Original Article ISSN (O):2395-2822; ISSN (P):2395-2814

Prenatal and Postnatal Developmental Changes of Testes of Albino Rats.

Eman Borai Mohammed[1], Ibrahim Amin Maher[1], Metwally Abdel-Bary Mansour[1], Youssef Hussein Abdel-atty[1], Rasha mohammed Sabry[1]

[1]Department of Anatomy & Embryology, Faculty of Medicine, Zagazig University, Egypt.

Received: December 2017
Accepted: December 2017

ABSTRACT

Background: The testis is the primary reproductive organ in the male, it is an ovoid organ covered from outside inwards by, the tunica vaginalis, tunica albuginea and tunica vasculosa. Each testis is separated from its fellow by a fibrous median raphe, which is deficient superiorly. The testis consists of numerous lobules, about 250-400, each lobule contains from one to three, or more convoluted tubules. Objectives: The aim of this work was to study the prenatal and postnatal development of the albino rat testes considering its structure and maturation. **Methods:** 35 healthy, non-pregnant female and 18 male albino rats weighing (200-250 g) were obtained from the animal house, Faculty of Medicine, Zagazig University. After mating and pregnancy, the rat embryos and offsprings were divided into 3 groups and 9 subgroups; Group A (13th, 16th &18th day fetal rats), Group B (1st, 10th, 15th &21st postnatal days rats) and Group C (2 months and 6 months old rats). The fetal rats (13th and 16th) were fixed as whole, while the remaining prenatal, postnatal and adult rats were dissected to obtain testes, which were processed for light and electron microscopic examinations, morphometric and immunohistochemical studies. **Results:** Albino rats at prenatal day 13 (E13) showed the gonads were formed of genital ridges which were present on the posterior abdominal wall on both sides of the developing dorsal aorta. Albino rats at prenatal day 16 (E16) showed The sex cords were transformed into fetal seminiferous cords in the form of compact structures without lumen. The testes at prenatal day 18(E 18) showed the seminiferous cords form the main bulk of the testis and the interstitial tissue nearly completely differentiated with small amount of connective tissue in the spaces between seminiferous cords especially in the center. In postnatal day 1,10,15(D1,D10&D15) showed The parenchyma of testis was consisted of multiple rounded and elongated seminiferous cords. In postnatal day 21 (D21), The parenchyma of testis was consisted of multiple closely packed ovoid shaped seminiferous tubules, some of them still cords with no lumina. In adult group, The parenchyma of testis consisted of large multiple seminiferous tubules, separated by relatively narrow interstitial spaces and attained lumina containing sperms. Immunohistochemistry showed positive reaction to proliferative cell nuclear antigen (PCNA) at E13, E16 & E18. Also positive reaction to (PCNA) showed in postnatal and adult groups. **Conclusion:** Sexual differentiation of the gonads in albino rats starts after 13 days of gestation and the development of testes in albino rats is completed postnatally reaching full maturation at about age of two months.

Keywords: Rat, testis, seminiferous cords, proliferative cell nuclear antigen, prenatal, postnatal, development.

INTRODUCTION

Sex differentiation is a complex process than involves many genes, including some that are autosomal. The key to sexual dimorphism is the y chromosome, which contains the testis-determining gene called the SRY (sex-determining region on y) gene on its short arm (yp11) (Sadler, 2012).

Name & Address of Corresponding Author
Dr. Eman Borai Mohammed,
Department of Anatomy & Embryology,
Faculty of Medicine,
Zagazig University,
Egypt.

In mammals, the sexual fate of the organism is revealed only during fetal development, when the gonads begin to differentiate as ovaries or testes (Wildelm et al., 2007).

Although the same histogenic buds and similar development processes are involved in the development of male gonads in various mammalian species, the dynamics of changes occurring in the course of development varies between species, this due to their different genstational periods (Russel et al., 1995).

Gonads appear initially as a pair of longitudinal ridges called genital ridges, they are formed by proliferation of the epithelium and condensation of underlying mesenchyme. Germ cells do not appear

in the genital ridges until the sixth week of the developing (Sadler,2012).

The testes are the primary reproductive organs in male. They have two major functions: The production of the male gamete, the spermatozoa in a process called spermatogenesis and the synthesis and controlled release of testosterone as the main androgen in a process called steroidogenesis (Comhaire et al., 2006).

The testis is divided into lobules. Lying within each lobule one to three coiled seminiferous tubules. The production of androgen and spermatozoa occurs in two discrete compartments within the testis. Spermatozoa develop within the seminiferous tubules in close association with the sertoli cells while the androgen production occurs in leydig cells located in the interstitial space between the tubules (Snell, 2008).

MATERIALS AND METHODS

Animal Model and group allocation

This study was performed on 35 healthy, non-pregnant female and 18 male albino rats of weighting 200-250 grams. They were obtained from the animal house, Faculty of Medicine, Zagazig University. The experiment was performed according to the norms of the ethics committee. All animals were kept under hygienic conditions. Standard food and water ad-libitium were allowed. The temperature was maintained at 23±2°C and 12 h. light dark cycles . The vaginal smears of all female rats were examined for detecting the time of ovulation (estrous stage). The groups of female rats that enter the estrous stage were isolated and then were mated with the male rats as two females for one male in each cage. The following morning, they were examined for the presence of sperms in their vaginal smears. Vaginal smears stained with Papanicolaou stain (Kiernan, 2008). The day in which the smear was sperm positive was considered as the zero day of pregnancy. The 35 pregnant animals were divided into three groups as follows: (Group A), (prenatal group): was divided into 3 subgroups (A1), (A2) and (A3) according to their embryonic days (E) (E13, E16 and E18). For each subgroup, the pregnancy of five female rats was terminated at these embryonic days. (Group B) (Postnatal group): 15 pregnant female albino rats were allowed to continue their pregnancy. Their offsprings were subdivided equally into 4subgroups (B1, B2,B3 and B4) at 1st , 10th, 15th and 21st postnatal day (D) respectively. (Group C) (Adult group): offsprings of 5 pregnant female albino rats were divided into 2 subgroups (C1,C2) and allowed to live for 2 months and 6 months under observation and in healthy environment to become mature adult.

Lower abdominal incisions were made to the pregnant females of the group (A) under light anesthesia with ether. The uteri were quickly removed and immersed in Ringer's solution. The individual embryos were extracted from the deciduas and fixed by immersion in formalin 10% for 4 hours at room temperature.

Fetuses aged 13, 16 days (subgroup A1&A2) were fixed as a whole after being injected by formalin 10%. Fetuses aged 18 days (subgroup A3), the offsprings of the group (B) and the adult rats of the group (C) were anesthetized with ether and were dissected to obtain testes. Specimens from all the age groups were processed for light microscopic examination, immunohistochemical and morphometric studies. Specimens from postnatal and adult groups (group B and group C) were processed for electron microscopic examination.

A- Light microscopic examination

Embryos and testes specimens were fixed in 10% buffered formalin (pH 7.2) for 24-72 hours. After routine histological laboratory procedures, tissues were blocked in paraffin.

1- Heamatoxylin and eosin (H&E) stains

Sections of 5µm thickness were obtained from all specimens and stained with heamatoxylin and eosin for general histological examination (Bancroft and Gamble, 2008) in the light microscopic unit, Department of histology, Faculty of Medicine, Zagazig University.

2- Immunohistochemical Techniques

Immunohistochemical reaction was carried out using the avidin biotin peroxidase system (Sternberger, 1986). Immunohistochemical reaction was carried out using the avidin biotin peroxidase system. Polyclonal proliferating cell nuclear antigen Ab-1 (Cat # RB-9055-R7 (7.0ml)) delivered from Sigma Laboratories was used. Universal kits used avidin biotin peroxidase system produced by Novacastra Laboratories in UK.

B- Electron microscopic examination

Preparation of the specimens for transmission electron microscopic examination (TEM) was according to (Glauert and Lewis, 1998). The semithin sections (1 µm) were stained by adding few drops of toluidine blue stain. The ultrathin sections (80 nm) were obtained and mounted on copper grids.

C- Morphometric study

The number of positive nuclei of immune reaction of PCNA is counted from an area of 19817.3 square micrometer using Digimizer 4.3.2. image analysis software (MedCalc Software bvba, Belgium).Non-overlapping fields from each rat were selected randomly and analyzed.The height of germinal epithelium was measured from x 200 photos using Digimizer 4.3.2. image analysis software (MedCalc Software bvba, Belgium(.

D- Statistical analysis

Statistical analysis was measured for positive nuclei of immune reaction of PCNA, and the height

of germinal epithelium. All the values of experiments were represented as mean ± Standard Deviation (SD). One-way analysis of variance (ANOVA) was used, followed by Post hoc least significant difference (LSD) test to evaluate the differences between the groups. For all comparison $P<0.05$ were considered as significant difference.All analyses were performed using the IBM SPSS 18.0 software.

RESULTS

A- Light microscopic examination

1- Heamatoxylin and eosin (H&E) stains

Subgroup A1 (E13): Section of albino rats at prenatal day 13 showed the gonads were formed of genital ridges which were present on the posterior abdominal wall on both sides of the developing dorsal aorta as shown in [Figure 1]. Gonocytes were noticed as relatively large pleomorphic cells in the form of clusters or scattered between epithelial cells of the genital ridge as shown in [Figure 2].

Subgroup A2 (E16): Section of albino rats at prenatal 16 showed the gonads were clearly histologically determined with orientation towards testis formation. They were located near the posterior abdominal wall [Figure 3]. The most important event in this age group was the formation of fetal seminiferous cords lacking lumens. At this developmental stage, the seminiferous cords revealed both Sertoli cells and numerous spermatogonia (large oval cells)

Gonocytes with pale polypoid nuclei were observed inside the seminiferous cords as shown in [Figure 4]. Subgroup A3 (E18): The seminiferous cords had more developed lamina propria containing myoid cells and fibroblasts. Within cords there were spermatogonia. Sertoli cells. Apoptotic germ cell.

Gonocytes with pale polypoid nucleus and pale cytoplasm inside the seminiferous cords, Leydig cells were increased in number and had acidophilic cytoplasm and large rounded vesicular nucleus , some Leydig cells ,other juvenile Leydig cells were small rounded or spindle –shaped cells as shown in [Figure 5].

