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Research Article

Ultrastructural Development Study of Testis in Golden Hamster at Different Postnatal Ages

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Abstract
The present study aimed to investigate the ultrastructures of the testis in golden hamster during 1, 14, 28, 42, 60 day of old. Animals were administered an intraperitoneal injection of ketamine at 50 mg/kg and xylazine at 5 mg/kg. Subsequently, the animals were surgically opened, and the testes were excised. The testes were sectioned into small 1 mm³ pieces and immediately fixed in 4% buffered glutaraldehyde for 25 to 30 minutes, after which they were trimmed with a sharp knife. Tissue samples with a thickness of 1-3 mm were obtained and subsequently fixed in glutaraldehyde. Observation of one-day-old specimens revealed a normal testicular structural design, characterized by a basal membrane encircled by myoid cells, along with a variety of spermatogonia of differing shapes and sizes. At 14 days old, the vacuolation of Sertoli cells was more dispersed and abundant. The Leydig cells exhibited spherical and oval nuclei containing testosterone. Primary and secondary spermatocytes were observed at 28 days, with some spermatocytes displaying large corkscrew-shaped vacuoles. At 42 days, the same fine structures were noted as in the previous stage. By 60 days, observations indicated a clear mature stage, with apoptotic cells identified near the spermatogonia. The midpiece of spermatozoa showed an axoneme encircled by an outer dense fibril, surrounded by a dense sheet of mitochondria. The principal tail of spermatozoa was distinctly visible, featuring an axoneme encircled by an outer dense fibril, covered by a fibril sheet, along with two central filaments and nine peripheral pairs of filaments.

Keywords: hamster, TEM, testis.

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Introduction

The embryonic phase is a chiefly important phase for reproductive system development of male [1]. Endocrine and paracrine indication play an important role in regulation the development as well as differentiation of Germ cells [2, 3]. The precursors of spermatogonia type A and spermatogonial stem cells are The gonocytes, this gonocytes establish the function of adult spermatogenic[4, 5]. 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 [6]. Maturation and spermatogenesis belong to testosterone influence[7], maturation distinguished by starting of meiosis and sperm creation [8]. 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[9].

Materials and Methods

Experimental animals Fifteen golden hamsters aged 1, 14, 28, 42, and 60 days were used. The animals were injected with ketamine 50 mg/kg and xylazine 5 mg/kg intraperitoneally[10, 11]. After opening the animals, the testes were fixed in 4% buffered glutaraldehyde for 25–30 min. The testes were then trimmed and 1–3 mm thick testicular tissue samples were taken[12]. The tissue samples were then fixed in glutaraldehyde for another hour. The samples were washed with phosphate buffer overnight. The samples were then transferred to a mixture of 1% osmium:1.25% potassium ferrocyanide for fixation and dehydrated with ethanol. The samples were then cultured in Epon 812-Araldite 502. After that, thin sections were prepared and suitable for microscopic examination. Thin sections of testicular tissue were placed on 200-mesh microgrids, stained with uranyl acetate and lead citrate, and transferred for examination using an EM-10 electron microscope (Zeiss, Oberkochen, Germany). Westville, South Africa[13-15].

Ethical approval

The present study design was accepted by the Animal Care and Use the team at the College of Veterinary Medicine, University of Baghdad. Baghdad, Iraq(P.G/2044).

Results

When observed by transmission electron microscopy **on the first day old**, a normal picture of the testicular structure appeared with a basement membrane surrounded by muscle cells and frequently set of different shape and size of spermatogonia with oval or spherical nucleus(fig1), single nucleolus appeared in type B but one or two nucleoli appeared in type A. sertoli cell appeared as tree that enveloping the spermatogonia was also noticed near the basement membrane which contained RER, free ribosome, abundant active mitochondria. Leydig cell was appeared in the interstitial tissue as well as collagen, fiber fibrocyts(fig2). **While at the 14 day** (undifferentiated phase) the same picture appeared as the previous stage, the vaculation of sertoli cell was more scattered and more abundant (fig3). Leydig cell was appeared had spherical and oval nuclei filled with secretion of testosterone hormone look like dark appearance(fig4). **At the 28 day** (differentiated phase) the same picture appeared as the previous stage, primary and secondary spermatocytes cleared, the primary appeared bigger than spermatogonia. (fig5), some spermatocytes have a large corkscrew-shaped vacuole on their head position called head nuclear vacuole (corkscrew-shaped). The initiation of mitotic division of spermatocytes was appeared as early stage. The connection between two cells was done by cytoplasmic bridges (fig 6. At day 42 of the differentiated phase, the same typical pyramidal shape of Sertoli cells with isolated chromatin was observed, along with varied mitochondrial shapes present in the cytoplasm of most cells. The Golgi apparatus was also identified within the cytoplasm. Leydig cells were observed near the basement membrane in the interstitium. These cells exhibited granular secretion and a significant quantity of lipid, which was distributed throughout the interstitial space (fig. 7, 8). **Finally the maturation phase at 60 day** showed several stage of spermatogenesis as well as revealed spermatozoa and spermatid (fig 9) the magnified cross section of mid piece of spermatozoa showed axoneme encircling by outer dense fibril that surrounding by heavy sheet of mitochondria (fig10). The axoneme of the sperm creates from the end of head and ended in the end piece. Its possess double nine microtubules as well as central pair microtubules (9+2 structure), microtubules connected with each other by nexin links and with central pair by protrusion as radial spokes,. Protrusion of double microtubules as inner and the outer was axonemal dynein arms protein. The principal of the tail of spermatozoa was appeared clearly and has axoneme which was encircling the outer dense fibril that covered by fibril sheet, two central filaments and nine peripheral pairs filaments (fig 11).

