International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 9; Issue 02 (A); February 2020; Page No.21152-21162
DOI: http://dx.doi.org/10.24327/ijcar.2020.21162.4150

Research Article

HISTOLOGICAL CHANGES OF HUMAN FETAL TESTIS IN SECOND TRIMESTER

Amandeep Kaur[1], Kanchan Kapoor[2], Mahesh K Sharma[3] and Jessy J P*[4]

[1]Vardhaman Mahavir Medical College & Safdarjung Hospital, New Delhi
[2,3]Government Medical College & Hospital, Chandigarh
[4]All India Institute of Medical Sciences, New Delhi

A R T I C L E I N F O

Article History:
Received 10th November, 2019
Received in revised form 2nd December, 2019
Accepted 26th January, 2019
Published online 28th February, 2020

Key words:

Testis, leydig cell, sertoli cell, spermatogonia, interstitium

A B S T R A C T

Objectives: Testis are reproductive and endocrine organs in males. Morphological, functional and maturational aspects of the human fetal testis are unique. Fetal testis produces testosterone, specifically fetal gonadal hormone: anti-mullerian hormone, plays important role in induction & regulation of male sexual differentiation.
Methods: Fetal testis of varying gestational ages was studied on 55 autopsied fetuses obtained from the Department of Obstetrics & Gynaecology, GMCH, Chandigarh, India.
Results: As gestational age advanced it capsule became thicker, more folded and compacted. Connective tissue septae invading the parenchyma of testis became deeper as age advanced and divided the testis into complete/incomplete lobules. The parenchyma divided into outer 1/4th dark zone and inner 3/4th light zone, differentiated into testicular cords and interstitium. As age advanced, the testicular cords started coiling with variable shapes and were surrounded by 2-3 layers of peritubular tissue. The number of Leydig cells increased upto 20 weeks, thereafter the number and size reduced progressively.
Conclusion: The literature available on histological changes of human fetal testis is scanty. The present work observed various cell populations at different gestational ages. The knowledge of histology of testis is important in case of undescended testis, cryptorchidism, hypospadias, ectopic testis, inguinal hernias, infertility, and testicular tumors.

INTRODUCTION

The latin word" testis" means witness; one of the defining characteristic features of living beings is their ability to continue the progeny which is emphatically the function of the testis with an additional endocrine function in human species[1]. The phylogenetic mechanism for the descent of testis into the scrotum continues through various stage of gestation. Testes are invested by three coats from outside inwards, the visceral layer of tunica vaginalis, tunica albuginea and tunica vasculosa. Each testis is separated from each other by fibrous median raphe, which is deficient superiorly[2]. The left testis usually lies lower than the right testis[3]. In the last 20 years, there has been an increased awareness in assessing aborted embryos and fetuses for evidence of developmental abnormalities. Knowledge of the developing testis is important in cryptorchidism, ectopic testis, hypospadias and inguinal hernias.

At birth, approximately 4.5% of new born have cryptorchidism - cause can be testicular dysgenesis and maldevelopment, which could be associated with infertility, malignancy, atrophy, torsion etc[4].

Corresponding author:* **Jessy J P
All India Institute of Medical Sciences, New Delhi

Histologically, the parenchyma of the testis contains parallel plates of cells and rounded sex cords which are separated by interstitial tissue. In cords two major varieties of cells - spermatogonic cells and sertoli cellsare recognizable. In fetal testis at 17 weeks Leydig cells are predominant, with many mesenchymal cells and few small mast cells in the interstitium. At 24 weeks the volume of the interstitial tissue reduces, with reduction of the Leydig cells in number and size. At term no distinct lumen was present in seminiferous tubules expect a central vacuolation[5].