Subgroup B1 (D1): Some seminiferous cords appeared as arched U-shaped, The seminiferous cords. Most cells became peripheral resting on basement membrane leaving centers filled with acidophilic material. The interstitial spaces was not crowded by cells as previous group and most cells were juvenile Leydig cells [Figure 6].

Subgroup B2(D10) & B3(D15): threre was no difference between these two age groups The seminiferous cords appeared lined by Spermatogonia and sertoli cells arranged in about one row resting on basal lamina. The centers of these cords were obliterated by faintly stained acidophilic cytoplasm and vacuolations appeared in this material.Fewer gonocytes located near the centers of the cords than in the previous group. Increase number of cords than the previous group with narrower interstial spaces.The interstitial spaces contained clusters of fusiform cells with oval nuclei and acidophilic cytoplasm (juvenile Leydig cells) [Figure 7].

Subgroup B4 (D21): The most important event in this age group was the beginning of spermatogenesis The parenchyma of testis was consisted of multiple closely packed ovoid shaped seminiferous tubules, some of them still cords with no lumina. The seminiferous tubules were lined by stratified germinal epithelium. The germinal epithelium revealed two types of cells; spermatogenic and sertoli cells. sertoli cells were detected in-between spermatogenic cells as pyramidal cells with pale basal oval or triangular nuclei. The spermatogenic cells were seen in regularly arranged rows at different stages of spermatogenesis. Spermatogenic cells were arranged from the basal compartment to the lumina of the tubules starting from spermatogonia, primary spermatocytes and secondary spermatocytes. Spermatogonia appeared as small rounded cells with spherical nuclei resting on the basement membrane. Towards the inner side of the tubule, primary spermatocytes appeared relatively larger in size with large rounded nuclei arranged in one or two layers. The seminiferous tubules were ensheathed by a single layer of myoid cells which appeared fusiform in shape with flattened nuclei. The narrow interstitial spaces showed leydig cells with oval vesicular nuclei and acidophilic cytoplasm [Figure 8].

Group C (adult): We had noticed that there was no difference between subgroups C1&C2 as the parenchyma of testis consisted of large multiple seminiferous tubules, separated by relatively narrow interstitial spaces and attained lumina containing sperms. The seminiferous tubules were lined by stratified germinal epithelium. The germinal epithelium revealed two types of cells; spermatogenic and Sertoli cells, the spermatogenic cells were seen in regularly arranged rows at various stages of spermatogenesis, they were arranged from the basal compartment to the lumina of the tubules starting from spermatogonia, primary spermatocytes, and spermatid, spermatogonia appeared as small rounded cells with spherical dark nuclei resting on the basement membrane, towards the inner side, primary spermatocytes appeared relatively larger in size with large rounded nuclei arranged in one or two layers, spermatids with pale stained rounded nuclei were seen towards the lumen, Sertoli cells were detected in-between spermatogenic cells as pyramidal cells with pale basal oval or triangular nuclei and prominent nucleoli. No evidence of presence of gonocytes

within the tubules. The interstitial spaces showed Leydig cells with oval nuclei and acidophilic cytoplasm shown in [Figure 9& 10].

1- Immunohistochemical examination for proliferative cell nuclear antigen (PCNA) stained sections:

At E13, the nuclei of proliferating germ cells in the testis showed positive reaction indicated by brown colour of their nuclei as shown in [Figure 11].

At E16, the nuclei of proliferating germ cells in the testis showed positive reaction indicated by brown colour of their nuclei also positive reaction showed in the interstitium and endothelial wall of blood vessel as shown in [Figure 12].

At E18, the nuclei of proliferating germ cells in the testis showed positive reaction indicated by brown colour of their nuclei and the reaction in this group stronger than in the previous groups and the interstitium showed positive reaction to (PCNA) but less than in the cords as shown in [Figure 13].

At D1, the proliferating spermatogonia showed positive reaction indicated by brown colour of their nuclei and gonocytes showed positive reaction to (PCNA). Also negative reaction to (PCNA) was noticed in the interstitium as shown in [Figure 14].

At D10&D15,: the proliferating spermatogonia showed positive reaction indicated by brown colour of their nuclei and negative reaction was noticed in the interstitium as shown in [Figure 15].

At D21, the proliferating spermatogonia and primary spermatocytes showed positive reaction indicated by brown colour of their nuclei and negative reaction in the interstitium as shown in [Figure 16].

At age of two months & six months the proliferating spermatogonia showed positive reaction to (PCNA) indicated by brown colour of their nuclei and the reaction was localized in spermatogonia at the basal compartment of seminiferous tubules and negative reaction in the interstitium .

But the reaction in group C2 was weaker than (group C1) and negative reaction in the interstitium as shown in [Figure 17 & 18].

B- Electron microscopic examination:

At D1, The ultrathin section of testis of newly born rats showed that the seminiferous cords were surrounded by two layers of myoid cells with flattened nuclei.The cords were lined by spermatogonia and Sertoli cells; some of spermatogonia were resting on the basement membrane and others away from it. Spermatogonia had different shapes of nuclei containing few clumps of heterochromatin against nuclear membrane (fig. 19). Sertoli cells had indistinct cell boundaries and rested on basal lamina of seminiferous cords; their nuclei were irregular in shape which were characteristic for Sertoli cells and some of them contained prominent nucleoli; their cytoplasm were scanty with high nucleocytoplasmic ratio contained many mitochondria of varying shapes (fig.20). The gonocytes were large round cells;The nuclei of gonocytes were large containing finely dispersed chromatin material with one or two nucleoli. Their cytoplasm was lightly stained and relatively free of organelles except for some round mitochondria as shown in (fig.21). In the interstitium, Leydig cells appeared in clusters; they contained highly electron dense nuclei clumps of heterochromatin; their cytoplasm contained many lipid droplets and large round mitochondria . A blood vessel also was present within the interstitial space and spindle shaped fibroblasts were also seen in the interstitial space [Figure 22].

At D10 &D15 most findings in this age group were the same as the prevous age group while we noticed migrating sermatogonia toward the basement membrane which were fusiform in shape with the narrow end toward the basement membrane [Figure 23]. Sertoli cells appeared more developed than in the previous group as had ill defined cell boundaries and settled on the basement membrane of the seminiferous epithelium;their nuclei were irregular in shape and had prominent nucleoli with increased amount of cytoplasm containing many mitochondria and decreased nucleocytoplasmic ratio [Figure 24]. Leydig cells appeared in clusters; they could be distinguished from the surrounding fibroblasts by their highly electron dense nuclei and cytoplasm; their cytoplasm contained many lipid droplets as shown in [Figure 25].

At D21, The ultrathin sections of 21 days aged rats showed that the seminiferous tubules were ensheathed by a single layer of flattened myoid cells and lined by sertoli cells. spermatogonia, primary spermatocytes and spermatids as shown in [Figure 26] with beginning of spermatogenesis. Spermatids were seen at different stages stages of development. The nuclei of early spermatids were rounded in shape and the cytoplasm contained numerous peripherally situated mitochondria. More developed spermatids showed acrosomal vesicle opposite one pole of the nucleus and mitochondria were displaced towards the opposite pole as shown in [Figure 27]. Leydig cells were seen in interstitium around the blood vessels. They were ovoid in shape with oval nuclei.Their cytoplasm contained small sized lipid droplets and many mitochondria [Figure 28].

Ultrastructurally there was no great difference between the adult groups (C1&C2) as examination of their testes showed that seminiferous tubules surrounded by single layer of flattened myoid cells. cells and lined by Sertoli cells, spermatogonia, primary spermatocytes and spermatids.

Sertoli cells had large, euchromatic and indented nuclei with prominent nucleoli, their cytoplasm contained many mitochondria as shown in [Figure 29].

Spermatids were seen at different stages stages of development.The nuclei of early spermatids were ovoid in shape contained finely dispersed chromatin and the cytoplasm contained numerous peripherally situated mitochondria as shown in [Figure 30]. More developed spematids had euchromatic nucleus with acrosomal cap and acrosomal granules as shown in [Figure 31]. Spermatogonia were totally separated from primary spermatocytes and spermatids by highly electron dense junction (blood testis barrier) as shown in [Figure 32].

Cross sections in the middle piece surrounded by mitochondrial sheath, principle piece surrounded by fibrous layers and end piece of the sperm tails surrounded by plasma membrane in the lumen of the tubules as shown in [Figure 33].

The interstitial cells of Leydig contained ovoid nucleus with aggregated clumps of chromatin against the nuclear membrane; their cytoplasm contained increased amount of lipid droplets of variable size as shown in [Figure 34].

While group C2 the cells were more mature and there was better arrangement of cells to each other. [Figure 35-37]

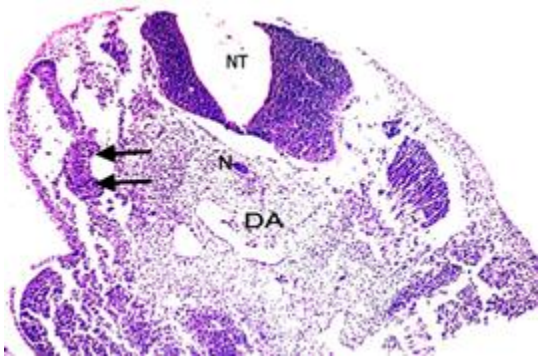

Figure 1: A photomicrograph of a transverse section of rat embryo at embryonic day 13(E13) showing the sexually undifferentiated genital ridge (arrows) on the side of primitive dorsal aorta (DA). The notochord (N) and the neural tube (NT) are also seen. (H & E × 100)

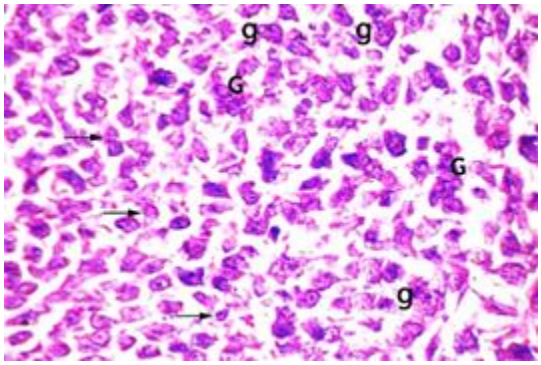

Figure 2: A photomicrograph showing dispersed gonocytes (g) and gonocytes in groups (G) infiltrating the undifferentiated mesodermal cells (thin arrows). (H & E × 1000).