Discussion

The primary function of the basement membrane is to maintain the structural and functional integrity of tissues, provide structural stability to organs, and facilitate signaling to cells through cell surface receptors [16]. Our opinion the sertoli cell appearance was needed to spermatogenesis regulation as well as production of spermatozoa, because lacking of material and metabolic support of the sertoli cells, germ cells cannot reproduce and survive as well as the sertoli cells are important in the production of special proteins which were make conform or maintain the release of hormone from the pituitary in addition to manage the spermatogonia by mitotic activity[17, 18]. Spermatogenesis requires motivation from the hypothalamus and pituitary gland, which signal the initiation of LH secretion influenced by GnRH. This process subsequently leads to the secretion of testosterone from Leydig cells, a hormone that significantly impacts spermatogenesis[19-21]. In contrast, FSH influences Sertoli cells, which are necessary for germ cell maturation[22, 23]. [24]Recorded the Leydig cells of rabbits had a great quantity of SER and lipid droplets, the cytoplasm contained heavy mitochondria which were had small granules in the matrix and tubular and vesicular cristae as our result mentioned. The suggestion of the current study Vacuolization regarded as mixture of organelle of intra cytoplasmic membrane like SER, these vacuoles may create modification of convey or elimination of proteins outside of SER, disturbance of ionic pump, alters in cytoskeleton sustaining the SER. otherwise possibly these vacuoles are remnants of needless cytoplasm and organelles that should have been removed during spermiogenesis[25-27].Probably, these residual contains of vacuoles were source of reactive oxygen species ROS, which were mad the sperm exposure to over oxidative stress, leading to DNA damage resulting in lowering fertility[28, 29] . The other opinion suggested that these large vacuoles appeared due to failure chromatin condensation [30-32]. The numerous small vacuoles refer to peripubertal condition and displace the spermatocytes in the direction of the seminiferous tubule lumen [33]. The result of Susheela and Arbind [24] were confided with our result who recorded that Leydig cells of rabbits had large quantity of SER in addition to lipid droplets. The germ cells apoptosis that happens in epithelia of testis is regarded as mechanism in order to provide to minimize population of germ cell to certain level that sertoli cell can be supported as well as in order to synchronized progression of cell death, which has a role in equilibrium of cell synthesis and differentiation [34, 35]. We

propose that the protrusion responsible for the arrangement and spacing of the double microtubules encircling the central pair, while axonemal dynein arms are considered essential for motility through the sliding mechanism of microtubule flagellar beating initiated by dynein from one double microtubule to another in the presence of ATP, causes axonemal dynein to shift towards the base of the flagellum to facilitate the sliding of adjacent double microtubules. The movement encountered resistance, resulting in the bending of the flagellum. Ultimately, dynein detached from the adjacent microtubule. This process occurs on one side of the axoneme, causing the flagellum to exhibit a zigzag motion, after which motility commences. This course of action occurs on one side of the axoneme while the opposite side remains inactive; consequently, the flagellar beating becomes apparent based on the activation and deactivation of the axonemal dynein arms. This process requires substantial ATPases, which are essential for the increased energy expenditure during sperm motility, as well as oxidative phosphorylation in the mitochondria (the site of ATP production) and glycolysis in both the flagellum and the head [36-39].

Conclusion: The results indicate that there are three stages in the development of the testis in hamsters: undifferentiated, differentiated, and maturation phases.

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Conflict of Interest: The authors declare no conflict of interest related to this work or the publication of this manuscript.