In adult testis, during sexual maturity spermatogenic cells proliferate and arrange into three ill- defined outer, intermediate and inner zone. The outer zone consists of direct descendants of primitive sex cells or gonocyte. The intermediate zone consists of outer layers of primary spermatocytes and inner layers of secondary spermatocytes. The inner zone consists of two or more rows of spermatids and some residual bodies. The polyhedral sertoli cells extend from basement membrane to lumen of seminiferous tubules. External to the seminiferous tubules interstitial cells of Leydig are present[3]. Therefore the structure of the testis undergoes a massive change during its life. In earlier stages, solid seminiferous cords are present. As, the age advances seminiferous tubules become more complex. The interstitial cells were abundant in fetal testis, disappear after birth,

reappear at puberty and persist throughout the reproductive life[3,5]. The seminiferous tubules have no lumen until puberty with its wall composed of sertoli cell, derived from the surface epithelium of the testis and spermatogonia derived from the primordial germ cell. In fetal life interstitial cell show marked activity in third, fourth & fifth month. The interstitial cells are derived from the mesenchymal cells of stroma[6,7].

Mantraratnam *et al*[1] observed the testicular cords within the mesenchymal tissue between 10-12weeks gestation , differentiation of leydig cells by 16 weeks with its prominence at 18weeks, distinct gestation lobulation with seminiferous tubules by the end of 24week, tunica albuginea, organisation of tubules into lobules, differentiation of sertoli cells precursors &prespermatogonial cell well marked during 28 weeks in 50 normal aborted fetuses from 10-40weeks.

Another study[5] conducted on 104 normal human male fetuses ranging from 9-40weeks revealed disposed sex cords at 9weeks. Tunica vasculosa and incomplete lobules became apparent at 17weeks, by 28weeks onwards tunica vaginalis and complete septa in the parenchyma were evident. From 30weeks seminiferous tubules became more complex with increase in number, coiling and tortusity. At term, the fetal testes still didn't attained the adult testes cytoarchitecture suggesting that testicular maturation continues postnatally.

Another study[8] conducted on 12 embryos and 6 fetuses of ovulational ages from 5-18weeks observed the main constituent cells of
gonads were derived from mesonephros and celomic epithelium. The author demonstrated that primordial sex cords were epithelial, not mesenchymal and were not identical with primary sex cords in regard to structure, location and origin. With the migration of primordial germ cells into gonadal ridge, the celomic epithelium became stratified to form a moderate protrusion of gonads into celomic cavity and the celomic epithelial cells developed into primary sex cords which were arranged into short pillars. The primordial sex cords were differentiating into seminiferous sex cords by elaborating a surrounding basal lamina.

Study[9] conducted on 70 goat embryo testes observed the formation of sex cords started on 44thday of gestation; the cords had small mesenchymal cells and large cells arranged in chain like manner. Asgestational age increased, the convolution of sex cords also increased. The sertoli cells were observed among the small cells of sex cords. Few mesenchymal cells started differentiating into Leydig cells which were located into interstitial space at 44thday of gestation.

Another study[10] done in six adult bulls showed that the basement membrane of tubules, spermatid and spermatozoa were PAS positive, whereas spermatogonia, primary spermatocytes, and secondary spermatocytes were PAS negative. The thickness of tunica albuginea and cross sectional length and breadth of seminiferous tubules was higher in the left testis whereas number of Leydig cells was higher in right testis.

A study[11] conducted on 60 human fetuses ranging in age from fetal, pubertal and adult period concluded that the fetal testis possessed two cellular lines, the spermatogonium and sertolian line. The germinal line showed three waves of differentiation and activation. The sertoli line showed only precursor types which evolve into immature and mature types during puberty. There were statistically significant variations both in germinal and sertolian line coinciding with three wave of differentiation. Not much literature is available on the prenatal histogenesis of testis to establish the exact gestational age for occurrence of various cell population. Therefore the present study was undertaken to determine the presence of various cell populations including germ cell, interstitial cell of Leydig, sertoli cells etc. at different gestational age.

MATERIAL AND METHODS

The present study was carried out in the Department of Anatomy, Government Medical College and Hospital, Chandigarh from 2013 to2015 on 55 aborted human fetal specimens with gestational ages ranging from 12-28weeks. The specimens were obtained from Department of Obstetrics and Gynaecology for routine fetal autopsy. Consent was taken from the parents to perform autopsy and to carry out additional studies. Fetuses with grossabnormality, macerated fetusresulting from IUD or spontaneous abortion and any maternal history of infections such as rubella, hepatitis, CMV, HIV were excluded from the study.

