Wasit Journal for Pure Science

Journal Homepage: https://wjps.uowasit.edu.iq/index.php/wjps/index
e-ISSN: 2790-5241 p-ISSN: 2790-5233

Histological study of testis of golden hamster (Mesocricetus auratus) in different ages during postnatal development

Qassem A. Mohammed[1] , Mohammed S. Dawood[2]

[1,2]Department of Anatomy and Histology, College of Veterinary Medicine, University of Baghdad, IRAQ

*Corresponding Author: Qassim Abbas Mohammed Al-Jebory

DOI: https://doi.org/10.31185/wjps.627
Received 21 November 2024; Accepted 24 December 2024; Available online 30 March 2025

ABSTRACT: The present study aimed to investigate the histology of the testis in golden hamster during 1, 28 and 60 days of old. Tissue samples were processed, sectioned and subjected to specific stains to reveal histological details. Observation of one day old showed the testicle enclosed by undifferentiated thin layer of testicle capsule which consisted of undifferentiated myoblasts and fibroblasts small size seminiferous tubules appeared, one layer of spermatogonium cells (Type-A) which revealed mitotic figures, at 28 day old the capsule was consisted of thick outer fibromuscular layer and thick inner vascular layer that revealed encouraged blood vessels and cells, Spermatogonial cells type-A & B appeared as large size with darkly stained nucleus and eosinophilic cytoplasm, as well as Primary and secondary spermatocytes appeared slightly small size cells comprised of small lightly stained nucleus at 60 day parenchyma was buildup by enlarged size seminiferous tubules that revealed complete differentiated spermatogenic cells and each tubule was surrounded by thin fibro cellular membrane with myoid cells. Spermatogonial cells types-A and B appeared enlarge size with darkly stained nucleus and eosinophilic cytoplasm, as well as Primary and secondary spermatocytes appeared slightly smaller in size cells, comprised of small lightly stained nucleus.

Keywords: hamster, histology, testis

1. INTRODUCTION

Mammals have very wide species but the rodents considered the major number of the mammalian species, it's about 40% of the mammals. The benefit of the rodents as source of animal protein and used as experimental animals, as well as, considered as zoonotic diseases transporter there for research interest was growing with Rodent biology [1]. The hamster considered as one of the rodent family relationship to the subfamily Cricetinae, the Cricetinae have 19 species distributing in 7 genera, the hamsters become popular small pets [2]. Golden hamster belong to seasonal animals for that its gonads are remain active through the long day in two season; spring and summer, and non-active through the winter season, for this reason it can be used as laboratory population in all day year by artificial alteration in photoperiod[3]. Generally, testis of mammals have two functions; spermatogenesis and sex hormones synthesis like prolactin, FSH, LH and testosterone[4].

Development of the testis belongs to several factors; hereditary, nutritional, mechanical, heat, biochemical and endocrine hormonal effect. The changes of any of these factors lead to disappear of spermatogenesis and causes infertility [5]. Maturation and spermatogenesis belong to testosterone influence[6], maturation distinguished by starting of meiosis and sperm creation [7]. Testis is a heterogeneous organ consisted of several partitions and cell types, the partition hold generally seminiferous tubules which is regarded the point of spermatogenesis process whereas the hole between the tubules named interstitium; endocrine function process that occurred by Leydig cells. The tubules delimited with stratum of cells called peritubular -myoid cells, which are alienated from tubular cells by a thin stratum extracellular matrix [8].

2. METHODOLOGY

2.1. Experimental animals:

fifteen animals golden hamster at 1, 28, 60 day of old were used, each group contain 5 animals, The animals were injected intra peritoneal by ketamine and xylazine [9, 10] .After scarified the animals, then the animals were opened up, and testis were separates of the animals, testis was washed by tap water then cut into small sections and instantly fixed in 10%formaline for 72 hour [11-13]. The samples were cut into thin section about 5-6μm by rotary microtome, then tissues have dealing according to the paraffin embedding histological method at 58-60C°[14]. The tissue sections have stained with Hematoxylin & Eosin stain, Masson's Trichrome stain combine alcian blue (pH2.5)-PAS stain [15-17]. Sections have investigated by bulletin Olympus microscope camera. All numerical data have displayed as mean±SE, one way (ANOVA) was used for detection an important difference at ($p < 0.05$).

2.2. RESULTS AND DISCUSSION

At 1st day, the figures of testes revealed that undifferentiated testicle capsule which revealed very thin layer composed of undifferentiated myoblasts and fibroblasts (Fig1& 2). Parenchyma was buildup by numerous small size seminiferous tubules each tubule was surrounded by thin fibro cellular membrane which consisted of myoid cells (Fig2&3). One layer of undifferentiated spermatogonium cells (Type-A) which revealed mitotic figures and showed clear cytoplasm, newly differentiated Sertoli cells that revealed intense eosinophilic cytoplasm and large dark nucleus (Fig2). The testicular interstitum was buildup by delicate layer of loose connective tissue that comprised of differentiated interstitial cells with inter tubular blood vessels (Fig2&3). Diameter of seminiferous tubules, thickness of tunica albuginea and height of germinal epithelium were recorded in Table1. Thickness of interstitial tissue and Numbers of interstitial cells were recorded in Table2.

