei in this stage were round and small in size (Fig. 6). In the second stage, chromatin flakes were found nearer to the center and some of these flakes may adhere to the nuclear spermatids (Fig. 6). The nuclear chromatin took the form of crusts which mostly adhere to the nuclear membrane in the third stage (Fig.6 and 8). The nuclear chromatin form more than three or four masses positioned in probable space nearer to the nuclear membrane in the fourth stage (Fig.7). The configuration of the nuclear spermatid appears long and the nuclear chromatin accumulates at one pole of the nucleus in the fifth stage (Fig.7 and 8). Two protrusions of nuclear chromatin in the form of two horns were formed in the sixth stage (Fig. 8). Another third horn of nuclear chromatin was formed in the seventh stage (Fig. 8). Slimy appearance of nuclear chromatin attached the condensed chromatin found at one pole was formed in the eight stage (Fig. 8).

In the ninth stage, the slimy nuclear chromatin loses its attachment with the condensed one. The nuclear spermatid was surrounded by a thick cellular cytoplasmic band (Fig. 8). Disappearance of the cytoplasmic band occurs in the tenth stage which lead that the nucleus becomes more elongated (Fig.7 and 8). Appearance of short flagellum in each spermatid in the eleventh stage (Fig. 8). In the twelfth stage, the nuclear chromatins become homogenous and occupy a wide space at the anterior pole and thinner at the posterior pole (Fig. 8). Each nucleus showed slimy extensions to the exterior within the cytoplasm in the thirteenth stage (Fig. 8). The spermatid in the fourteenth stage possesses delicate flagellum and the nuclear spermatid may assume exterior of sperm (Fig.10). The fifteenth stage showed that the chromatin assume the form of elongation at anterior tip (Fig. 10). The nuclear spermatid showed the acrosomes substantial extend to anterior tip sixteenth stage (Fig. 11). The mature spermatozoa were indicated

within the lumen of the seminiferous tubules. These spermatozoa were divided into three main segments; head, mid piece, and tail in the seventeenth stage (Fig. 5). The architectural of spermatogenesis and Spermiogenesis was also reported by (Al-Hamery, 2008)^[21] in goat fifteenth stage, (Pawar and Wrobel, 1991)^[19] and by (Singh and Wrobel, 1991)^[18] in buffalo but the lineage was divided into sixth stage only. The germinal seminiferous epithelial lineage was fixed as twelfth stages in mouse, rat, guinea pig, and hamster^[3], and in dairy bull, the lineage was fixed as eight stages (Amman,1962)^[7]. Indeed, the fact of these different lineage registered the variability of cellular observations present in the epithelium of seminiferous tubules in the testes of the donkey in this study may be related to the reproductive season or may be due to access number of Sertoli cells^[20-22].



FIGURE 1: show external appearance of donkey testis



FIGURE 2: shows the donkey testis (Epididymis border).

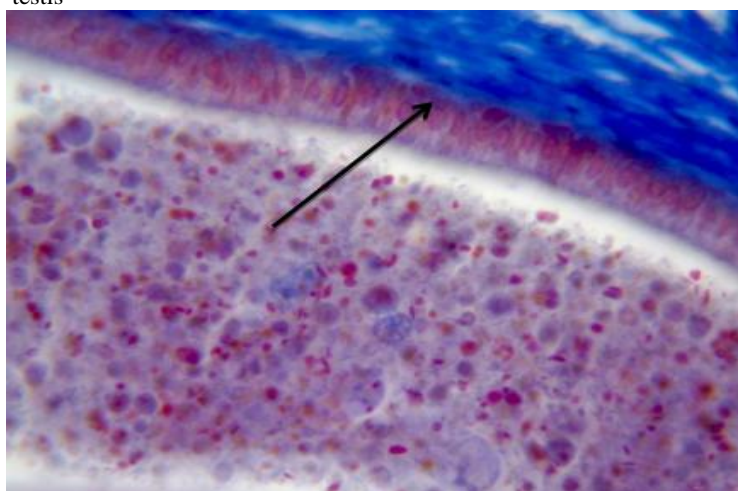


FIGURE 3: a-connective tissue capsule H & E. stain (x40).

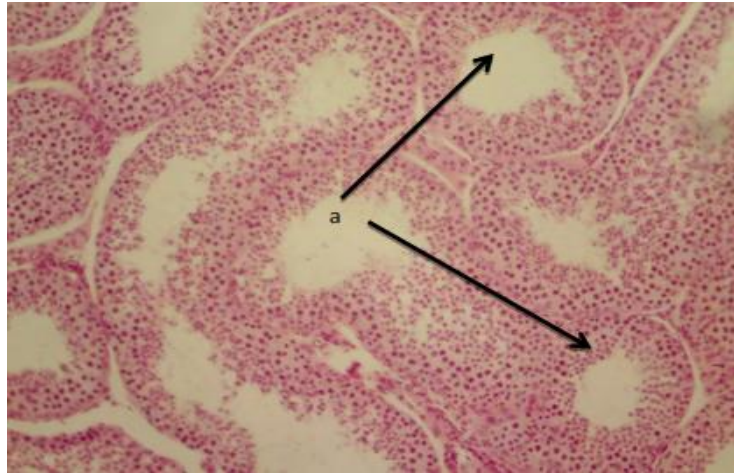


FIGURE 4: a-seminiferous tubule, H&E. stain (x40).