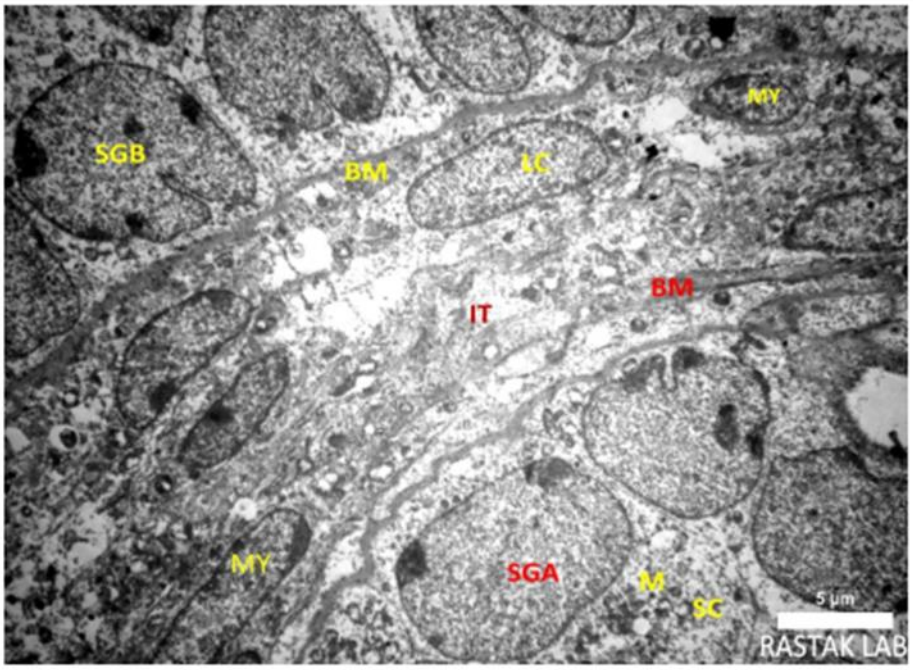

Fig (1), Electron micrograph images (TEM) of hamster testis at one day old showed: SG, spermatogonia, SC, sertoli cell, BM, basement membrane, M, mitochonderia, MY myoid cell, IT, interstitial tissue, LC, leydig cell,

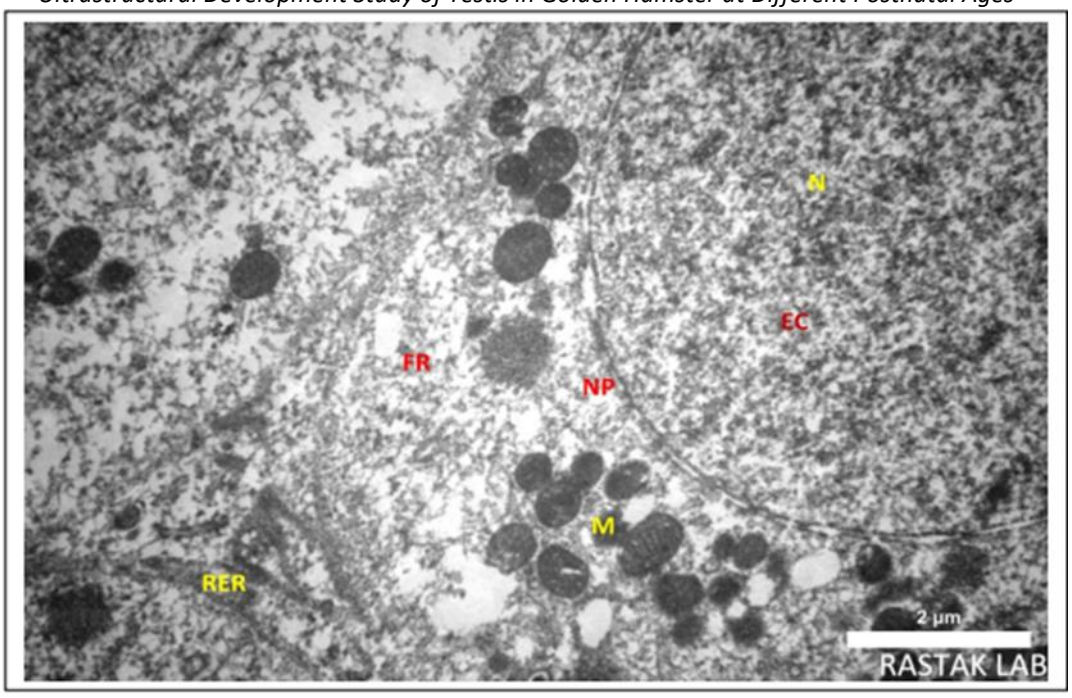

Fig (2), Electron micrograph images (TEM) of hamster testis at one day old showed :NP, nucleoplasm, M, mitochonderia, FR, free ribosom, RER, rough endoplasmic reticulum,EC, euchromatin,