The result of the current study was In contrast with Dorostghoal, *et al.* [18], who demonstrated that seminiferous tubules of Wister rat at one day of age seem as solid cords, without a central lumen, while mentioned one type of spermatogonium cells as the present study, as well as Sertoli cells appearance. On the other hand our result also was coincided with Ismail and kareem Atyia [19], who recorded that the rabbit tunica albuginea was consisted of connective tissue without muscle fibers, inner part of this capsule is loose and highly vascularized but the outer part is dens and surrounded by simple squamous epithelium of mesothelium, as well as The seminiferous diameter was more three times than hamster it about 70.33±1.59µm. Germ cell mitoses are not seen in this stage as compared with the mitotic figure that seen in the current study at one day of age, moreover the interstitial tissue had the same structure of the hamster interstitial tissue, but the diameter of seminiferous tubules were 48.20 ± 0.24µm reach to two time more than of the current study.Tsunenari and Kast [20] in Himalayan rabbit recorded elevation of four time of tubules diameter, which were 83± 2µm. The significant differences between these diameter were due to the level of testosterone (Leydig cell synthesis), FSH, LH value between these spices, these hormones have influence on the tubule diameter and process of spermatogenesis as well as influence on the thickness of tunica albuginea, Height of germinal epithelium, Thickness of interstitial tissue, Numbers of interstitial cells. Cordeiro Jr, *et al.* [21] have a dissimilar data of the present study except the similarity with the Leydig cell number who reported that the tubular diameter, Seminiferous epithelium height, Leydig cell and Tunica albuginea. While [22] declared testis of rats at the same day of age revealed seminiferous cord surrounded by several layers of concentrically arranged mesenchymal cells (undifferentiated myoblasts) as the current study mentioned, as well as Leydig cells were seen scattered in the interstitium like a large clusters of cells. But at 6 day of age of golden hamster [23, 24] showed that the testis seminiferous tubules diameter was two time compared as the result of the current study at one day it was about 46µm, the tubule bounded by one row of small basal mitosis epithelial cells as our result.

At 28th day the figures of the testicle revealed that the testicle tunica albuginea was very thick, composed of thick outer fibromuscular layer and thick inner vascular layer that revealed encouraged blood vessels and cells (Fig4). Parenchyma was buildup by numerous large sizes of seminiferous tubules that revealed differentiated cells each tubule was surrounded by very thin fibro cellular membrane which comprised of myoid cells (Fig5). The spermatogenic cells revealed uncomplete differentiation of spermatogonia cells that consisted of Spermatogonial cells type-A & B those appeared large size with darkly stained nucleus and eosinophilic cytoplasm, as well as, Primary and secondary spermatocytes those appeared slightly small size cells comprised of small lightly stained nucleus. The testicles interstitum composed of thick interstitial tissue with furthermore (Leydig cells)

and inter tubular blood vessels (Fig6&7). Diameter of seminiferous tubules were, thickness of tunica albuginea, height of germinal epithelium were recorded in Table1,Thickness of interstitial tissue Numbers of interstitial cells recorded in Table2. França, *et al.* [25]reported that the pig testis seminiferous diameter elevated, at 28 day of age the diameter of seminiferous tubules grow less than the current study its being 57± 2µm. Dorostghoal, *et al.* [18] reported that the primary spermatocytes in Wister rat at 21 days of age, were noticed close to all tubules, in contrast, the present study mentioned that there are two types; primary and secondary spermatocytes. As well as previous authors stated the diameter of tubules more than half time of the present result reach to approximately 90.50 ± 0.51 µm, while at 28 day of age reach to about twice time approximately244.40 ± 1.0 µm.This indicated to that the spermatogenesis in hamster was more active than rat due to rat needed 52 days to reach at this stage, according to hormonal influence [26]. But Lee, *et al.* [27], stated that the seminiferous tubules diameter and the epithelial height in wild mice at 18 to 35 day of age were 20 µm, 50 µm,42, µm, 60 µm respectively.

At 60th day The figures of the testicle revealed that the testicle tunica albuginea was very thick, composed of thick outer fibromuscular layer and thick inner vascular layer that revealed encouraged blood vessels and cells (Fig8&9). The testicular parenchyma was buildup by numerous markedly enlarged size seminiferous tubules that revealed complete differentiated spermatogenic cells and each tubule was surrounded by very thin fibro cellular membrane with myoid cells (Fig10).The spermatogenic cells revealed uncompleted differentiation of spermatogonial cells that consisted of (1) Spermatogonial cells types-A and B those appeared enlarge size with darkly stained nucleus and eosinophilic cytoplasm, Primary and secondary spermatocytes those appeared slightly smaller in size cells, comprised of small lightly stained nucleus. And these series of spermatogenesis revealed the presence of spermatids (Fig10). The testicles interstitum composed of loose connective tissue with furthermore (Leydig cells) that revealed highly eosinophilic cytoplasm (glycoprotein) and inter tubular blood vessels (Fig11&12). Diameter of seminiferous tubules , thickness of tunica albuginea , Height of germinal epithelium recorded in (Table1),Thickness of interstitial tissue were Numbers of interstitial cells (Table2). França, *et al.* [25] mention that in Pig, the Thickness of interstitial tissue were 14.951±0.394, Numbers of interstitial cells were 4.4±0.244, the diameter of seminiferous tubules that recorded at 112 to 140 day of age, reach to 84±3 µm, and stabilized at 196 day, this value approximately near the value of our result at 60 day of age this indicated that the sexual activity of hamster was more active than pig, as well as the hormones. While Lee, *et al.* [27] demonstrated that the diameter of seminiferous tubules, height of germinal epithelium at 60 day of age of wild mice reach to 60µm, 75µm respectively as compared with the current study in the same old. On the other

hand, Prakash, *et al.* [28] noticed that the testis of bonnet monkey at Postnatal the spermatogonial cells spread via seminiferous tubules and the spermatogonial cells increasing accompanied with increasing of these tubules for the duration of adulthood as our result.

Table1.-Showing Diameter of seminiferous tubules, Thickness of tunica albuginea and Height of germinal epithelium during different ages.

Parameter / Age (days)	Diameter of seminiferous tubules/400x	Thickness of tunica albuginea/400x	Height of germinal epithelium/400x
1st	26.625 ± 0.380 A	8.094±0.086 A	6.064±0.313 A
28th	68.026 ± 3.146 B	14.811±0.436 B	20.268±0.600 B
60th	82.736 ±1.894 C	12.988±0.540 C	26.473±0.486 C

Values represent mean ±S.E

Different capital letters in same column mean significant differences (P≤0.05) between different ages.

Table2.-Showing Thickness of interstitial tissue and Numbers of interstitial cells in male Hamster during different ages.

Parameter / Age (days)	Thickness of interstitial tissue/400x	Numbers of interstitial cells/400x
1st	5.201±0.336 A	2.6±0.244 A
28th	8.194±0.409 B	3.8±0.200 B
60th	14.951±0.394 C	4.4±0.244 C

Values represent mean ±S.E

Different capital letters in same column mean significant differences (P≤0.05) between different ages.