After routine autopsy, testes were dissected out and histological observations were made using Haematoxylin and eosin to demonstrate the normal structure of the testis, PAS for basal membrane of seminiferous tubules, reticular and collagen fibrils, Masson's trichrome stain for connective tissue examination. The fetuses were divided into 4 groups: Group A- 12-16weeks, Group B- 16^{+}- 20weeks, Group C- 20^{+}- 24weeks, Group D- 24^{+}- 28weeks.

RESULTS

Group A

Haematoxylin and eosin stain
Capsule

Before 12 week, testes were covered by thin layer of simple squamous with patches of cuboidal epithelium. Cells of the germinal epithelium were not continuous. In between the epithelium large primordial germ cells were also seen. The thickness of surface epithelium was 8.4 micron. Beneath the surface epithelium mesenchymal cells, immature red blood cells, differentiating fibroblasts were present (Fig:1).

After 12weeks the outer 2-4 layers of mesenchymal cells laid down thick tunica albugineajust beneath the germinal epithelium, composed of mesenchymal cells, fibroblasts and small capillaries. From 12-16weeks the average thickness of tunica albuginea was 17.32 micron and started differentiating into two layers. The outer layer of tunica albuginea composed of circumferentially arranged spindle shaped fibroblast like cells with elongated nuclei and densely packed collagen fibres. Whereas inner layer contained several blood vessels and loosely packed connective tissue.

Table 1 Histological changes seen in group A (12-16 weeks)

Age	Capsule	Parenchyma		Mediastinum	Rete testis
		Testicular cord	Interstitium		
Between 11-12 week	Single layer of simple squamous epithelium. Primordial germ cells seen in between cells of epithelium	Few primordial germ cells with vesicular nucleus, small cells and spindle shaped fibroblast seen in undifferentiated mesenchyme of testis. Cords and interstitium were not differentiated.		Not differentiated	Not differentiated
12-16 weeks	Tunica albuginea composed of outer densely packed collagen fibres and inner loosely packed connective tissue and blood vessels. Average thickness measured 17.32 micron. Beginning of septa formation.	Arranged near periphery in chain like manner. Spermatogonia and sertoli cell could not be distinguished from each other.	Mesenchymal cells, differentiating fibroblast, appearance of Leydig cells, capillariesseen.	Connective tissue core of mediastinum placed centrally.	Clusters of rete testispresent.

Table 2 Histological changes seen in group B (16^{+} -20 weeks)

Age	Capsule	Parenchyma		Mediastinum	Rete testis
		Testicular cord	Interstitium		
16^{+}-20weeks	Surface epitheliumcuboidal or squamous at some places. The tunica albuginea consisted of outer thick fibrous layer. Inner loosely arranged connective tissue with blood vessels. Average thickness was about 49.58 micron. Septae became well defined.	Process of convolution with distal ends starting to coil. Solid cords surrounded by 1-2 layer of well defined peritubular cells. Large and small spermatogonia and sertoli cells identified. Number of large cell spermatogoniamore than the small cells.	Interstitiumexpanded. Leydig cellsmore prominent in size and number. As age advanced vascularity increased.	Shifted posteriorly. Solid clusters of rete tubules along with collagen fibres, blood vessels and fibroblast present. Straight tubules started at the end of testicular cords and forming connection with the rete testis.	Rete tubules visible, anastomosing the testicular cords to efferent tubules. Process of lumen formation started in the tubules.