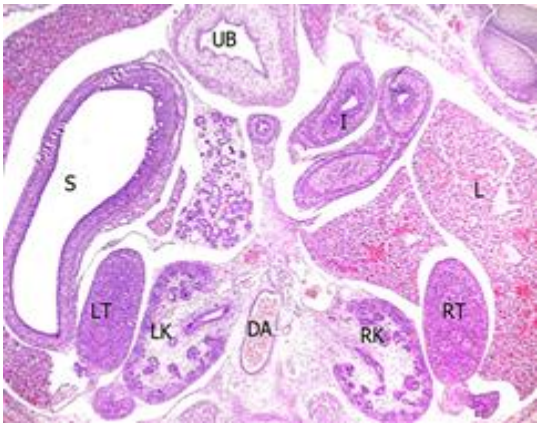

Figure 3: A photomicrograph of a transverse section of a rat fetus at embryonic day 16 (E16) showing dorsal aorta (DA),kidneys (RK & LK),testes (RT & LT),liver (L),stomach (S),intestine (I) and urinary bladder (UB). (H & E × 100).

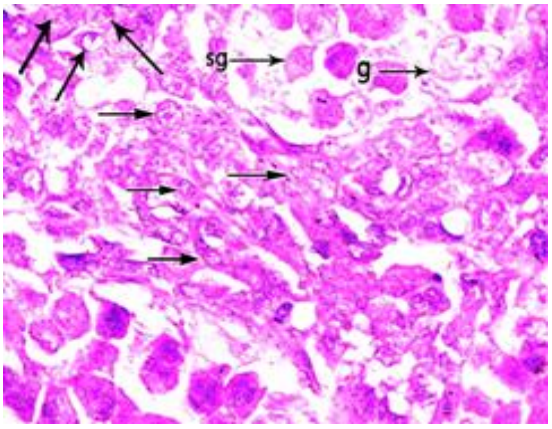

Figure 4: A photomicrograph of a transverse section of a rat fetus aged 16 days post coitum showing parts of seminiferous cords containing spermatogonia (sg) and gonocyte inside the cords (g). The interstitial tissue shows large number of fetal Leydig cells (arrow). (H & E × 1000).

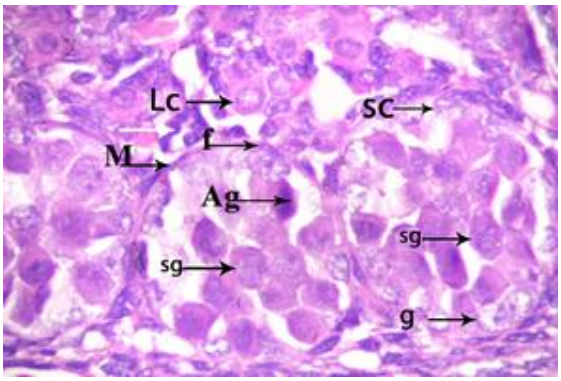

Figure 5: A photomicrograph of testis of a rat fetus at embryonic day 18 (E18) showing multiple seminiferous cords containing spermatogonia (sg), apoptotic germ cell (Ag), Sertoli cells (sc), gonocyte (g) and in between interstitial cells of Leydig (LC) and juvenile Leydig cells (white arrow), the cords are surrounded by well-formed tunica propria with myoid cell (M) . fibroblast (f) (arrows). (H & E × 1000).

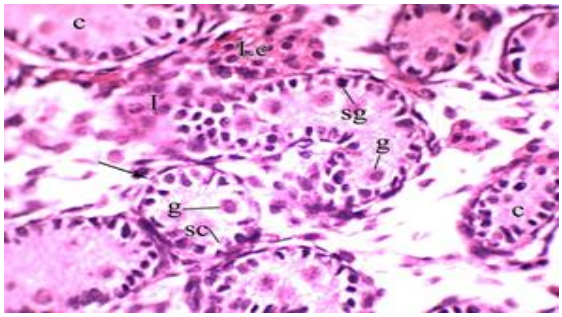

Figure 6: A photomicrograph of a testicular section of PND1 albino rat showing one row of spermatogonia (sg) and Sertoli (sc) cells lining the seminiferous cords(c). Large gonocytes (g) with single large nucleus are shown near the center of the cords. Notice the myoid cells (arrow) with flattened nuclei. Clusters of Leydig (Lc) are seen within the interstitium (I) between the cords. (H&E X 400).

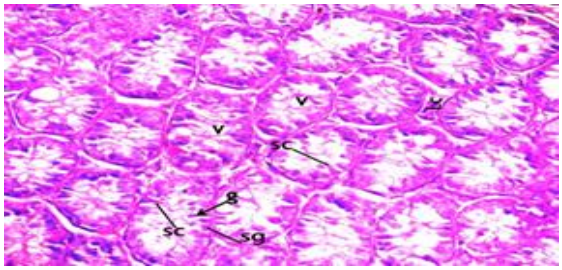

Figure 7: A photomicrograph of a testicular section of 10 days old albino rat showing many rounded seminiferous cords that are lined by spermatogonia (sg) and Sertoli cells (sc) and few gonocytes (g) , vacuolations (V) in the centers of the cinterstitial space contains clusters of Leydig cells (LC). (H&E X 400).

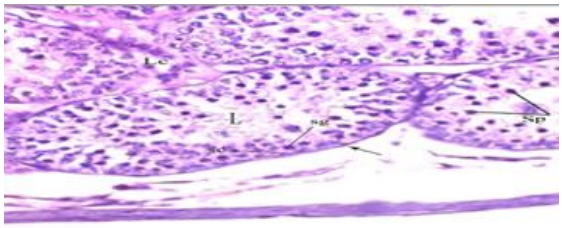

Figure 8: A photomicrograph of a testicular section of 21 days old albino rat showing ovoid seminiferous tubules had lunen (L), lined by multiple layers of germinal epithelium.The germ cells are namely spermatogonia (sg) and spermatocytes (sp).Sertoli cells (sc) with pale basal nuclei are present inbetween germ cells.Leydig cells (LC) are present between the tubules. Myoid cells with flattened nuclei ensheath the seminiferous tubules (arrow). (H&E X 400).

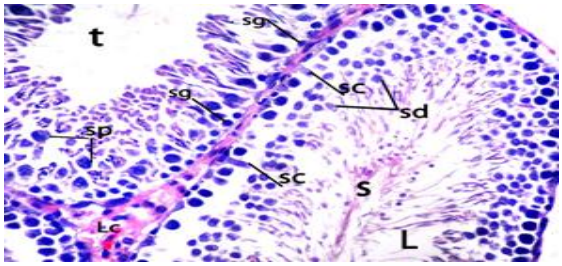

Figure 9: A photomicrograph of a testicular section of 2 months old albino rat showing the seminiferous tubules lined by multiple layers of germinal epithelium.The germinal epithelium consisting of Sertoli cells (sc) that appears as pyramidal cells with pale basal nuclei, spermatogonia (sg) appears as small rounded cells resting on the basement membrane, primary spermatocytes (sp)appears larger in size with large rounded nuclei, and spermatids (sd) that appears rounded cells with pale stained nuclei seen towards the lumen.Lumen (L) of seminiferous tubules filled with sperm tails (S). The interstitial spaces shows Leydig cells (LC) with oval nuclei and acidophilic cytoplasm. (H&E X 400).

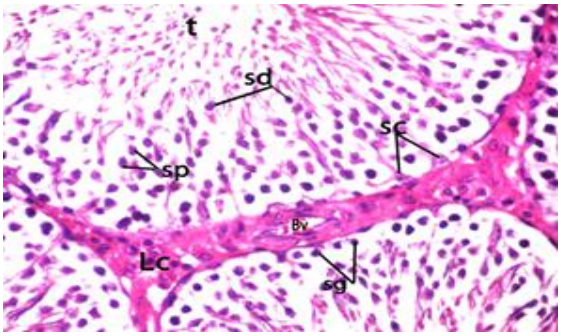

Figure 10: A photomicrograph of a testicular section of 6 months old albino rat showing the seminiferous tubules (t) lined Sertoli cells (sc) that appears as pyramidal cells with pale basal nuclei, spermatogonia (sg) appears as small rounded cells with spherical nuclei resting on the basement membrane, primary spermatocytes (sp)appears larger in size with large rounded nuclei, and spermatids (sd) that appears rounded cells with pale stained nuclei seen towards the lumen. The interstitial spaces shows Leydig cells (LC) with oval nuclei and acidophilic cytoplasm. (H&E X 400).

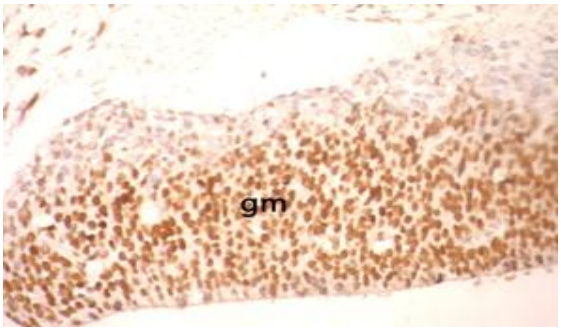

Figure 11: A photomicrograph of testis at embryonic day 13 (E13) showing positive reaction in the nuclei of proliferating germ cells (gm) to (PCNA) indicated by brown colour of their nuclei.(PCNA × 400)

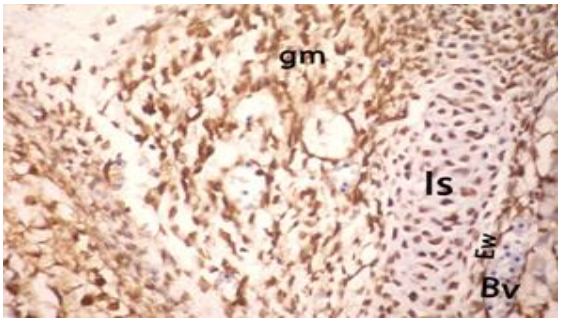

Figure 12: A photomicrograph of proliferating cell nuclear antigen (PCNA) stained testicular section at

prenatal day 16 (E16)(group A2) showing positive reaction in the nuclei of proliferating germ cells (gm) indicated by brown colour of their nuclei.Positive reaction to (PCNA) was noticed in the intestitium (Is) and in endothelial wall (Ew) of blood vessel (Bv).(PCNA × 400).