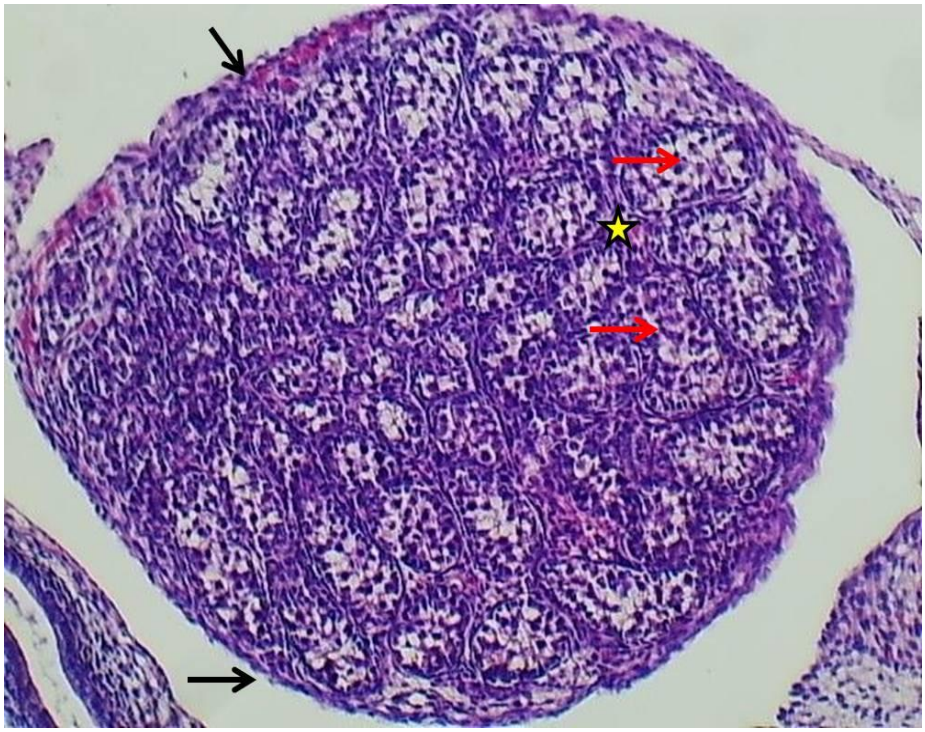

FIGURE1.- **section of testis (1 day) shows:** very thin tunica albuginea (Black arrows) with small size seminiferous (Red arrows) & delicate loose connective tissue of testicular interstitum (Asterisk). H&E stain. 100x.

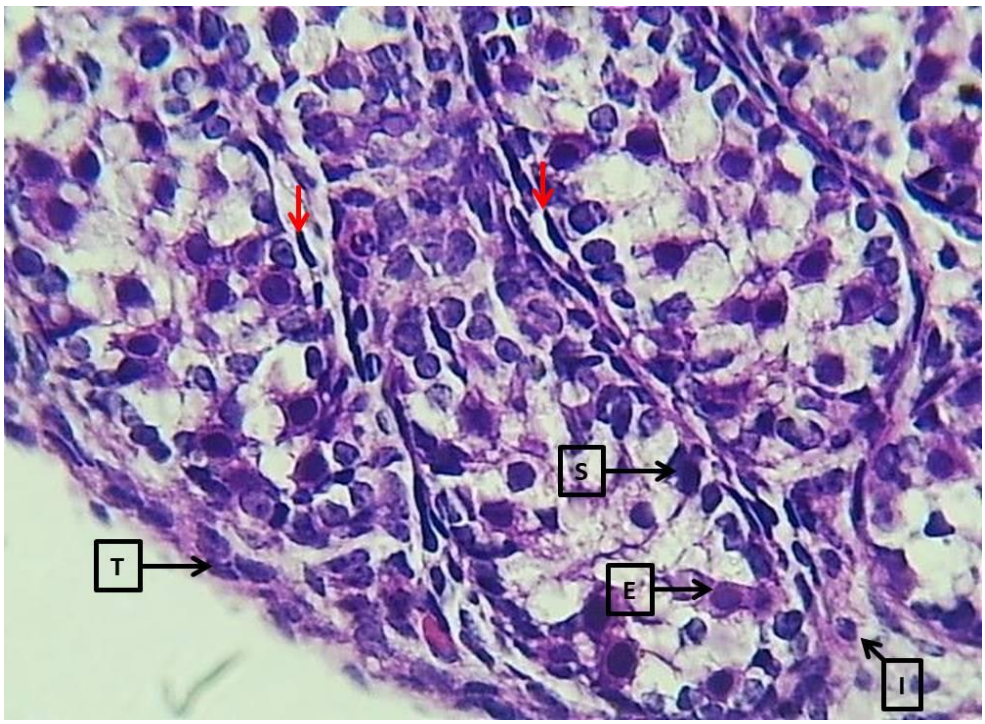

FIGURE2.- **section of testis (1 days) shows:** Undifferentiated myoblasts and fibroblasts of tunica albuginea (T), small size seminiferous composed of spermatogonium cells (S) & newly differentiated sertoli cells (E), furthermore myoid cells (Red arrow), & differentiated lydic cells (I). H&E stain. 400x.