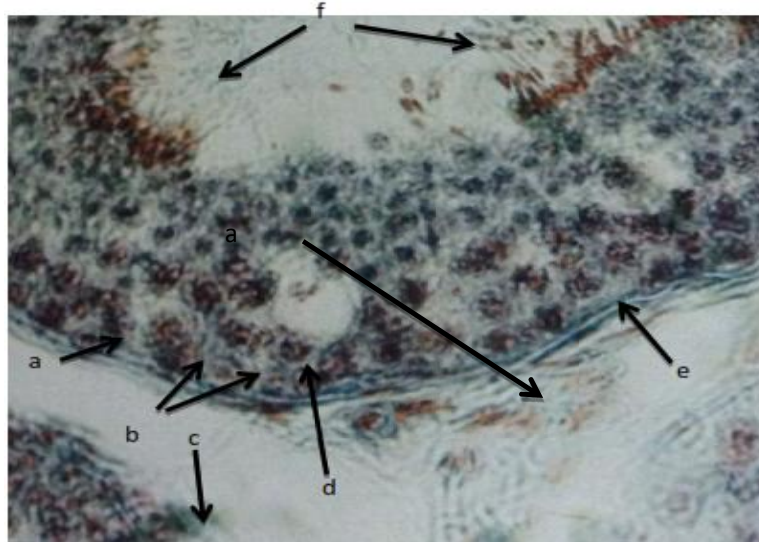


FIGURE 5: show a-site of spermatogenesis on seminiferous tubule. b- type A-spermatogonia. c-B-spermatogonia. d-I-spermatogonia. e-germinal stratified epithelium of the seminiferous tubules in the testis of the donkey.f- 17th stage. H & E. stain (x 40).

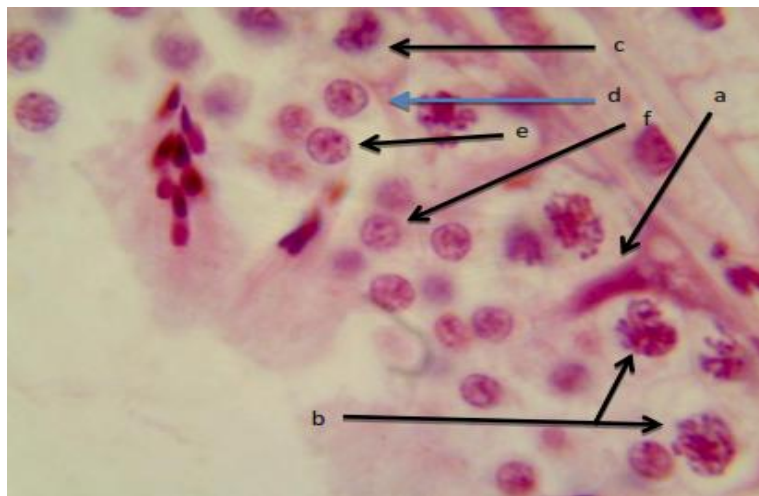


FIGURE 6: show a-Sertoli cell. b- primary spermatocytes. c- secondary spermatocyte d- 1st stage . e- 2nd stage. f- 3rd stage. H&E. stain (x100).



FIGURE 7: a- germinal stratified epithelium of the seminiferous tubules in the testis of the donkey. b- 4th stage. c- 5th stage. d-10th stage. H & E. stain (x100).

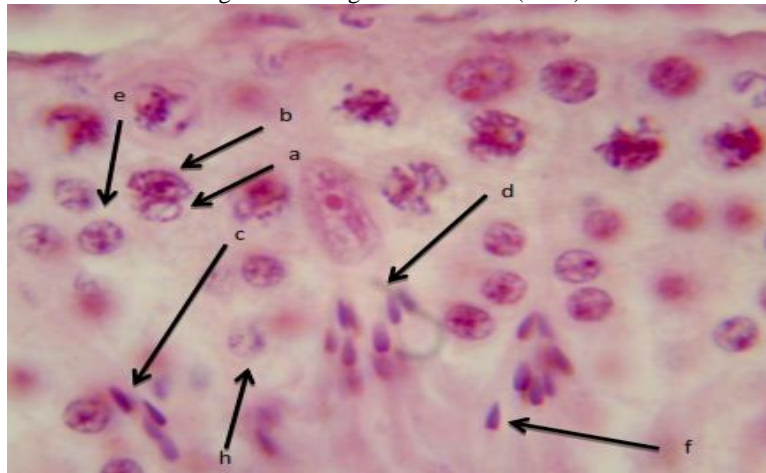


FIGURE 8: shows a- 5th stage. b- 6th stage. c-10th stage. d-11 stage. e- 7th stage. f- 12th stage. g-13th stage. h- 3rd stage. H & E. stain (x40).