Table 3 Histological changes seen in group C (20^{+} -24 weeks)

Age	Capsule	Parenchyma		Mediastinum	Rete testis
		Testicular cord	Interstitium		
20^{+}-24weeks	Surface epitheliumsquamous. Capsule became more folded, compact and compressed. Tunica vasculosavisible. The average thickness of tunica albuginea was 100.79 micron. Connective tissue septae divided the parenchymainto incomplete or complete lobules.	Numerous. Various shapes noticed- C shaped, horse shoe shaped or reverse question mark. Large cells decreased and small cells increased. Sertoli cells were in close contact with the membrane	Fetal Leydig cell started regressing in size and number. More blood vessels seen.	Shifted more posteriorly. Size of mediastinum became small.	Rete testis luminised with fibroblast, elastic and collagen fibres.

Table 4 Histological changes seen in group D (24^{+} -28 weeks)

Age	Capsule	Parenchyma		Mediastinum	Rete testis
		Testicular cord	Interstitium		
24^{+}-28weeks	Surface epithelium was simple squamous type with patches of cuboidal epithelium. Tunica albugineamore folded. Tunica vasculosa was more vascular. The average thickness of tunica albuginea was 141.74 micron. Interlobular septa extended more deeply.	More crowded. Showed central cell degeneration with appearance of lumen in solid cords. Large cell population compressed further.	Fetal Leydig cell regressed more. Leydig cell dedifferentiated into spindle shaped mesenchymal cells. Greater amount of fibres seen in interstitium.	Straight tubules and mediastinal portion were anastomosing.	Became more recognizable. Lumen became more prominent.

Few mesenchymal cells from the capsule along with primordial germ cells and small blood vessels started invaginating to form the incomplete connective tissue septa in between the developing testicular cords. These septae containing blood vessels divided the testicular parenchyma into ill-defined lobule. Concentric arrangement of collagen fibres around the blood vessel were seen; along with fibroblast and mesenchymal cells.

Parenchyma

Between 11-12weeks large cells were observed in between the mesenchymal cellsknown as primordial germ cells.

Primordial germ cells were round to spherical in shape with vesicular nucleus and 2-3 nucleoli towards the periphery. The cytoplasm of primordial germ cell were acidophilic and were very few in number. Mesenchymal cells were small, spherical or oval in shape with eosinophilic cytoplasm. Few mesenchymal cells were spindle shaped and had slightly eosinophilic cytoplasm and densely stained nuclei. These cells were known as differentiating fibroblast. Few immature red blood cells were also observed among the mesenchymal cells. The testicular capsule or tunica albuginea was not well defined along with testicular cords and interstitium of testis. (Fig:2). From 12week the parenchyma of testis was differentiated into testicular cords and interstitium.

Testicular cords

At 12weeks parenchyma of the testis was divided into an outer $1/4^{th}$ of darker zone and inner $3/4^{th}$ of lighter zone. The outer zone consisted of newly developed testicular cord, connective tissues and blood vessels. The large primordial germ cells and small mesenchymal cells were arranged in chain like manner (Fig:3).

The large cells were spherical or oblong in shape with distinct cell boundaries. The vacuolated cytoplasm was less eosinophilic. The cells were placed peripherally close to basement membrane. Few cells were presents towards the centre of cords. This may be an indication of the moment of the cells towards the future lumen of the cords. The small cells were spherical, oval or irregular in shape, located at peripherally close tobasement membrane of developing testicular cords. These cells were compactly arranged with cytoplasm eosinophilic than the large cells. The small cells formed a layer along the basement membrane. After a short distance from the tunica albuginea these large and small cells grouped together and formed clusters of irregular shape.

The testicular cords were arranged as elongated near the periphery. The ends of the tubules were not coiled. The inner zone was nearly a cord free zone comprised of loosely arranged mesenchymal cells. The testicular cords were approaching the central area and were not delineated out from the rest of the parenchyma. At 40x in the testicular cords, the spermatogonia and sertoli cells could not be distinguished from each other. Whereas dark and pale spermatogonia cells were identified (Table 1). At 14weeks, the testicular cords were seen toward the centre of the testis. The testicular cords were surrounded circumferentially by 1-2 layers of peritubular myoid cells composed of collagen fibres and fibroblast cell (Fig:4).