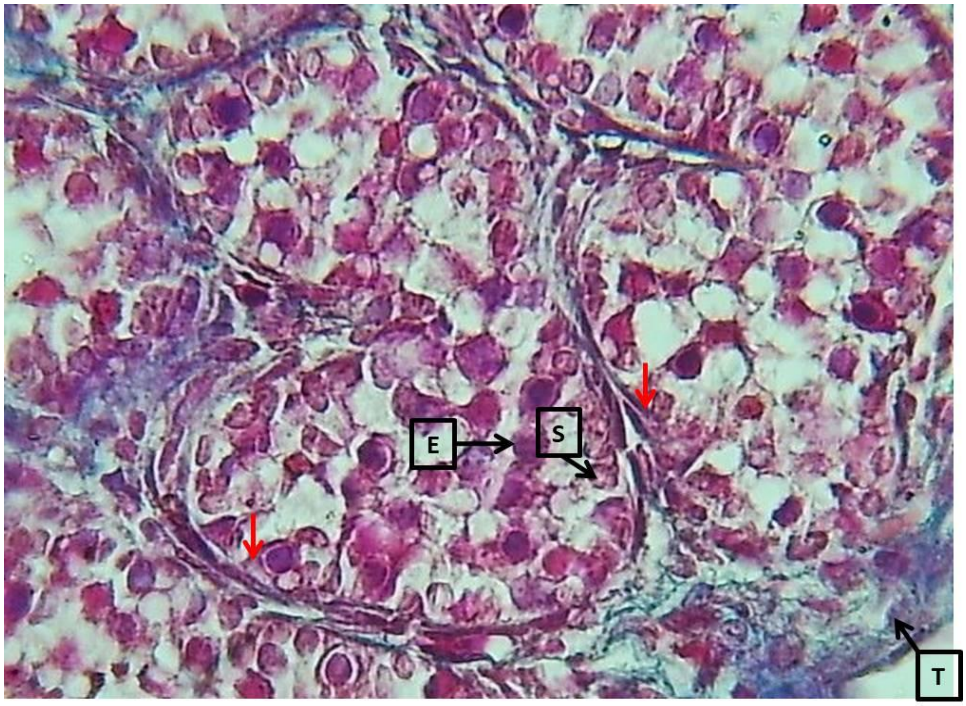

FIGURE3.- **section of testis (1 days) shows**: Undifferentiated tunica albuginea (T), small size seminiferous composed of spermatogonium cells (S) & newly differentiated sertoli cells (E), furthermore myoid cells (Red arrow).M.T. stain. 400x.

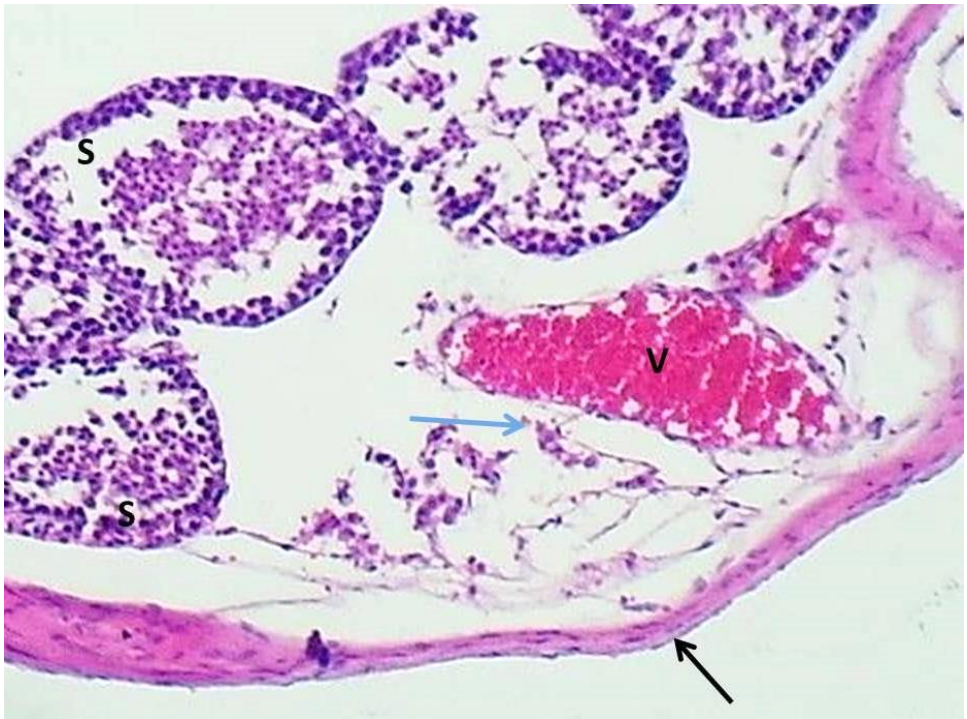

FIGURE4.- **section of testis (28 days) shows:** slightly thick outer fibromuscular layer of tunica albuginea (Black arrow), thick inner vascular layer of tunica albuginea (Blue arrow), enlarge size seminiferous with differentiated spermatogenic cells (S) & capsular blood vessels (V). H&E stain. 100x.

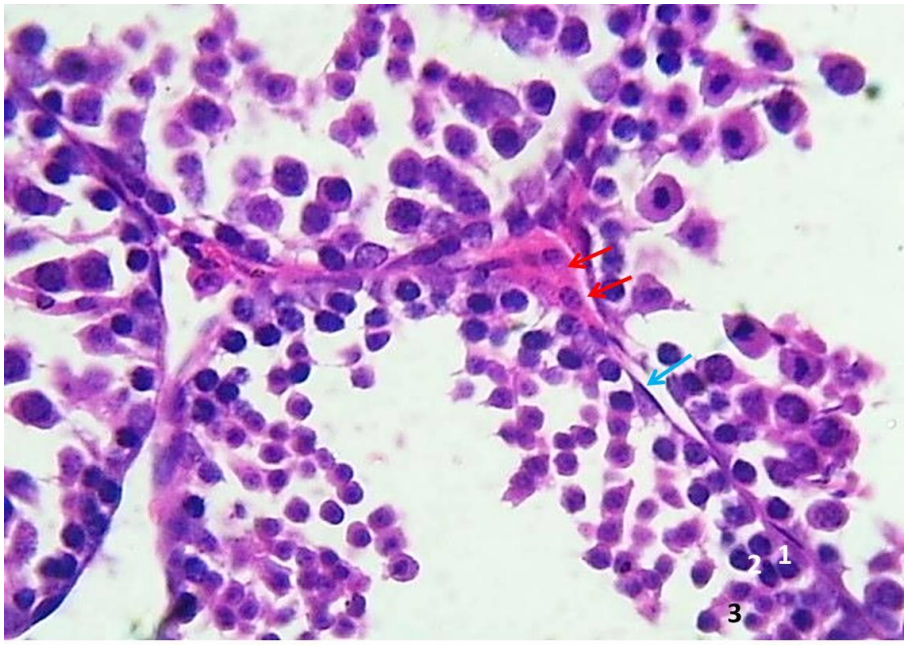

FIGURE5.- **section of testis (28 days) shows:** spermatoginial cells type-A (1), spermatoginial cells type-B (2), interstitial cells (Red arrow), and myoid cells (blue arrow). H&E stain. 400x.