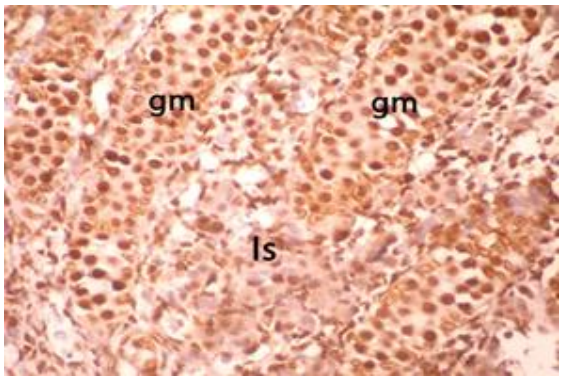

Figure 13: A photomicrograph of proliferating cell nuclear antigen (PCNA) stained testicular section at embryonic day 18 (E18)(group A3) showing positive reaction in the nuclei of proliferating germ cells (gm) indicated by brown colour of their nuclei. Positive reaction to (PCNA) showed in the interstitium (Is).(PCNA × 400)

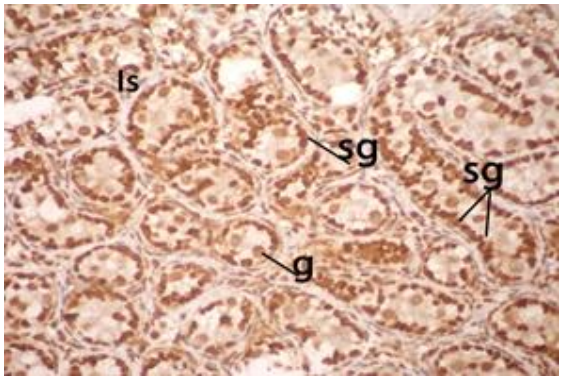

Figure 14: A photomicrograph of PCNA stained testicular section of PND1 albino rat showing spermatogonia (sg) in one layer giving positive reaction to PCNA.Positive reaction to (PCNA) is noticed in gonocytes (g)and weak positive reaction in the interstitium (Is). (PCNA X 400)

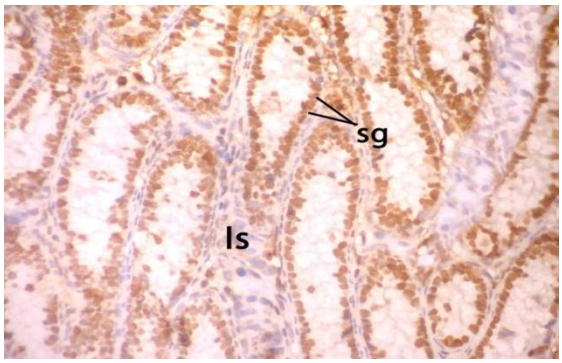

Figure 15: A photomicrograph of PCNA stained testicular section of 10 days old albino rat showing spermatogonia (sg) arranged in one layer lining the seminiferous cords giving positive reaction to PCNA.Negative reaction to (PCNA)noticed in the interstitium (Is). (PCNA X 400)

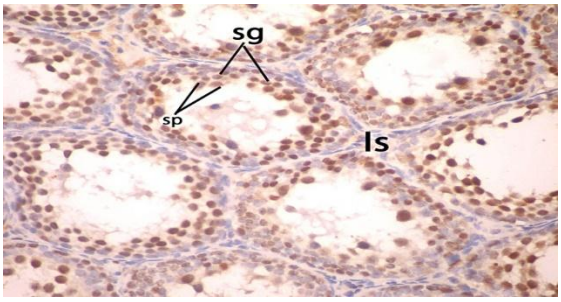

Figure 16: A photomicrograph of PCNA stained testicular section of 21 days old albino rat showing spermatogonia (sg) and primary spermatocytes (sp) giving positive reaction to PCNA.Negative reaction to PCNA is noticed in the interstitium (Is). (PCNA X 400)

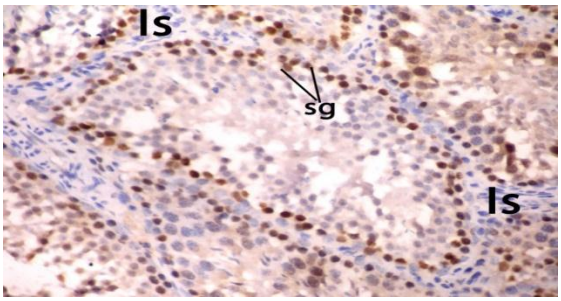

Figure 17: A photomicrograph of PCNA stained testicular section of 2 months old albino rat showing basal spermatogonia (sg) giving positive reaction to PCNA. Negative reaction is noticed in the intrestitium (Is). (PCNA X 400)

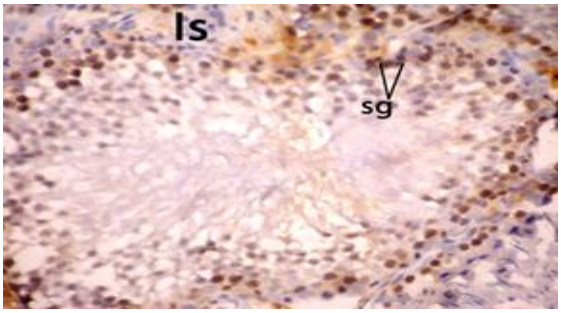

Figure 8: A photomicrograph of PCNA stained testicular section of 6 months old albino rat showing basal spermatogonia (sg) giving positive reaction to PCNA.Negative reaction to PCNA noticed in the interstitium (Is). (PCNA X 400)

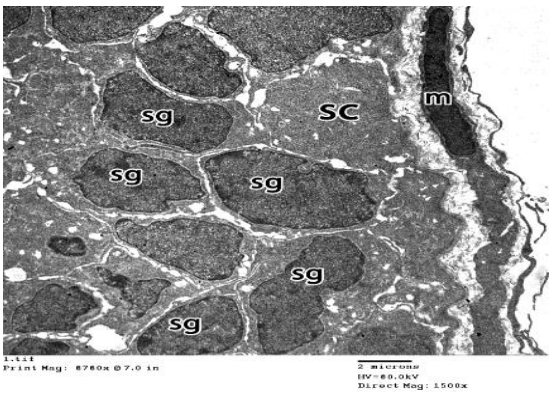

Figure 19: An electron micrograph of one day old albino rat testis showing the seminiferous cord are

surrounded by myoid cells (m) with flattened nuclei.The cords are lined by spermatogonia (sg) have predominantly rounded nuclei containing few clumps of heterochromatin and also lined by Sertoli cells (sc), they have indistinct cell boundaries and euchromatic nucleus rested on basal lamina of seminiferous cord.(TEM X 1500)

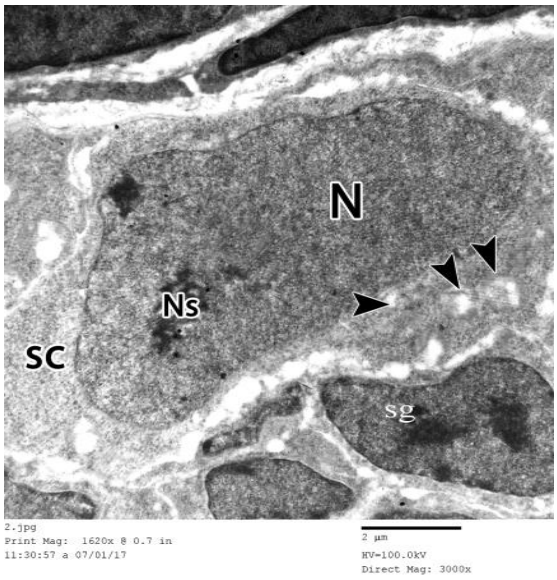

Figure 20: An electron micrograph of one day old albino rat testis showing Sertoli cell (sc) rests on basal lamina of seminiferous cord with irregular large nucleus (N) nucleus and prominent nucleolus (Ns). Its cytoplasm contains many mitochondria of varying shapes (arrow heads).Spermatogonia (sg) is seen .(TEM X 3000)

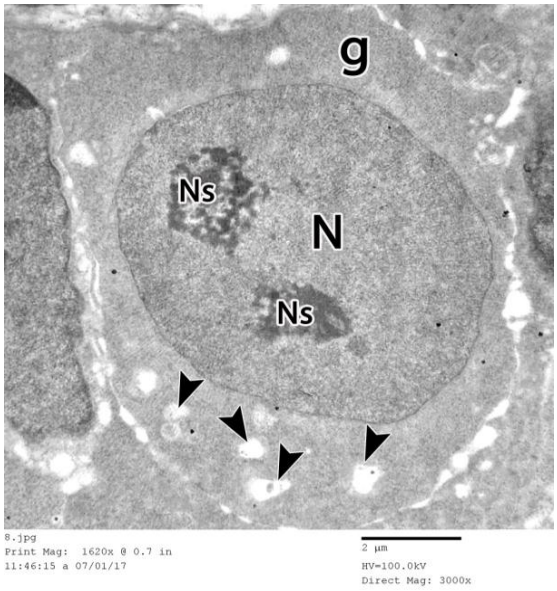

Figure 21: An electron micrograph of one day old albino rat testis showing gonocyte (g) which is large rounded cell with spherical nucleus (N) containing dispersed chromatin material and two nucleoli (Ns). Its cytoplasm is free of organelles except from some round mitochondria (arrow heads). (TEM X 3000)

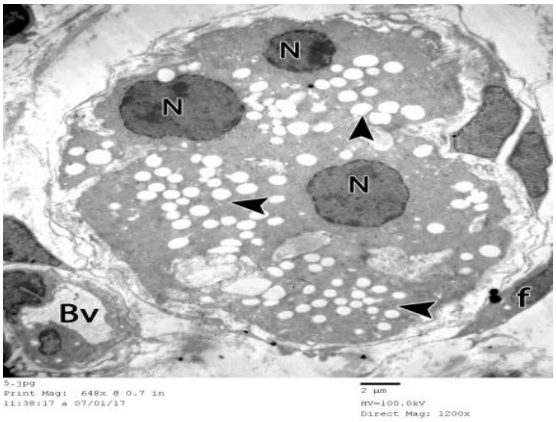

Figure 22: An electron micrograph of one day old albino rat testis showing Leydig cell in clusters and their nuclei (N) are electron dense and slightly irregular with clumps of heterochromatin. Their cytoplasm shows many lipid droplets (arrow heads). A blood vessel (Bv) and spindle shaped fibroblasts (f) also present in the interstitial space. (TEM X 1200)