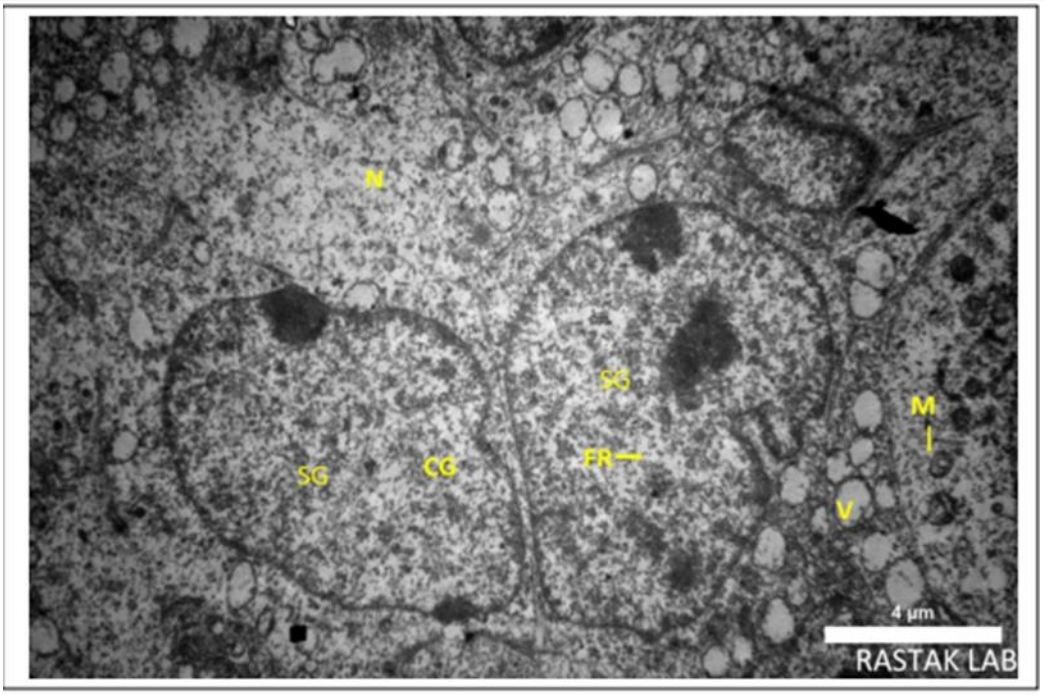

Fig (3), Electron micrograph images (TEM) of hamster 14 day old showed: SG, spermatogonia, SC, sertoli cell, V, vacoule, N, nucleus OF SERTOLI, CC, condense chromatin. M, mitochonderia, RER, rough endoplasmic reticulum, MY myoid cell