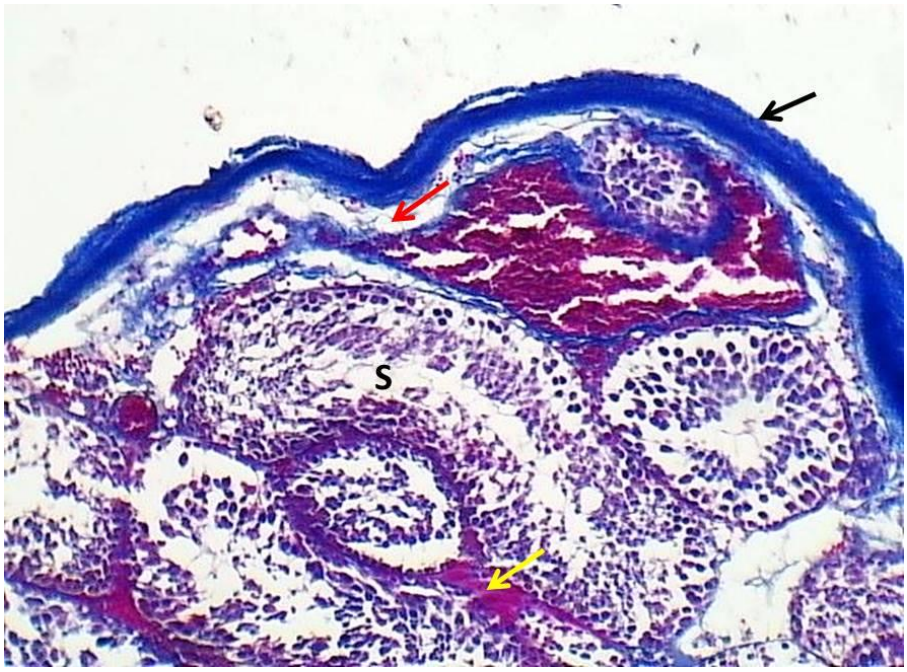

FIGURE6.- **section of testicle tunica albuginea (28 days) shows:** outer fibromuscular layer of tunica albuginea (Black arrow), inner vascular layer of tunica albuginea (Red arrows), large size seminiferous (S) & thick interstitum (yellow arrow). M.T. stain. 100x.

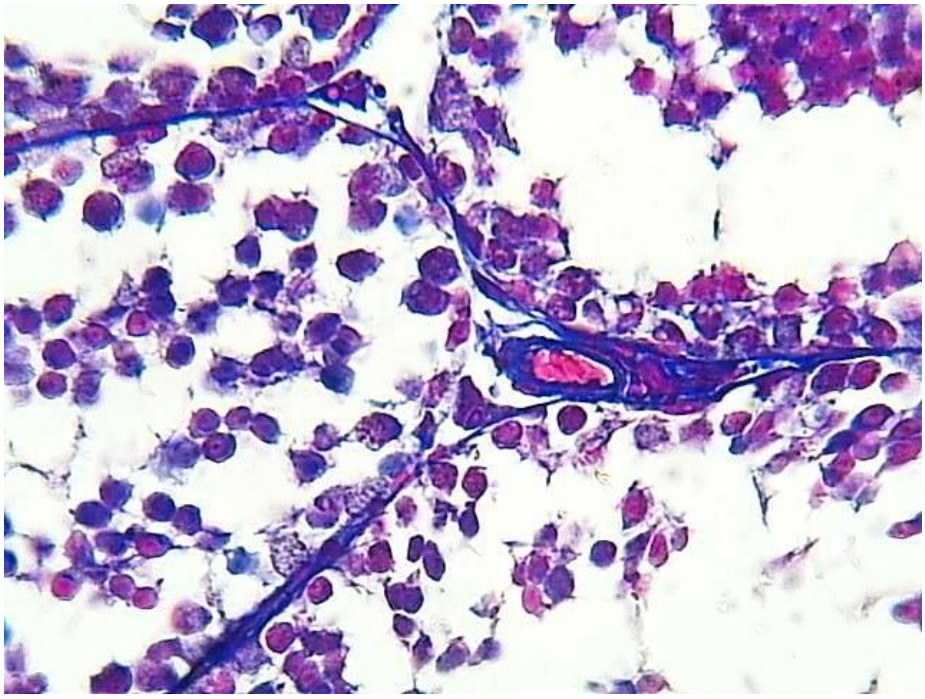

FIGURE7.- **section of testis parenchyma (28 days) shows:** blood vessels, fibrocellular membrane of seminiferous tubules, myoid cells & interstitial cells with blood vessels. M.T. stain. 400x.