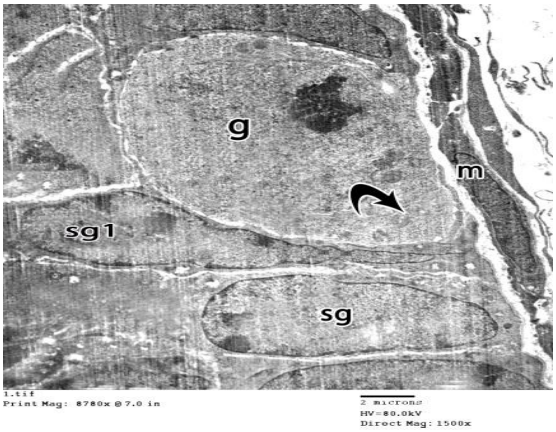

Figure 23: An electron micrograph of 10 days old albino rat testis showing the seminiferous cord are encheathed by two layers of myoid cells (m) with flattened nuclei. Also the cords are lined by spermatogonia (sg) which are rounded cells resting on the basement membrane and some of them migrating towards the basement membrane (sg1). Gonocytes (g) are large rounded cells with large nucleus containing prominent nucleolus, its cytoplasm free of organells except from some round mitochondria (curved arrow). (TEM X 1500)

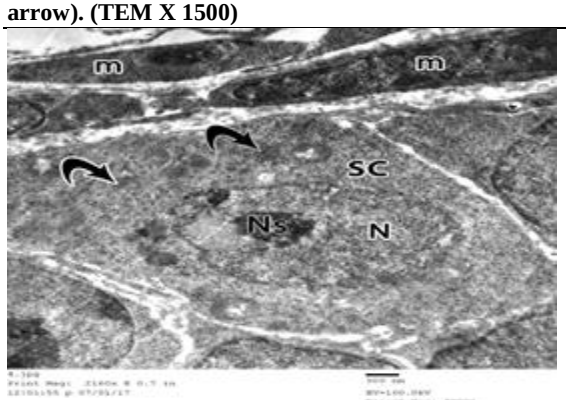

Figure 24: An electron micrograph of 10 days old albino rat testis showing Sertoli cell (sc) has ill defined

cell boundaries and euchromatic nucleus (N) with prominent nucleolus (Ns) and settled on the basement membrane and it has prominent nucleolus (Ns) and cytoplasm containing many mitochondria (curved arrows).The seminiferous cord surrounded by two layers of myoid cells (m). (TEM X 4000)

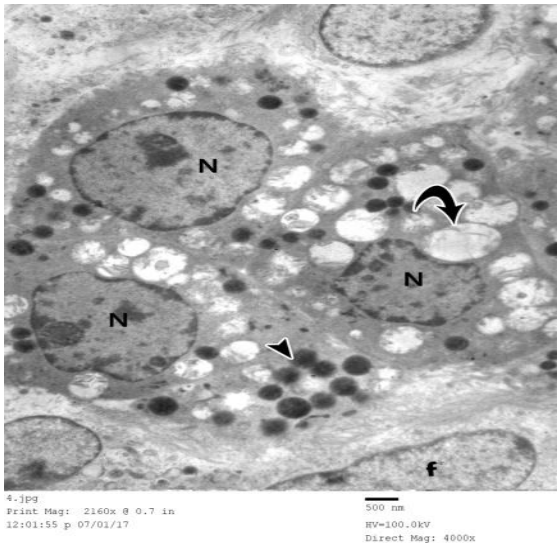

Figure 25: An electron micrograph of 10 days old albino rat testis showing highly electron dense nuclei (N) of Leydig cells and their cytoplasm contain many mitochondria (curved arrow) and lipid droplets (arrow head). Fibroblasts (f) also appears in the interstitial space. (TEM X 4000)

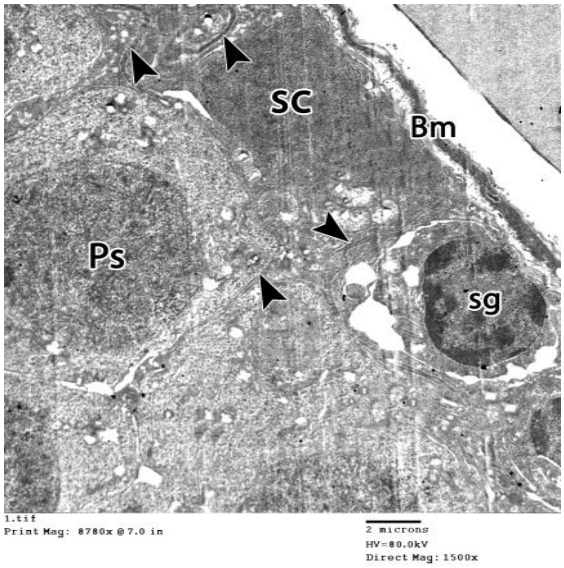

Figure 26: An electron micrograph of 21 days old albino rat testis showing the seminiferous tubule lined by Sertoli cell (sc) resting on the basement membrane (Bm), it has euchromatic nucleus and its cell boundaries are hardly followed and short strands of electron densities representing tight junction are identified at its cell membrane (blood testis barrier) (arrow heads). Also the tubule is lined by spermatogonia (sg) and primary spermatocyte (ps). (TEM X 1500)

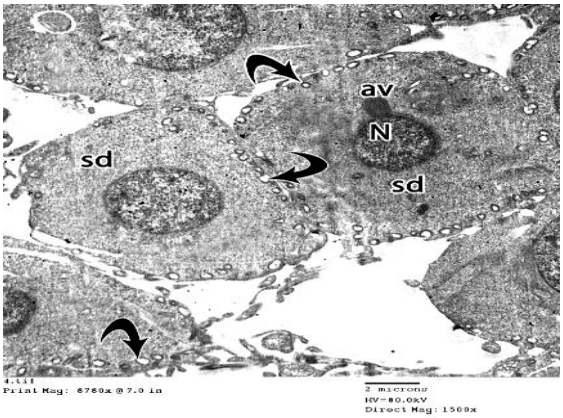

Figure 27: An electron micrograph of 21 days old albino rat testis showing spermatids (sd) at different stages of development. The nuclei (N) of early spermatid rounded in shape and the cytoplas contains numerous peripherally situated mitochondria (curved arrows). More developed spermatid shows acrosomal vesicle (av) opposite one pole of the nucleus.(TEM X 1500)

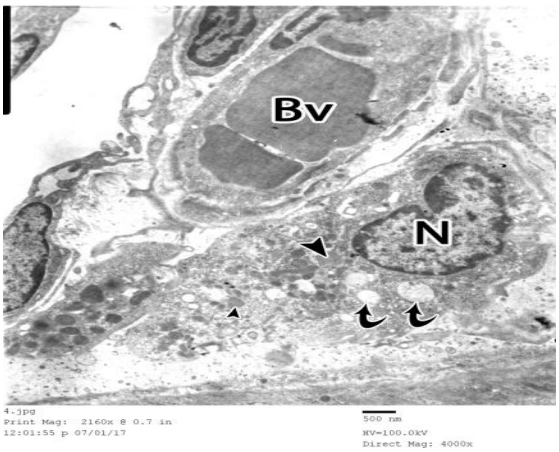

Figure 28: An electron micrograph of 21 days old albino rat testis showing nucleus (N) of Leydig cell appears highly electron dense with peripheral heterochromatin. Its cytoplasm contains small sized lipid droplets (arrow heads) and many mitochondria (curved arrows). (TEM X 4000)

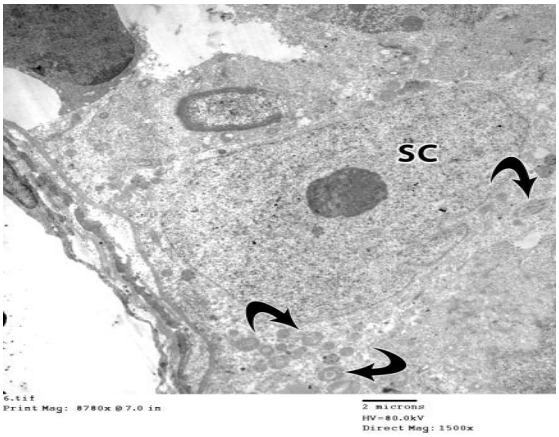

Figure 29: An electron micrograph of 2 months old albino rat testis showing Sertoli cell (sc) has large

euchromatic and indented nucleus with prominent nucleolus; its cytoplasm contains many rounded mitochondria (curved arrows). (TEM X 1500)

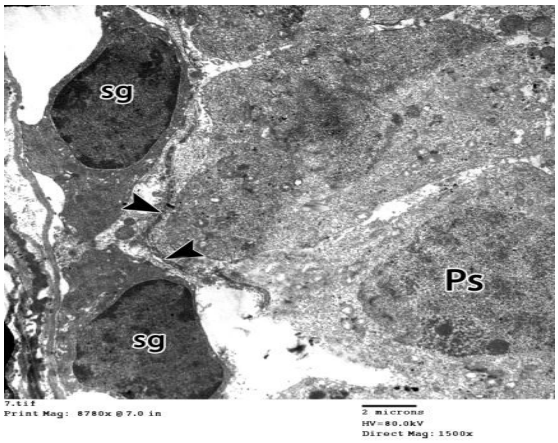

Figure 30: An electron micrograph of 2 months old albino rat testis showing spermatogonia (sg) are lying on the basement membrane, they have ovoid nuclei with finely granular nucleoplasm. Also primary spermatocytes (ps) appears larger in size than spermatogonia with rounded shape and spherical nucleus. Highly electron dense junctions (blood testis barrier) (arrow heads) between Sertoli cell processes separating spermatogonia (sg) and other spermatogenic cells.(TEM X 1500)