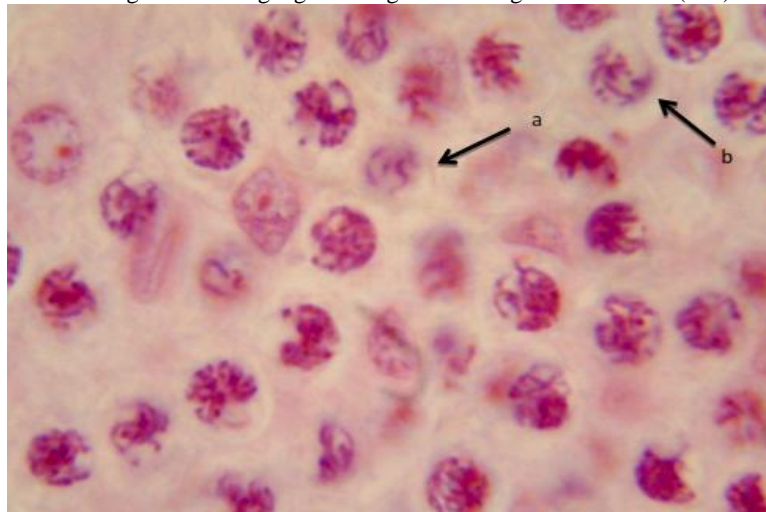


FIGURE 9: a- 8th stage. b- 9th stage H&E. stain (x100).

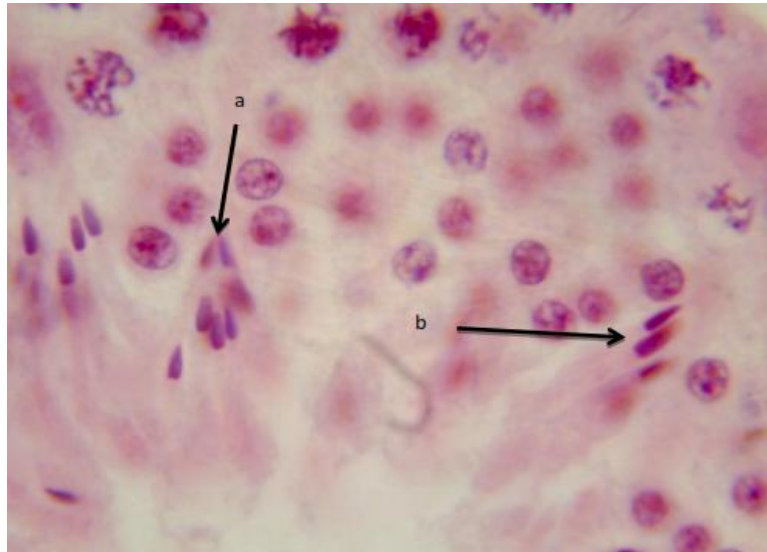


FIGURE 10: a-14th stage. b- 15th stage. H&E. stain (x100).

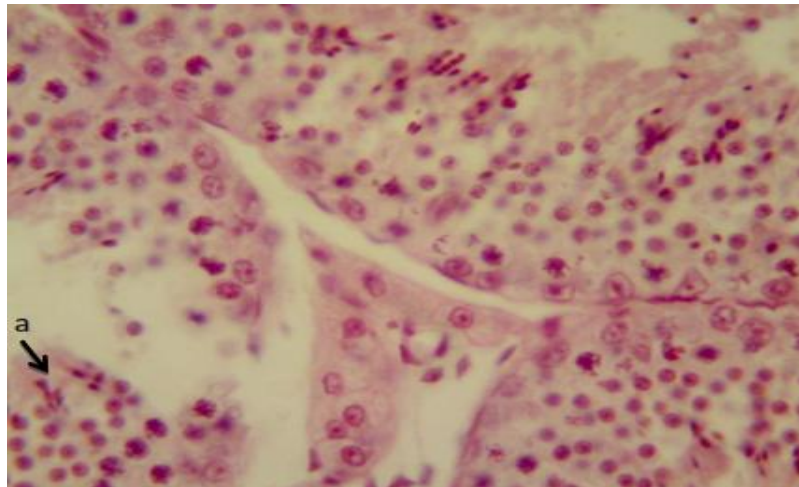


FIGURE 11: shows a- 16th stage H & E. stain (x40).

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