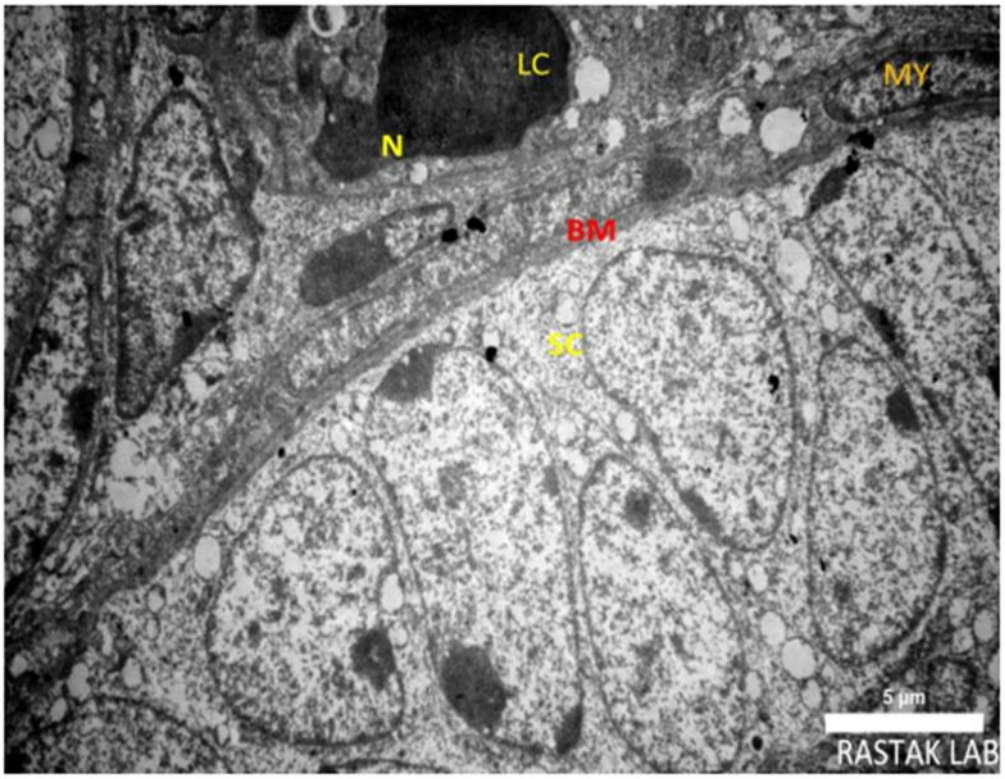

Fig (4), Electron micrograph images (TEM) of hamster 14 day old showed: LC, leydig cell, MY myoid cell, N, nucleus

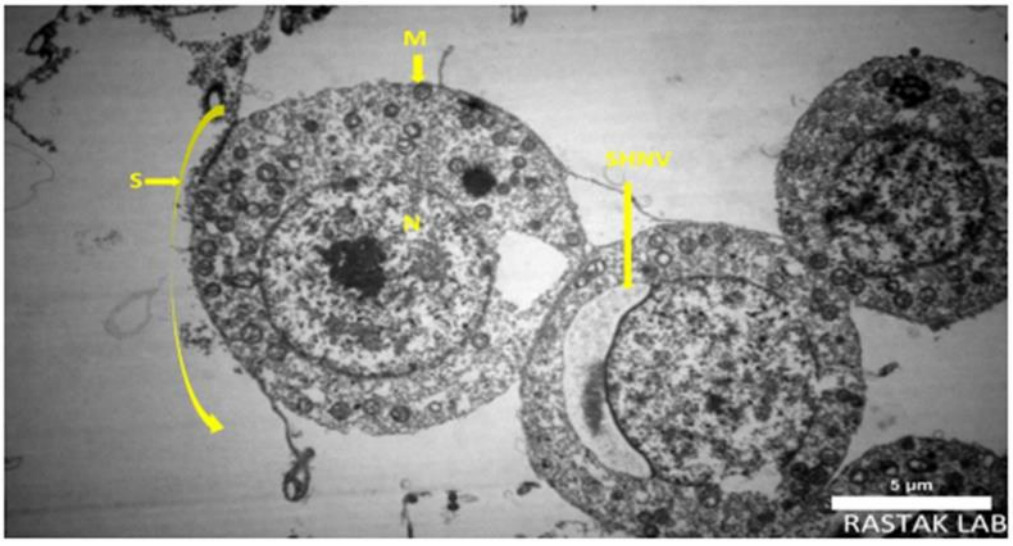

Fig (5), Electron micrograph images (TEM) of hamster testis at 28 day old showed: SHNV, Seprm head nuclear vacuole, S spermatocyte, M, mitochondria.