Interstitium

The space between developing testicular cords were comprised of mesenchymal cells, differentiating fibroblast, capillaries and Leydig cells. Few of the mesenchymal cells started developing into future interstitial cells, characterised by their polygonal shape and highly eosinophillic cytoplasm with vesicular nucleus and eccentrically placed 2-3nucleoli, and were present singly. Differentiation of Leydig cells started after the appearance of tunica albuginea.

In later stage of gestation, the central area showed branching and anastomosing pattern of spindle shaped fibroblast, blood vessels, nerves and collagen fibre. The Leydig cells were distributed singly or in groups.

Mediastinum testis

At this stage the mediastinum testis identified as an area devoid of testicular cords. The arterioles, differentiating spindle shaped fibroblast with fusiform nuclei, mesenchymal cells and fine fibres with branching pattern were distinctly visible (Fig:3).

Rete testis

The rete testis tubules had clusters, containing 5-6cells in the area of mediastinum testis and they were not connected with the testicular cords. The rete testis were solid with no lumen formation.

PAS stain

The basal lamina of testicular cords showed mildly positive PAS reaction because basal lamina was very thin. Some cells inside the testicular cords showed intense positive affinity toward PAS stain. Small cells and large irregular Leydig cells with large nuclei showed mild to moderate affinity to PAS. (Fig:5&6).

Masson's trichrome stain

The collagen fibres of tunica albugineastained blue. The peritubular layer around the testicular cord were weakly bluish stained. The cytoplasm of germ cells, large and small cells, and mesenchymal cells stained red (Fig:7).

Group B

Capsule

At 16weeks the surface epithelium was mainly cuboidal but at some places squamous. As the gestational age advanced, the epithelium was mainly squamous. The outer thick fibrous and inner vascular layer of tunica albuginea were well demarcated separately. In the outer thick fibrous layer, mesenchymal cells and differentiating fibroblast were more compactly arranged and were running parallel to germinal epithelium. Inner layer contained blood vessels with erythrocytes, mesenchymal cells. The inner layer was sharply demarcated from the underlying Leydig cells. The average thickness of tunica albuginea was 49.58 micron (Fig:8).

Septa became well defined and lobules of testis were clearly visible. Upto 19-20weeks the connective tissue septae along with large blood vessels were invaginated in between the testicular cords and interstitium of testis and complete septa upto the mediastinum of testis were also noticed.

Parenchyma

Testicular cords

Foramtion of cluster further progressed. Due to increased length of cords the distal end of cords slightly started coiling indicating the beginning of process of convolution of testicular cords (Table 2). The testicular cords contained large, small spermatogonia and differentiating sertoli cells. The solid testicular cords were demarcated by indistinct basal lamina surrounded by 3-4 layers of well-defined peritubular cells (Fig:9). In few spherical testicular cords located peripherally, the basement membrane was discontinuous. The size of large cells also increased, with eccentric densely stained nucleus. Nucleus of small cells showed intense staining with eosin. Few small cells were observed differentiating into sertoli cells containing elongated nuclei with eccentric nucleolus and nuclei located perpendicular to basal lamina. These cells were irregular in shape and few had pyramidal nuclei.

Interstitium

Large numbers of Leydig cells were present along with undifferentiated mesenchymal cells. The interstitium expanded due to differentiation into Leydig cells, peritubular cells, undifferentiated mesenchymal cells and connective tissue cells. Testicular cords widely separated due to increased number of Leydig cells and mesenchymalt issue within the interstitium. As age advanced, presence of blood vessels and number of Leydig cells progressively increased. (Fig:9)

Mediastinum testis

The connective tissue core of mediastinum testis, which was placed centrally in the earlier age group now shifted posteriorly (Fig:11). This area was poorly stained with H and E as compared to surrounding parenchyma. Some of the mesenchymal cell of this area located in well vascularised zone formed solid clusters, the forerunner of rete tubules. The appearance of collagen fibres along with blood vessels and fibroblasts were also noticed.

Straight tubules

The ends of few solid testicular cords near rete tubule began to form straight tubules. These tubules were making connections with rete tubules (Fig:12). Solid straight tubules were mainly composed of small cells with indiscernible boundaries. At certain parts straight tubules showed degeneration of cells in the process of lumen formation. The tubuli recti and rete tubules connections further progressed but lumen was not formed.