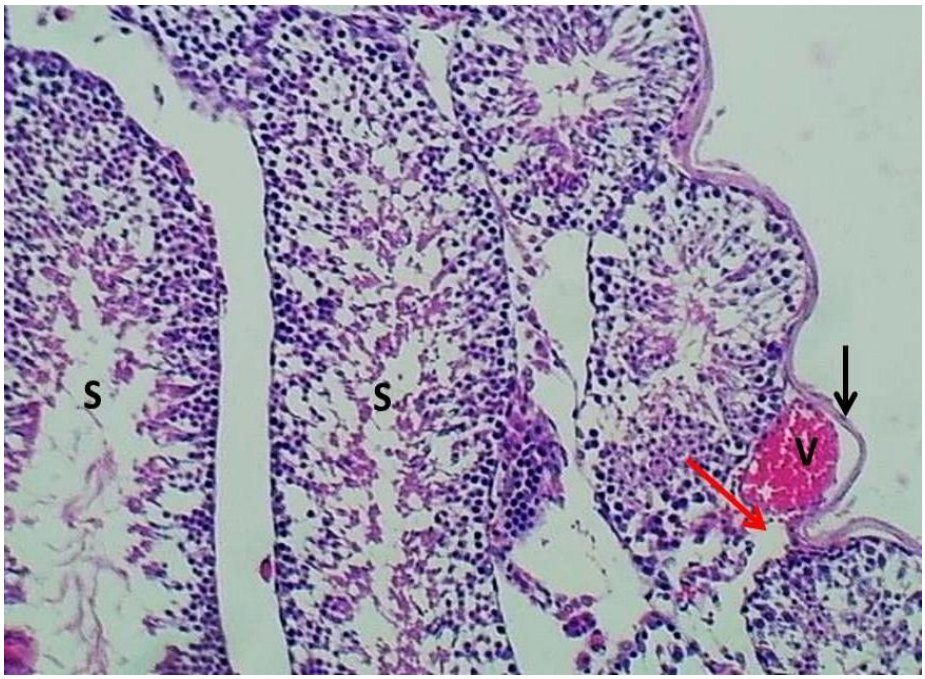

FIGURE8.- **section of testis (60 days) shows:** thick outer fibromuscular layer of tunica albuginea (Black arrow), inner vascular layer of tunica albuginea (Red arrow), marked enlarge seminiferous with completion differentiated spermatogenic cells (S) & thick interstitum. H&E stain. 40x.

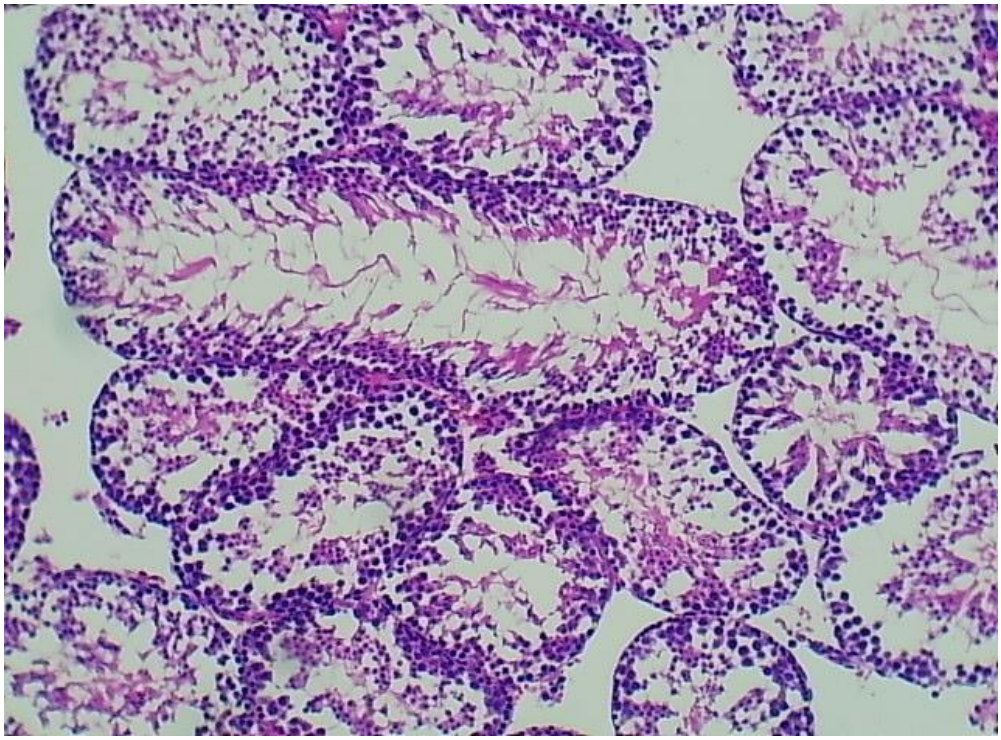

FIGURE9.- **section of testicle parenchyma (60 days) shows:** marked enlarge seminiferous with completion differentiated spermatogenic cells and thick interstitum. H&E stain. 100x.

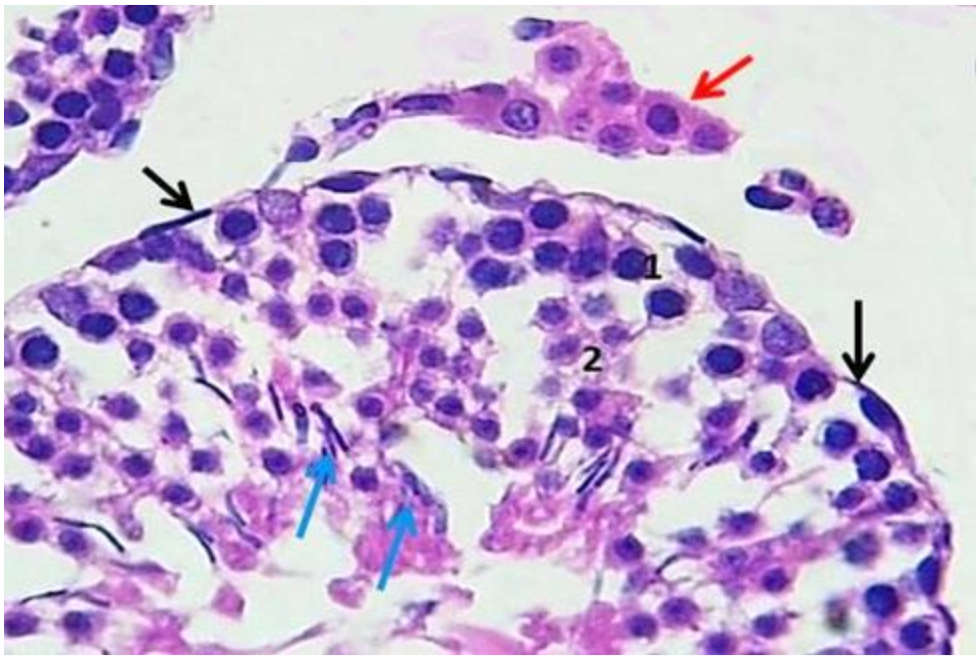

FIGURE10.- **section of seminiferous tubule (60 days) shows:** spermatoginial cells type-A & B (1), primary & secondary spermatocytes (2), spermatids (Blue arrows), interstitial cells (Red arrow), and myoid cells (Black arrows). H&E stain. 400x.