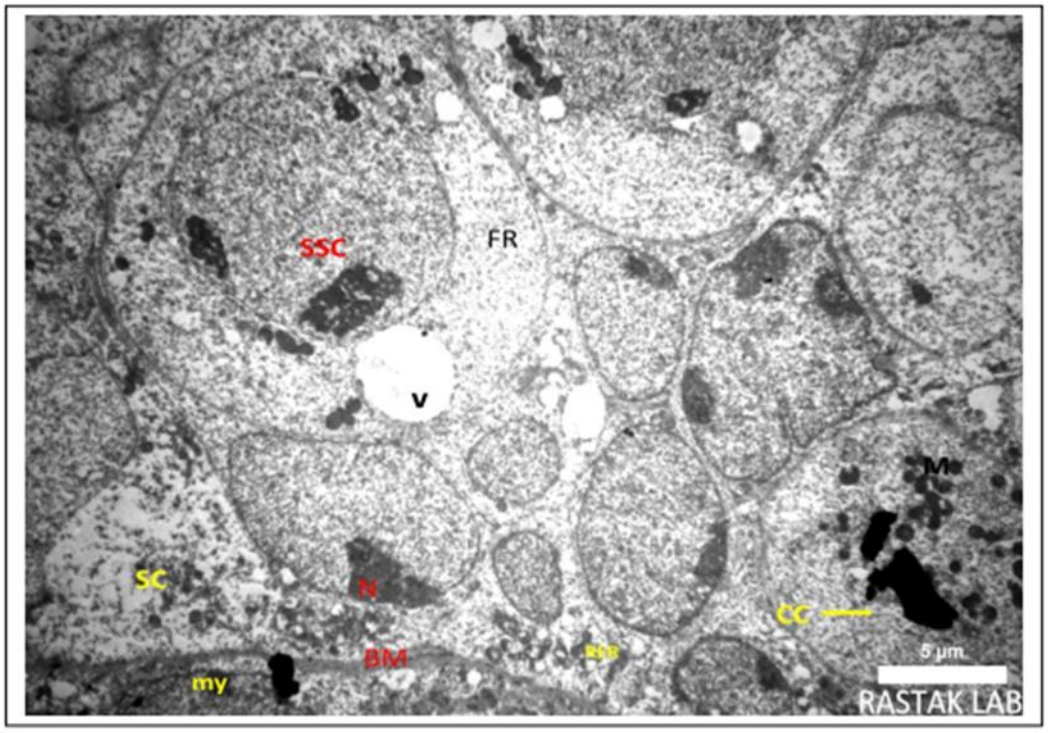

Fig (6), Electron micrograph images (TEM) of hamster testis at 28 day old showed: SCY, spermatocyts, SC, sertoli cell, V, vacoule, BM, basement membrane, N , nucleus , CC, condense chromatin. M, mitochondria, RER, rough endoplasmic reticulum, MY myoid cell.

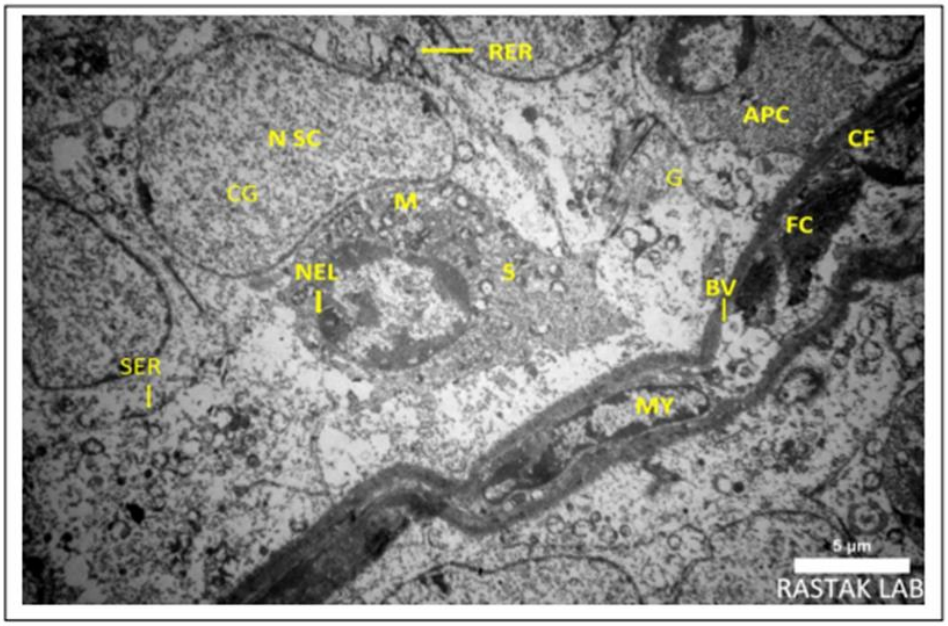

Fig (7), Electron micrograph images (TEM) of hamster testis at 42 day old showed: NEL, nucleolus, SER, smooth endoplasmic reticulum, RER, rough endoplasmic reticulum, APC, apoptotic cell, G ,Golgi apparatus, CF, collagen fiber, MY , myiod cell, S, spermatid, BV, blood vessel, FC, fibrocyte, NCS,