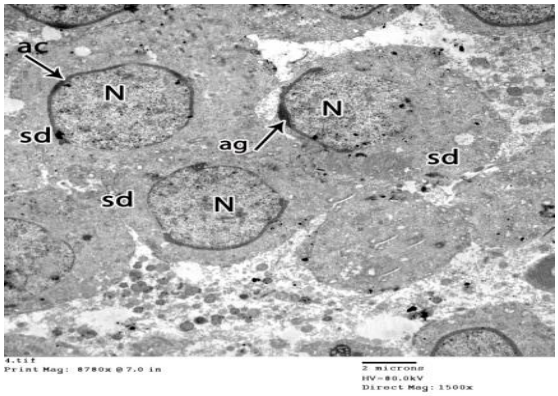

Figure 31: An electron micrograph of 2 months old albino rat testis showing more developed spermatids (sd) with euchromatic nuclei (N) developing acrosomal cap (ac) and acrosomal granule (ag). (TEM X 1500)

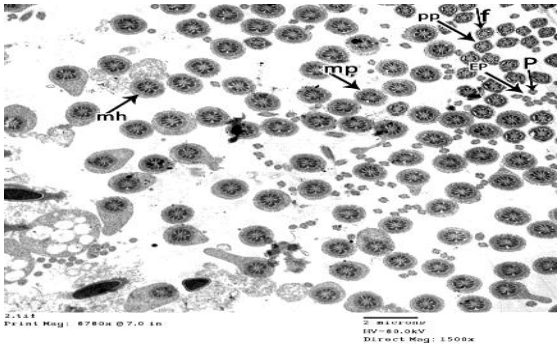

Figure 32: An electron micrograph of 2 months old albino rat testis showing cross sections in the middle piece (mp) surrounded by mitochondrial sheath (mh), principle piece (pp) surrounded by fibrous sheath (f), and end piece (Ep) surrounded by plasma membrane (p) of sperm tails in the lumen of seminiferous tubule.(TEM X 1500)

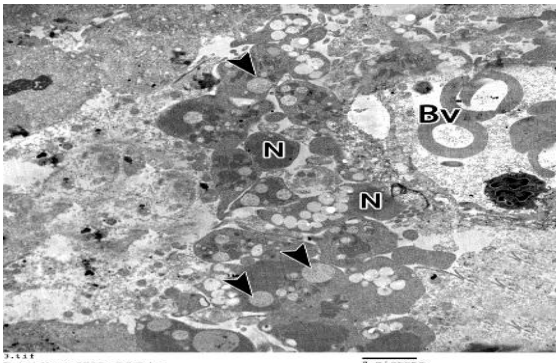

Figure 33: An electron micrograph of 2 months old albino rat testis showing nuclei of Leydig cells (N). Their cytoplasm contain variable sized lipid droplets (arrow heads). Also a blood vessel (Bv) appears in the interstitium. (TEM X 1500)

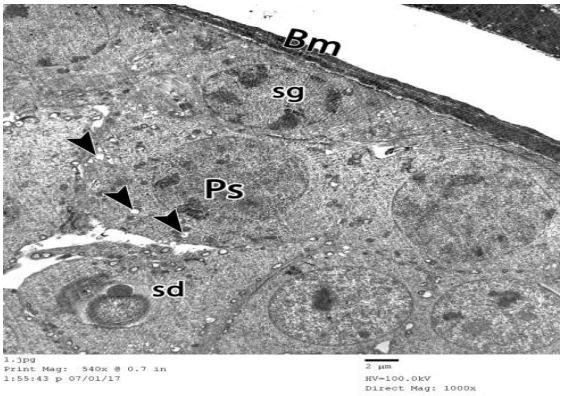

Figure 34 : An electron micrograph of 6 months old albino rat testis showing the seminiferous tubule lined by spermatogonia (sg) resting on the basement membrane (Bm), primary spermatocytes (ps) lying inner to spermatogonia and larger in size with spherical nucleus and its cytoplasm contain moderate number of mitochondria (arrow heads). Spermatid (sd) (TEM X 1000).

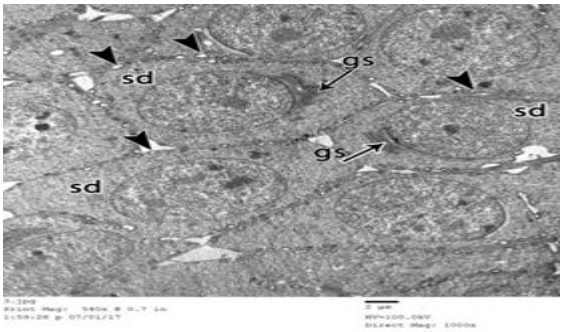

Figure 35: An electron micrograph of 6 months old albino rat testis showing spermatids (sd) at different stages of differentiations. Developed spermatids show flattened Golgi saccules (gs) opposite one pole of the nucleus. (TEM X 1000)

Table 1: Statistical analysis of number of positive nuclei to PCNA reaction by one-way ANOVA test.

Parameter	A1 Mean ±SD	A2 Mean ±SD	A3 Mean ±SD	B1 Mean ±SD	B2 Mean ±SD	B3 Mean ±SD	B4 Mean ±SD	C1 Mean ±SD	C2 Mean ±SD	F	P-value
number of positive nuclei to PCNA REACTION	37.8 ± 5.95	41.6 ± 7.6	42.7 ± 9.4	43.2 ± 4.7	38.8 ± 5.97	43 ± 10.7	38 ± 9.6	17.8 ± 4.7	13.7 ± 5.2	20.83	0.000**

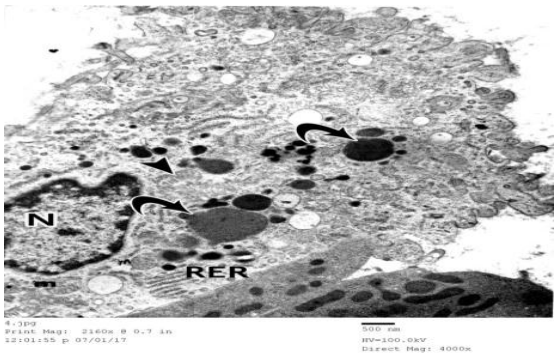

Figure 36: An electron micrograph of 6 months old albino rat testis showing nucleus of Leydig cell (N) appears euchromatic, large and rounded. Its cytoplasm shows many lipid droplets (curved arrows) and many mitochondria (arrow head)rough endoplasmic reticulum (RER) .(TEM X 4000)

Morphometric study and statistical analysis
Morphometrical results for different age groups:
Results of ANOVA test:
There was highly significant difference between groups.

Table 2: The least significant difference of number of positive nuclei to PCNA reaction between groups.

LSD	A1	A2	A3	B1	B2	B3	B4	C1	C2
A1		0.28 NS	0.17NS	0.12NS	0.78NS	0.14NS	0.95NS	0.000**	0.000**
A2	0.28 NS		0.75NS	0.64NS	0.43NS	0.68NS	0.31NS	0.000**	0.000**
A3	0.17NS	0.75NS		0.87NS	0.27NS	0.92NS	0.19NS	0.000**	0.000**
B1	0.12NS	0.64NS	0.87NS		0.21NS	0.95NS	0.14NS	0.000**	0.000**
B2	0.78NS	0.43NS	0.27NS	0.21NS		0.23NS	0.82NS	0.000**	0.000**
B3	0.14NS	0.68NS	0.92NS	0.95NS	0.23NS		0.16NS	0.000**	0.000**
B4	0.95NS	0.31NS	0.19NS	0.14NS	0.82NS	0.16NS		0.000**	0.000**
C1	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**		0.24NS
C2	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.24NS	

Comments: -
**: highly significant
*: significant

NS: Non significant
From the above results we noticed that from the statistical analysis the number of positive nuclei to (PCNA) reaction is higher in the prenatal groups (A1,A2,A3) and in the postnatal groups (B1,B2,B3,B4) than in the adult groups (C1,C2).

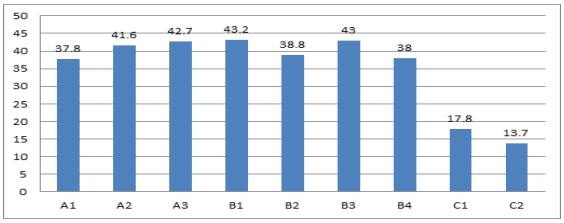

Figure 37: Bar chart for number of positive nuclei to PCNA reaction in all age groups.

This histogram showed the mean and SD of number of positive nuclei to PCNA reaction in all age groups.
From this histogram we noticed the great difference and decrease in PCNA positive reaction between age groups B4 and C1groups.

Table 3: Statistical analysis of height of germinal epithelium (µm)by one-way ANOVA test.

Parameter	B1 Mean ±SD	B2 Mean ±SD	B3 Mean ±SD	B4 Mean ±SD	C1 Mean ±SD	C2 Mean ±SD	F	P
height of germinal epithelium (µm)	19.3 ± 3.3	31 ± 4.5	33.6 ± 7.1	40 ± 5.7	117.2 ± 10.9	144.1 ± 15.6	383.9	0.000**

Results of ANOVA test:
There was highly significant difference between groups

Table 4: The least significant difference of germinal epithelium height between all postnatal groups.

LSD	B1	B2	B3	B4	C1	C2
B1		0.003*	0.000**	0.000**	0.000**	0.000**
B2	0.003*		0.502 NS	0.021*	0.000**	0.000**
B3	0.000**	0.502 NS		0.095 NS	0.000**	0.000**
B4	0.000**	0.021*	0.095 NS		0.000**	0.000**
C1	0.000**	0.000**	0.000**	0.000**		0.000**
C2	0.000**	0.000**	0.000**	0.000**	0.000**	

Comments
**: highly significant
*: significant

NS: Nonsignificant

From the above results we noticed that from the statistical analysis the height of germinal epithelium is higher in adult groups (C1, C2) than in the postnatal groups (B1,B2,B3,B4).

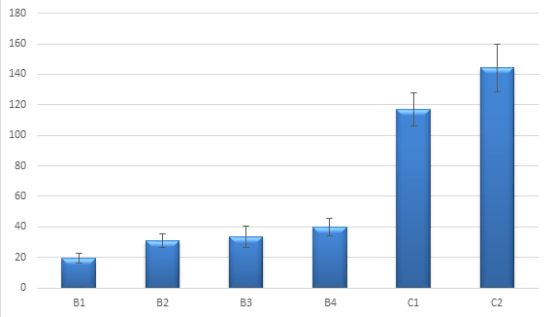

Figure 38: Bar chart for germinal epithelial height in all postnatal groups.