Rete testis

In few tubulescentral cells started degeneration to form the lumen. The epithelium appeared simple cuboidal. Lumen formation further progressed. Rete testes were interconnecting the testicular cords to the efferent ductules.

PAS stain

Capsule of testis showed weakly positive with PAS stain, whereas some cells inside the testicular cords were still PAS positive. Leydig cells were showing PAS positive affinity which were irregular large cell having large nucleus with nucleoli containing dispersed chromatin network. Basal lamina of testicular cords exhibited intense PAS affinity (Fig:13).
Masson's trichrome stain Capsule of testis, basement membrane of blood vessels, collagen fibres present in the interlobular septa and in between the rete testis have taken blue stain (Fig:14). While the cell population within the testicular cords were more proliferated and darkly red stained.

Group C

Capsule

The surface of testis were covered by flattened cells andas inprevious group. The cell boundaries were unnoticeable. The outer layer of tunica albuginea became more fibrous with formation of loose vascular tissue deep to the tunica albuginea and became the tunica vasculosa (Fig:16). The capsule became more folded, compact and compressed. The average thickness of tunica albuginea was 100.79 micron. The connective tissue septae radiated from the hilum of testis, divided the parenchyma of testis intolobules. The blood vessels along the septa were larger in size.

Parenchyma

Testicular cords

Process of coilingfurther progressed due to increased length of testicular cords. The shape of testicular cords was in form of reverse question mark or c shaped or horse shoe shaped due to convolution, surrounded by 2-3layers of peritubular tissuecomprised of differentiating fibroblasts (Table 3).
The large cells decreased in number and showed degenerative changes in their nucleus. Few large cells located close to the basement membranehad similar character as in previousgroups. Cytoplasm of most of the degenerative cells showed large sized vacuoles. The number of small cells further increased, with few having ovoid nucleus, placed horizontal to the basement membrane. The sertoli cells were in close contact with basement membrane.

Interstitium

Fetal Leydig cells dedifferentiated and regressed in size and number. The column of Leydig cell between the tubules became progressively narrower. There were few cells between the tip of the cords and the tunica albuginea. Some of the Leydig cells became smaller and spindle shaped. As Leydig cells regressed, testicular cords became more crowded. (Fig:17)

Mediastinum testis

They shifted more posterior and became smaller in size. The vascularity increased rapidly especially towards the posterior border of testis.(Fig:18).

Straight tubules
The tubuli recti exhibited similar findings as in above. Lumen appeared in very few tubules and had simple cuboidal epithelium.

Rete testis

Most of the rete tubules were luminised. A layer of darkly stained fibroblasts, elastic, collagen and coarse reticular fibres covered the rete tubules.

Masson's trichrome stain

The collagen fibres in tunica albuginea stained blue,whereas in the interstitium and walls around the testicular cords were weakly bluish stained. Cytoplasm of all cell population stained red (Fig:20).

Group D

Capsule

The surface epithelium was simple squamous with patches of cuboidal epithelium. The fibrous layer of tunica albuginea were more folded with further increase in vascularity of tunica vasculosa, fibrovascular interlobular septa extended more deeply into the parenchyma. The average thickness of tunica albuginea was 141.74 micron.

Parenchyma

Testicular cords

The peripheral loops of the sex cords tightly coiled back to back. As the fetal life advanced the testicular cords became more and more crowded. Most of the testicular cords showed central cell degeneration in the process of formation of seminiferous tubules (Fig:22).

The large cell populationfurther compressed, number of degenerating cells increased. Such cells contained centrally placed nucleus with densely staining chromatin granules (Table 4).

Interstitium

Some Leydig cells regressed in size and few dedifferentiated into spindle shaped mesenchymal cells. Beside Leydig cells, greater amount of fibres, mast cells, mesenchymal cells, fibroblast cells and more blood vessels were seen (Fig:23).