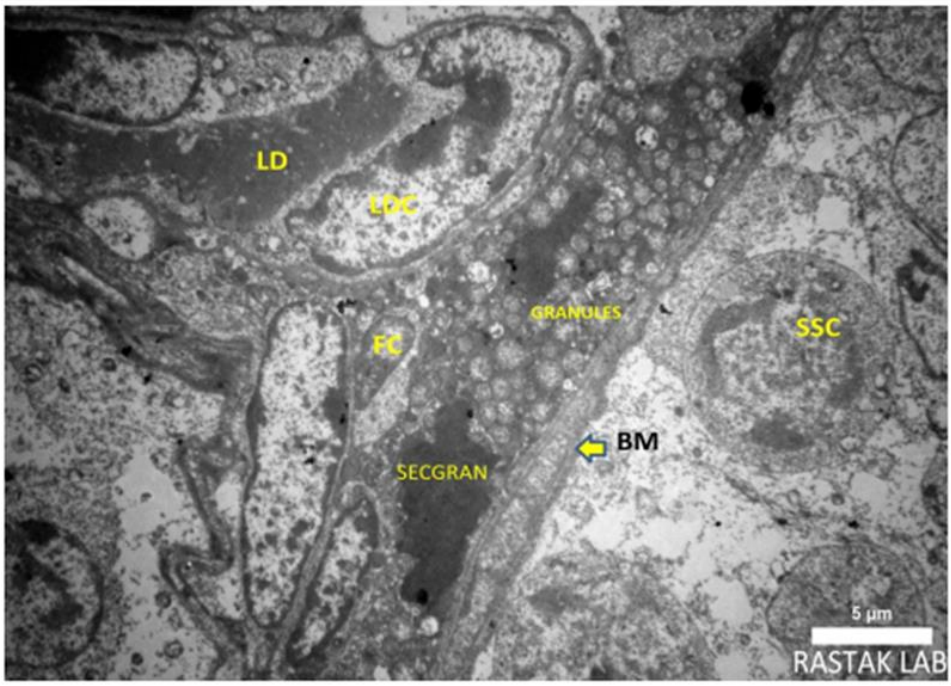

Fig (8), Electron micrograph images (TEM) of hamster testis at 42 day old showed: LD, lipid droplet, FC, fibrocyte, SSC, secondary spermatocyte, BM, basement membrane

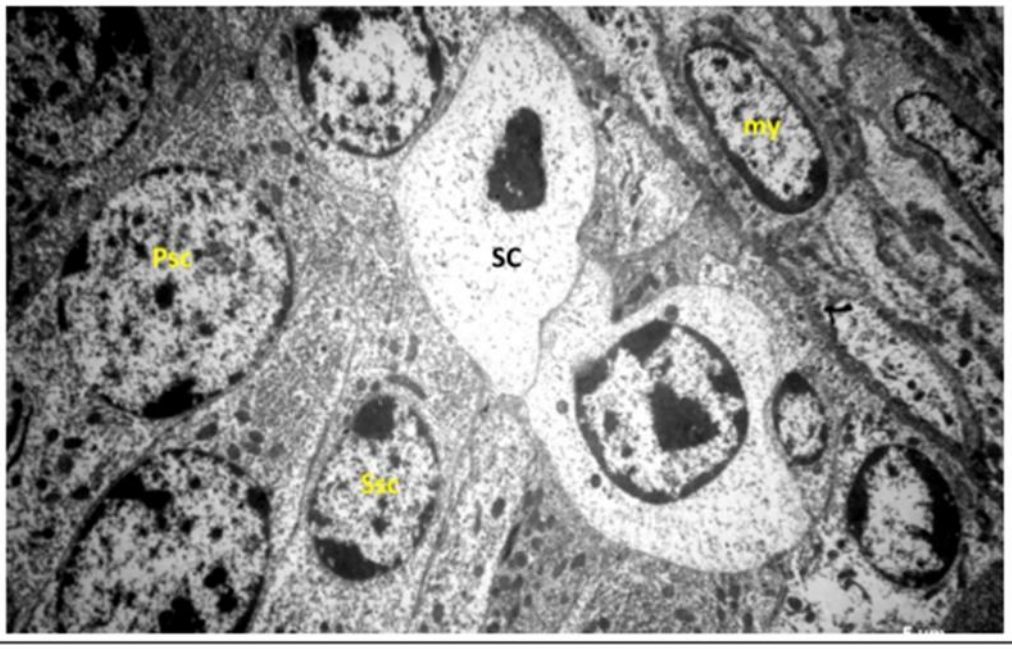

Fig (9), Electron micrograph images (TEM) of hamster testis at 60 day old showed: different stage of spermatocytes, SC, sertoli cell ,MY, myiod cell.