This histogram showed the mean and SD of germinal epithelial height of all postnatal groups.

There was marked increase in germinal epithelial height between groups B4 and C1 and this was concomitant with with the marked decrease in PCNA reaction between these two groups and was also concomitant with the beginning of spermatogenesis at B4.

DISCUSSION

Rat is considered to be more complex and informative than that of the mouse because it is a closer mammalian model for human regarding neurobehavioral, toxicological and organ manipulation studies (Hew and Keller, 2003). Laboratory rat was used to aid in the extrapolation to human assessment because in these small laboratory animals; such morphological changes were happening faster than human (Polla et al., 2004). For these reasons this study used the laboratory rat as a mammalian model for studying the development of the testes. In the present work, in 13 days rat fetuses, the gonads were still sexually undifferentiated and were formed of genital ridges. These ridges were formed of few layers of mesodermal epithelium with few gonocytes.

These gonocytes were either scattered or arranged in clusters among epithelial cells. This finding is supported by (Zolnowska et al., 1998) who also mentioned that the gonads of albino rats at day 13 post coitum are still sexually undifferentiated.

In the present study in 13 days rat fetuses, the gonads were sexually undifferentiated in contrary to our findings, Jost, (1972) revealed that at day 12 post coitum the gonads are sexually differentiated and can detect cylindrical cords which are composed of clusters of germ cells enclosed by a layer of Sertoli cells, which is in turn surrounded by a layer of peritubular myoid cells at the day 12 post coitum.

In this study, at day 16 post coitum, the gonads of albino rats were clearly histologically determined with orientation towards testis formation. They were located near the posterior abdominal wall on both sides of the developing kidneys and medial to the liver in the right side and to the stomach in the left side. This finding is supported by Zolnowska et al., (1998) who reported that the gonads are histologically determined at the day 16 post coitum.

In the present study, in rat fetuses aged 16 days post coitum, the gonads were clearly histologically determined with orientation towards testis formation but Brennan et al., (2003) reported that development of the testis starts when testis determining factor is expressed during a narrow window of time between 10.5 and 12.5 days post coitum in the gonad, resulting in rapid morphological changes that produce characteristic testis morphology by 12.5 days post coitum.

In this work at the day 18 post coitum the testes showed variable degree of differentiation between the center and periphery with more condensation of the seminiferous cords in the peripheral part. The seminiferous cord shows a clearly identified semicircular shape which matched the findings of (Zolnowska et al., 1998).

In accordance with our findings Zolnowska et al., (1998) revealed that germinal epithelium covering the fetal testes was in places two-layered and tunica albuginea was very thin with complete absence of interstitium. Small amount of connective tissue in the space between seminiferous cords. The main bulk of the fetal testis was made up of fetal seminiferous cords.

Zolnowska et al., (1998) revealed that fetal Leydig cells were observed in embryonic days 16 and 18 in the interstitium of testes and they appeared as large cells with vacuolated acidophilic cytoplasm and they were scarce at E18 and these findings not detected in our study. Fetal Leydig cells in rats were described by (Kerr and Knell, 1988).

As in this study we found large numbers of fetal Leydig cells which large vesicular nucleus and acidophilic cytoplasm in early fetal life at E16 then at E18 juvenile Leydig cells appeared which had small basophilic nucleus this was in accordance with (Waters & Trainer ,1996) who described the difference and function of fetal and juvenile Leydig cells.

Before birth, Vergouwen et al., (1991) revealed that, the seminiferous cords were surrounded by myoid cells that were cuboidal to fusiform and were in intimate contact with the basal lamina of the cords. These findings were not detected in our study.

In the present study, examination of PND1, PND10 and PND15 old albino rat testis revealed that the testicular parenchyma was formed of seminiferous cords and interstitium. These cords were small in size, round in shape and devoid of lumen. They

were surrounded by 2-3 layers of fusiform myoid cells and lined by spermatogonia, gonocytes and Sertoli cells. These data were in agreement with (Leeson and Forman, 1981; Codesal et al., 1990 and Xie et al., 1996).

In PND10 and PND15 we observed an increase in number seminiferous cords in both peripheral and central parts of testicular parenchyma and also we observed the occurrence of vacuolation in the acidophilic material that present at the centers of seminiferous cords as apreparatory step to lamination or canalization of seminiferous cords. In the present work, examination of 21days old albino rat testis showed that the majority of the seminiferous cords developed small lumina converting them into seminiferous tubules. These tubules were larger in size than those of young ages, lined by spermatogonia, primary spermatocytes and Sertoli cells and ensheathed by a single layer of flat myoid cells with beginning of spermatogenesis and also in PND21 actual seminiferous tubules became evident for the first time in our study in PND21 and these findings were supported by (Marcela et al., 2013).

Russell et al., (1989) reported that the developed lumina usually occurred by the action of tubular fluid that usually arouse from the apical part of Sertoli cells. With the development of occluding junctions between Sertoli cells, paracellular back-flow was prevented and this fluid was pushed towards the center of the cords in an obligatory manner to canalize it.

In the present study, examination of PND1, PND10 and PND15 old albino rat testis revealed that gonocytes appeared as round pale-stained cells with clearly defined cell boundaries. These gonocytes originated from primordial germ cells (Paniagua and Nistal,1984) which in turn ,migrated from vetilline sac to the gonodal ridge (Fujimoto et al., 1977). In the present study , the presence of spermatogonia in seminiferous cords, early posnatally, supported by the opinion of Roosen-runge and leik, (1968) who claimed that some newborn gonocytes contacted the basement membrane at birth and mitotically divided to give spermatogonia.

In the present study, we observed most of gonocytes in PND1, PND10 and PND15 moved to the peripheral part of the seminiferous cords and were seen separated from the basement membrane by Sertoli cell cytoplasm. These findings were supported by Vergouwen et al., (1991) who revealed that after birth the gonocytes moved towards the basal lamina of the cords.

In the present study, testicular interstitium consisted of leydig cells connective tissue cells and blood vessels leydig cells were relatively small, present in clusters,having oval nuclei and acidophilic cytoplasm.

In this work, examination of the testis in adult rat showed that each testis contained large multiple convoluted seminiferous tubules. Each tubule was surrounded by a basal lamina and a single layer of flat myoid cell. The myoid cells were believed by Goyal and Williams, (1987) to be responsible for the rhythmic shallow contraction of the seminiferous tubules. Gartner and Hiatt, (2014) reported that the neighboring myoid cells exhibited junctional complexes which not detected in our study that retarded but do not entirely prevented the passage of macromolecules from the interstitial spaces to the seminiferous epithelium. In the present work we found that in the adult group the seminiferous tubules were separated by relatively narrow interstitial space and attain wide lumina, the tubules were lined by stratified germinal epithelium consisting of Sertoli cells and spermatogenic cells which were seen in regurally arranged raws and the lumina of the tubules filled with sperm tails.these data were in agreement with Hagar,(2015).

The proliferating cell nuclear antigen(PCNA) is required during DNA replication and is expressed in the nuclear matrix of cells during DNA replication. Its application in immunohistochemistry directly reflects the proliferative state of the cell. PCNA had been extensively used in the identification of proliferating spermatogonia and spermatocytes in anumber of species, including human (Sterger et al., 1998).

In our work we found that all ages of the prenatal group(A) the proliferating germ cells gave positive reaction to PCNA. These data were in agreement withAngelopoulou et al.,(2008).also in our study all the postnatal group(B) and adult group(C) the proliferating spermatogonia gave positive reaction to PCNA and the reaction was more strong in PND21 and we attributed that to appearance and proliferation of primary spermatocytes which were abundant at that age.

In the present work, statistically we found that the number of positive nuclei to PCNA reaction was lower in the adult groups (C1,C2) than in the prenatal groups (A1,A2,A3) and in the postnatal groups (B1,B2,B3,B4) and we attributed that to increase in the layers of germinal epithelium due to appearance of additional layers formed of secondary spermatocytes and spermatids which did not contain replicated DNA as PCNA stain only replicated DNA also from our statistical results we attributed the increase in the germinal epithelial height in adult groups (C1,C2) to the appearance of additional layers formed of secondary spermatocytes and spermatids. The TEM findings of our study revealed that the seminiferous cords were surrounded by two layers of flat myoid cells in PND1,PND10 and one layer of myoid cells in PND15,PND21 and one layer in the adult group. Wilhelm et al., (2007) explained the function of

these cells is to promote the movement of mature sperm through the seminiferous tubules of the adult testis.

The TEM findings of the present study revealed spermatogonia had predominantly round nuclei containing few clumps of heterochromatin against nuclear membrane.These data were in agreement with Arighi et al., (1987) who explained that to be indicative of mitotic activity.

In the present work, we observed that in PND1 Sertoli cells had indistinct cell boundaries and their nuclei were irregular with prominent nucleoli also their cytoplasm was scanty with high nucleocytoplasmic ratio. Sertoli cells settled on the basement membrane also in our study we observed that Sertoli cells in the rest of postnatal and adult groups attained its mature characteristics as it appeared elongated cell settled on the basement membrane of seminiferous tubuls, had ill defined cell boundaries with euchromatic nucleus and prominent nucleolus also it had an increase in amount of cytoplasm with decreased nucleocytoplasmic ratio. We observed in PND21 and in adult groups the development of short strands of electron densities between cytoplasmic prcesses of Sertoli cells representing blood testis barrier separating spermatogonia from other spermatogenic cells.

This blood-testis barrier was descrbed by Byers et al.,(1991) to play many important roles e.g. permitting formation of specialized luminal environment and preventing potentially non-self molecules shed from postmeiotic germ cells, from passing into systemic circulation. Pelletier, (1986) showed that the appearance of blood-testis barrier occurred and developed at the time of meiosis. Junqueira et al., (1995) explained that differentiation of germ cells led to the appearance of sperm specific protein Since sexual maturity occurred long after the development of immunocomptence, differentiating sperms could be recognized as foreign bodies and provoke an immune response that would destroy the germ cells. Therefore, blood-testis barrier eliminated any interaction between developing sperm and the immune system.