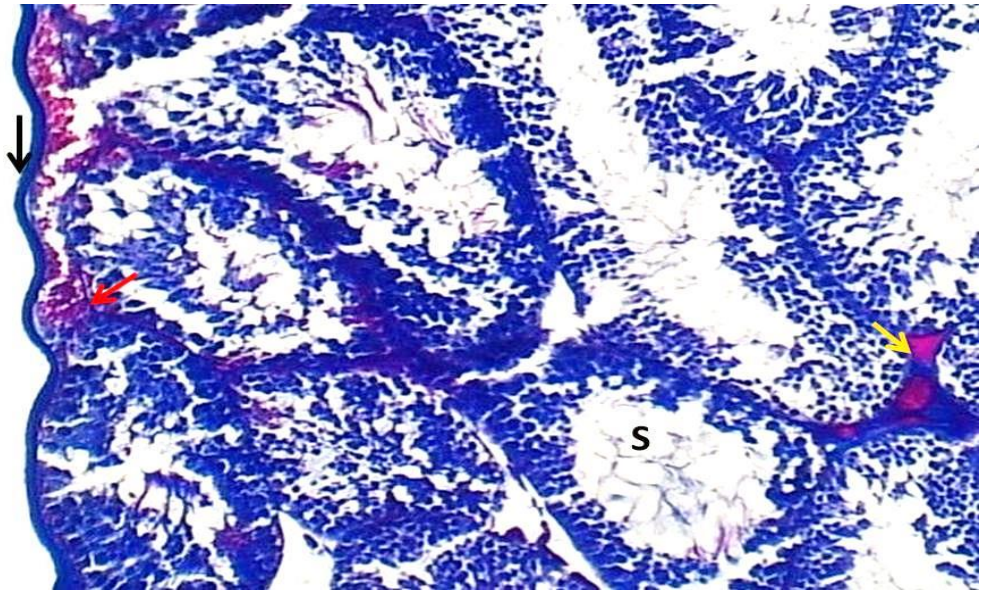

FIGURE11.- **section of testicle (60 days) shows:** outer fibromuscular layer (Black arrow) and inner vascular layer (Red arrow) of tunica albuginea, seminiferous tubule (S), & loose interstitum connective tissue (yellow arrow) .M.T.stain. 40x.

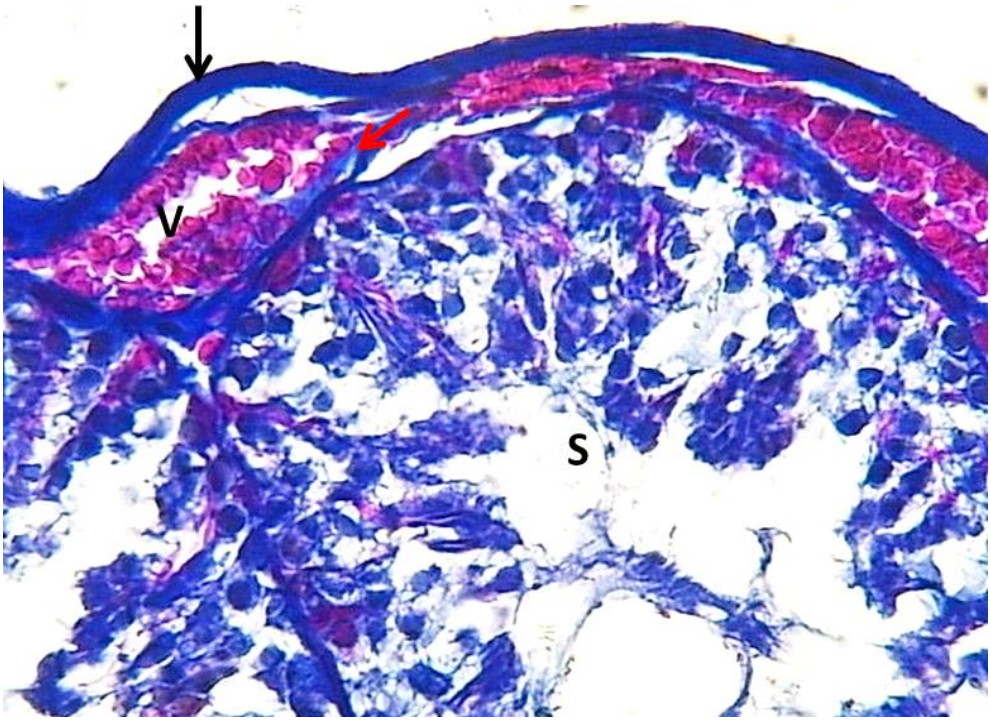

FIGURE12.- **section of testicle tunica albuginea (60 days) shows:** outer fibromuscular layer (Black arrow) and inner vascular layer (Red arrow) of tunica albuginea, seminiferous tubule (S). M.T.stain. 400x.

3. CONCLUSION

The results referred that there is three stages, undifferentiated, differentiated and maturation phase in development testis of hamster.

REFERENCES

[1] M. A. Suckow, K. A. Stevens, and R. P. Wilson, *The laboratory rabbit, guinea pig, hamster, and other rodents*: Academic Press, 2011.

[2] K. Neumann, J. Michaux, V. Lebedev, N. Yigit, E. Colak, N. Ivanova, *et al.*, "Molecular phylogeny of the Cricetinae subfamily based on the mitochondrial cytochrome b and 12S rRNA genes and the nuclear vWF gene," *Molecular phylogenetics and evolution,* vol. 39, pp. 135-148, 2006.

[3] A. P. Sinha Hikim, A. Bartke, and L. D. Russell, "Morphometric studies on hamster testes in gonadally active and inactive states: light microscope findings," *Biology of reproduction,* vol. 39, pp. 1225-1237, 1988.