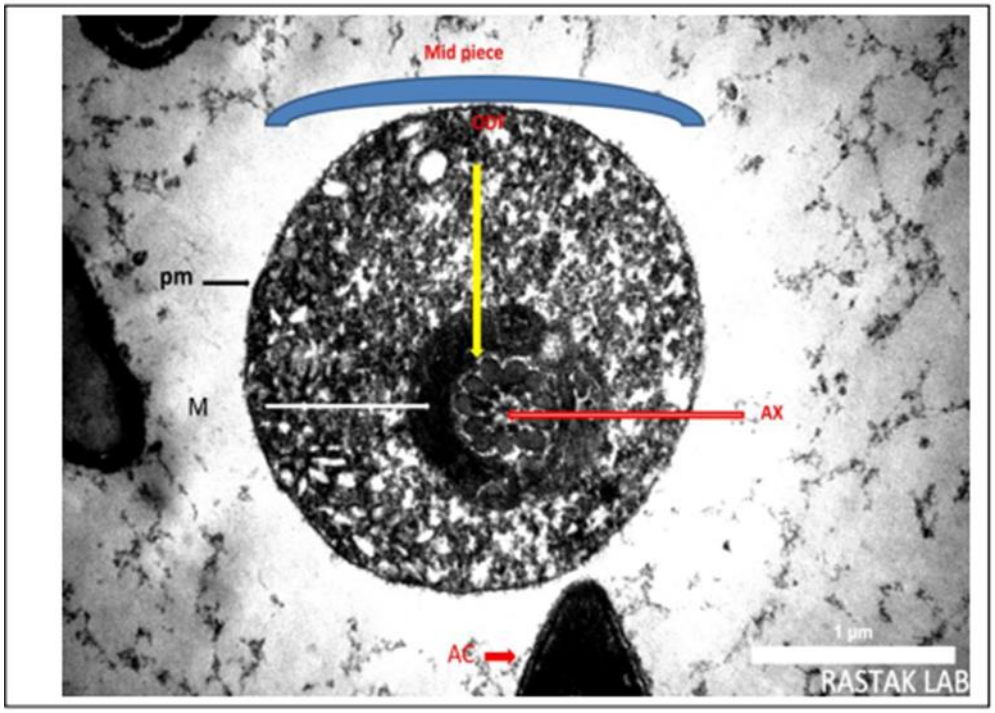

Fig (10), Electron micrograph images (TEM) of hamster testis at 60 day old showed: ODF ,outer dense fibril, PM , plasma membrane, AC, acrosome, AX, axoneme (central microtubule),M, mitochondria.

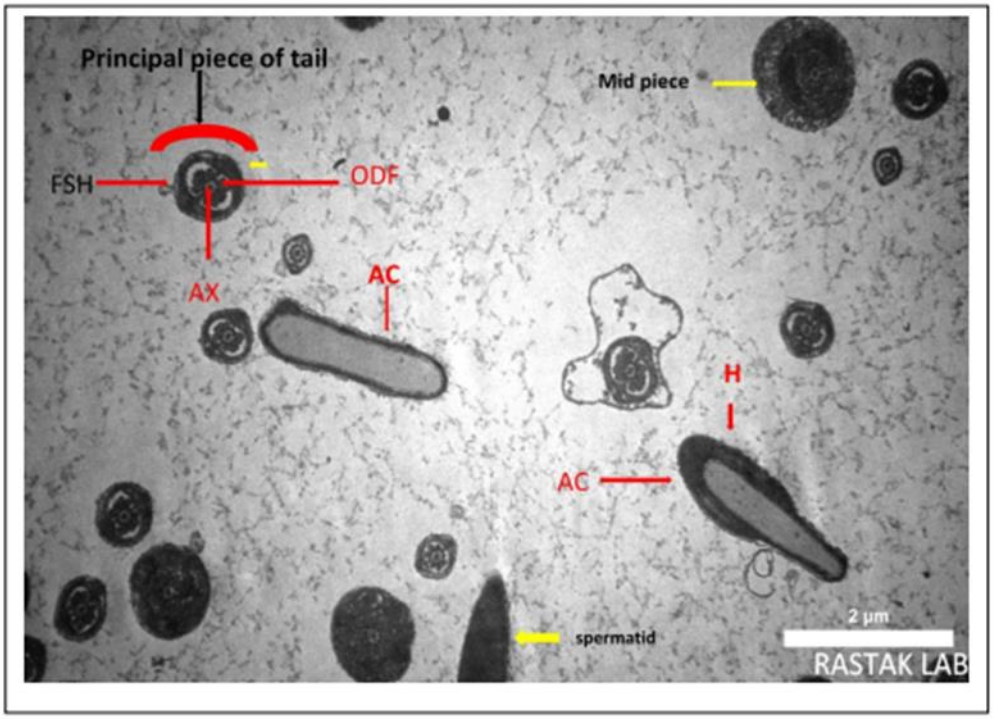

Fig (11), Electron micrograph images (TEM) of hamster testis at 60 day old showed: ODF, outer dense fibril, head, AX, axoneme, FSH, fibril sheet, AC, acrosome.

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