In disagreement with our study, Cavicchia and Sacerdote, (1991) described the postnatal development of blood-testis barrier in the rats to be on day 15-16 and its completion in all tubules prior to day 20 with the appearance of spermatocytes. George and Wilson, (1994) stated that Sertoli cell barrier was widely presumed to have a marked influence on spermatogenesis as the Sertoli cells were regarded as a principal regulator of materials delivered to germ cells.

In the present study,at the inner aspect of blood-testis barrier, primary spermatocytes divided meiotically giving rise to spermatids . these cells were round or ova with round nuclei containing euchromatin. Their cytoplasm showed round or oval mitochondria arranged at the periphery of the cells immediately below the cell membrane. This was explained by Burgos and Gutierrez, (1986) to the high requirement of spermatids for energy and some special enzymatic activity for their final maturation and detachment from Sertoli cells. In more developed spermatids, the present study showed flattened Golgi saccules at one pole and mitochondria that migrated to the other pole. This was coincident with the findings of Lin and Jones, (1993) that the proximal centriole was surrounded by Golgi lamellae and located half-way between the nucleus and the distal centriole.

In the present study in PND21 and in adult groups we observed that spermatids at different stages of differentiation as they underwent spermiogenesis with appearance of acrosomal cap, acrosomal granule and Golgi saccule. these spermatides were filling the lumina of the tubules Junqueira et al., (1995) explained that the acrosomes contained several hydrolytic enzymes, such as hyaluronidase, neuroaminase, acid phosphatase and protease. Thus, these acrosomes served as a specialized type of lysosome and the enzymes were known to dissociate the cells of corona radiate and also digested the zona pellucid surrounding the ovum. The final stage of sperm production was the formation of the tail that projected in the lumen of the tubules. The sperm head embedded themselves in the apical processes of Sertoli cells for the process of nutrition.

In this study, interstitial tissue contained blood vessels and Leydig cells. These cells were large in size with electron dense nuclei. Their cytoplasm showed lipid droplets, mitochondria. These data were in accordance with Ezeasor, (1985) and Heyns, (1997). Lipid droplets were believed by the same author to function as storage depots of precursors used in the synthesis of androgen.

CONCLUSION

In the mammalian embryo, most organs are formed locally. The gonad needs both germ cells, which generate the gametes, and somatic cells, which provide a matrix to support the gametes.

The indifferent gonad is identical in males and females and composed of bipotential precursor cells that can follow one of two possible fates.Sexual differentiation of the gonads in albino rats starts after 13 days of gestation and the development of testes in albino rats is completed postnatally reaching full maturation at about age of two months.

REFERENCES

1. Angelopoulou R, Balla M, Lavranos G, Chalikias M, Kitsos C, Baka S, Kittas C. Sertoli cell proliferation in the fetal and neonatal rat testis: A continuous phenomenon? Acta histochemica; 2008; 110: 341-347.

2. Arighi M, Bosu WTK, Horney FD. Histology of normal and retained Equine testis. Acta Anatomica; 1987; 129:127-130.
3. Bancroft JD, Gamble A. Theory and practice of histological techniques. 6th edition; 2008; Churchill Livingstone, New York, London; 165-175.
4. Brennan J, Tilmann C, Capel B. Development in the XY gonad pdgfr-a mediates testis cord organization and fetal Leydig cell. Genes Dev; 2003; 17: 800-810.
5. Burgos MH, Gutierrez LS. The Golgi complex of the early spermatid in guinea pig. Anatomical Record; 1986; 216:139-145.
6. Byers S, Graham R, Dai HN, Hoxter B. Development of Sertoli cell junction specializations and the distribution of the tight-junction associated protein ZO-1 in the mouse testis. American Journal of Anatomy; 1991; 191:35-47.
7. Cavicchia GC, Sacerdote FL. Correlation between blood-testis barrier development and onset of the first spermatogenic wave in normal and in Busulfan-treated rats.A lanthanum and freeze fracture study. Anatomical Record; 1991; 230:361-368.
8. Codesal G,Regadera J, Nistal M, Panigagua R. Involution of human fetal Leydig cells. An immunohistochemical, ultrastructural quantitative study. Journal of. Anatomy; 1990; 127:103-114.
9. Comhaire F, Hargreave T, Meinhardt A. Andrology for clinician, springer berlin Heidelberg; 2006; 259.
10. Ezeasor DN. Light and electron microscopic observations on the Leydig cell of the scrotal and abdominal testes of naturally unilateral cryptorchid West African dwarf goats. Journal of Anatomy; 1985; 141:27-40.
11. Fujimoto T, Miyayama Y, Fuyuta M. The origin, migration and fine morphology of human primordial germ cells.Anatomical record; 1977; 188:315-340.
12. Gartner LP, Hiatt GL. Colour Textbook of Histology. 6th edition; 2014; W.B.Saunders Company, Philadelphia, Pennsylvania.
13. George FW, Wilson JD. Sex Determination and Differentiation. In the physiology of Reproduction. 2nd edition; 1994; Raven Press, New York.
14. Glauert AM, Lewis PR. An introduction to fixation and embedding procedures and the safe use in the laboratory. In Biological Specimen Preparation for Transmission Electron Microscopy; 1998; volume 17, Portland Press, London, pp. 1-18.
15. Goyal HO, Willam CS. The rete testis of the goat: a morphological study. Acta Anatomica; 1987; 130:151-157.
16. Hagar A. Evaluation of histopathological and ultrastructural changes in the testis of tadalafil treated adult male albino rats. Journal of histology and histopathology; 2015; Volume(2), article (18).
17. Hew KW, Keller KA. Postnatal anatomical and functional development of the heart: A species comparison. Birth Defects. Res. B. Dev. Reprod. Toxicol; 2003; 68: 309-320.
18. Heyns R. Microarchitecture of the cat testis with special reference to Leydig cells: A three- dimensional study by alkali maceration method and scanning electron microscope. Archives of Andrology; 1997; 39: 135-145.
19. Jost A. A new look at the mechanisms controlling sex differentiation in mammals. Johns Hopkins Med. J; 1972; 130: 38–53.
20. Junqueira LC, Carneiro J, Kelley RO. Basic Histology. 9th edition; 1995; Appleton & Lange, Norwork, San Mateo, California.
21. Kerr JB, Knell CM. The fat of Leyding cell during the development of fetal and postnatal rat testis. Development; 1988; 103:535-544.
22. Kiernan JA. Histological and Histochemical Methods: Theory and Practice, 4th edition; 2008; Scion, Bloxham, 190−213.
23. Leeson CR, Forman DEPostnatal development and differentiation of contractile cells Within the rabbit testis. Journal of Anatomy; 1981; 132(4):491-511.
24. Lin M, Jones RC. Spermiogenesis and spermiation in the Japanese quail . Journal of Anatomy; 1993; 183:525-525.
25. Marcela AS, Rosa MVV, Socorro RM, Marisela HG, Herlind BJ, Xochitl GG, Jose" LCM. Testosterone levels and development of the penile spines and testicular tissue during the postnatal growth in Wistar Rats: Advances in Sexual medicine; 2013; (3):1-9.
26. Paniagua R, Nistal M. Morphological and histometric study of human spermatogonia from birth to the onset of puberty. Journal of Anatomy; 1984; 139:535-552.
27. Pelletier RM. Cyclic formation and decay of blood-testis barrier in mink, a seasonal breeder. American Journal of Anatomy; 1986; 175: 91-117.
28. Polla B, D'Antona G, Bottinelli R, Reggiani C. Respiratory muscle fibers: Specialization and plasticity. Thorax; 2004; 59: 808-817.
29. Roosen-Runge EC, Leik J. Gonocyte degeneration in the postnatal male rats. American Journal of anatomy; 1968; 189: 393-406.
30. Russel LD, Defranca LR, Hess R, Cooke P. characteristics of mitotic cells in developing and adult testes with observation on cell lineages. Tissue cell; 1995; 27(1): 105-128.
31. Russell LD, Bartke A, Goh JC. Postnatal development of the Sertoli cell barrier, tubular lumen, and cytoskeleton of Sertoli and myoid cells in the rat, and their relationship to tubular fluid secretion and flow. American Journal of anatomy; 1989; 184: 179-189.
32. Sadler WT. Longman's medical embiylogy loppincoH wdilliams and wilkins, Baltimore. Hong Kong, London and Tokyo, 11th edition; 2012; 246-251.
33. Snell RS. Clinical anatomy by Regions 8th edition, lippincott william's and wilkins; 2008; 169.
34. Steger K, Aleithe I, Behre H, Bergmann M. The proliferation of spermatogonia in normal and pathological human seminiferous epithelium: an immunohistochemical study using monocolonal antibodies against Ki-67 and proliferating cell nuclear antigen. Mol. Hum; 1998; (4):227-233
35. Sternberger L. Immunohistochemistry . 3rd edition; 1986; John Wiley medical. New York Pp: 190- 209.
36. Vergouwen RPFA, Jacobs SGPM, Huiskamp R, Davids JAGde, Rooij DG. Proliferative activity of gonocytes, Sertoli cells and interstitial cells during testicular development in mice. J Reprod Fertil; 1991; 93:233-243.
37. Waters BL, Trainer TD: Development of the human fetal testis. Pediatr Pathol Lab Med 1996, 16:9–23.
38. Wildelm D, Palmer S, Koopman P. Sex determination and gonadal development in mammals physiological review; 2007; 87: 1-28.
39. Wilhelm D, Palmer S, Koopman P. Sex Determination and Gonadal Development in Mammals. Physiol Rev; 2007; 87: 1-28.
40. Xie Q, Mackay S, Ullman SL, Gilmore DP, Payne AP. Testis development in the Opossum Monodelphis domestica. Journal of Anatomy; 1996; 189:393-406
41. Zolnowska T, Sopinski M, Fortak W. Some cytological and histological aspects of the development of fetal and neonatal male gonads of the albino rat. Zoologica Poloniac; 1998; 43/1-4:35-54.

How to cite this article: Mohammed EB, Maher IA, Abdel-Bary MM, Abdel-atty YH, Sabry RM. Prenatal and Postnatal Developmental Changes of Testes of Albino Rats. Ann. Int. Med. Den. Res. 2018; 4(1):AT04-AT18.

Source of Support: Nil, **Conflict of Interest:** None declared