[4] L. A. Lodhi and Z. lqbal Qureshi, "The Bovine Testes-II: Role of Hormones During Pre and Post Natal Development," *Pakistan Journal of Biological Sciences,* vol. 3, pp. 1929-1934, 2000.

[5] B. Guerra-Carvalho, D. F. Carrageta, L. Crisóstomo, R. A. Carvalho, M. G. Alves, and P. F. Oliveira, "Molecular mechanisms regulating spermatogenesis in vertebrates: Environmental, metabolic, and epigenetic factor effects," *Animal Reproduction Science,* vol. 246, p. 106896, 2022.

[6] A. Castro, W. E. Berndtson, and F. Cardoso, "Plasma and testicular testosterone levels, volume density and number of Leydig cells and spermatogenic efficiency of rabbits," *Brazilian Journal of Medical and Biological Research,* vol. 35, pp. 493-498, 2002.

[7] T. J. Parkinson, "Reproductive physiology of male animals," *Arthur's Veterinary Reproduction and Obstetrics-E-Book,* p. 35, 2018.

[8] L. D. Russell, H. P. Ren, I. S. Hikim, W. Schulze, and A. P. S. Hikim, "A comparative study in twelve mammalian species of volume densities, volumes, and numerical densities of selected testis components, emphasizing those related to the Sertoli cell," *American Journal of Anatomy,* vol. 188, pp. 21-30, 1990.

[9] M. D. Salman and M. S. Dawood, "Morphological and morphometrical study of penis in indigenous dog," *Biochemical & Cellular Archives,* vol. 22, 2022.

[10] N. Al-Falahi, "Comparative evaluation of bovine pericardial membrane and amniotic membrane in wounds skin healing in rabbits: NH Al-Falahi[1]; Dhyaa. Ab. Abood[2] and MS Dauood2," *The Iraqi Journal of Veterinary Medicine,* vol. 41, pp. 137-145, 2017.

[11] M. S. Dawood, D. A. Abood, and A. Y. Hameza, "The histological and histochemical features of the esophagus in local breed dogs (Canis familiaris)," *Iraqi Journal of Veterinary Sciences,* vol. 36, pp. 1069-1074, 2022.

[12] A. F. Reshag, D. A. Abood, and M. S. Dawood, "Anatomical and histological study of the kidneys and salt glands in great flamingos (Phoenicopterus roseus)," *The Iraqi Journal of Veterinary Medicine,* vol. 40, pp. 140-146, 2016.

[13] S. J. Noori and S. M. Mirhish, "Histomorphological study of epididymis, ductus deferens and phallus in adult male guinea fowl (Numida meleagris)," 2018.

[14] L. E. Mohammed, "Morphological and histochemical features of the cloaca of Turkey hen Meleagris Gallopavo," *The Iraqi Journal of Veterinary Medicine,* vol. 41, pp. 28-33, 2017.

[15] K. S. Suvarna, C. Layton, and J. D. Bancroft, *Bancroft's theory and practice of histological techniques*: Elsevier health sciences, 2012.

[16] D. A. Abood, M. S. Dawood, L. E. Mohammed, and A. J. Karim, "Histological and histochemical characteristics of the esophagus in local breed donkey (Equus asinus)," *Journal of Advanced Veterinary and Animal Research,* vol. 10, p. 14, 2023.

[17] S. D. Mohammed, "Histological features of penis in indigenous tom cat," *Indian Journal of Natural Sciences,* vol. 8, pp. 13720-13729, 2018.

[18] M. Dorostghoal, F. Sorooshnia, and A. Zardkaf, "Stereological analysis of wistar rat testis during early post‐natal development," *Anatomia, Histologia, Embryologia,* vol. 40, pp. 89-94, 2011.

[19] A. Ismail and M. A. kareem Atyia, "Morphological changes of descending testes during postnatal developmental stages in the Rabbit (Oryctolagus cuniculus)," *The Iraqi Journal of Veterinary Medicine,* vol. 37, pp. 237-243, 2013.

[20] I. Tsunenari and A. Kast, "Developmental and regressive changes in the testes of the Himalayan rabbit," *Laboratory animals,* vol. 26, pp. 167-179, 1992.

[21] D. A. Cordeiro Jr, G. M. Costa, and L. R. França, "Testis structure, duration of spermatogenesis and daily sperm production in four wild cricetid rodent species (A. cursor, A. montensis, N. lasiurus, and O. nigripes)," *Plos one*, vol. 16, p. e0251256, 2021.
[22] I.-S. Kim and H.-H. Yang, "Morphometric study of the testicular interstitium of the rat during postnatal development," *Korean Journal of Anatomy*, pp. 849-858, 1999.
[23] E. Ortiz, "The postnatal development of the reproductive system of the golden hamster (Cricetus auratus) and its reactivity to hormones," *Physiological zoology*, vol. 20, pp. 45-66, 1947.
[24] M. W. Hance, "Leydig Stem Cell Differentiation in the Prepubertal Hamster (Mesocricetus auratus)," 2005.
[25] L. R. França, V. A. Silva Jr, H. Chiarini-Garcia, S. K. Garcia, and L. Debeljuk, "Cell proliferation and hormonal changes during postnatal development of the testis in the pig," *Biology of Reproduction*, vol. 63, pp. 1629-1636, 2000.
[26] R. I. McLachlan, L. O'Donnell, S. J. Meachem, P. Stanton, D. De Kretser, K. Pratis, *et al.*, "Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man," *Recent progress in hormone research*, vol. 57, pp. 149-179, 2002.
[27] K. H. Lee, J. H. Park, D. Bunick, D. B. Lubahn, and J. M. Bahr, "Morphological comparison of the testis and efferent ductules between wild‐type and estrogen receptor α knockout mice during postnatal development," *Journal of anatomy*, vol. 214, pp. 916-925, 2009.
[28] S. Prakash, E. Prithiviraj, and S. Suresh, "Developmental changes of seminiferous tubule in prenatal, postnatal and adult testis of bonnet monkey (Macaca radiata)," *Anatomia, histologia, embryologia*, vol. 37, pp. 19-23, 2008.