Rete testis

The rete testis became more recognizable. The straight tubules and mediastinal portion were anastomosing. Lumen of rete tubules became prominent. Canalization of rete testis and efferent ductules junction was not completed.

PAS stain

The basement membrane of blood vessels and testicular cords showed intense affinity toward PAS stain. Cells of tubules also showed positive affinity to PAS stain.

Masson's trichrome stain

The thick tunica albuginea and interlobular septa were well stained blue. Cytoplasm of cells stained red. (Fig:24).

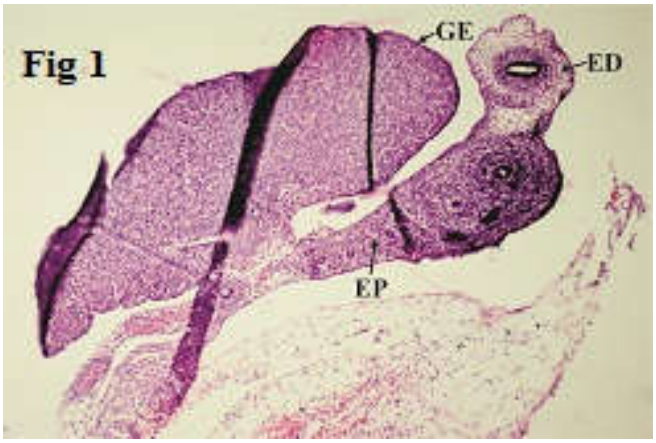

Figure 1 Fetal testes along with epididymis showing germinal epithelium (GE), efferent ductule (ED) at 11-12weeks. (H& E; X 40)

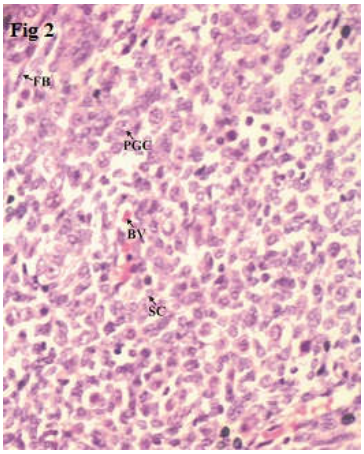

Figure 2 Primordial germ cell (PGC), small cell (SC), blood vessel (BV) and fibroblast (FB) at 11-12weeks. (H &E; X 400)

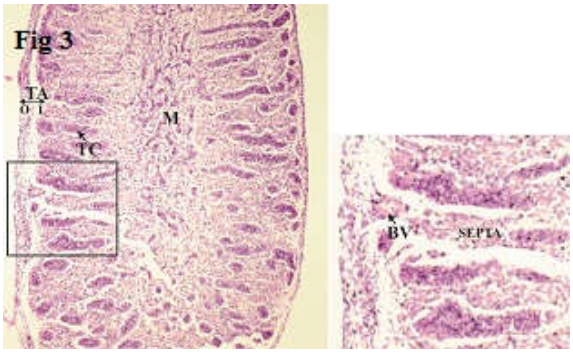

Figure 3 Tunica albuginea (TA), testicular cords (TC) and loosely arranged mesenchyme (M) at 12weeks of gestation. (H& E; X 100)

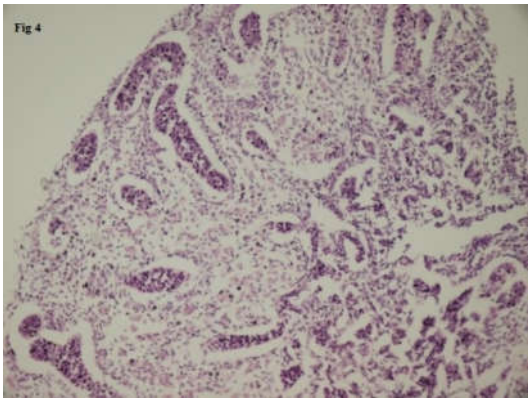

Figure 4 Reverse coma shaped testicular cord with 1-2 layers of peritubular tissue. (H &E; X 200)

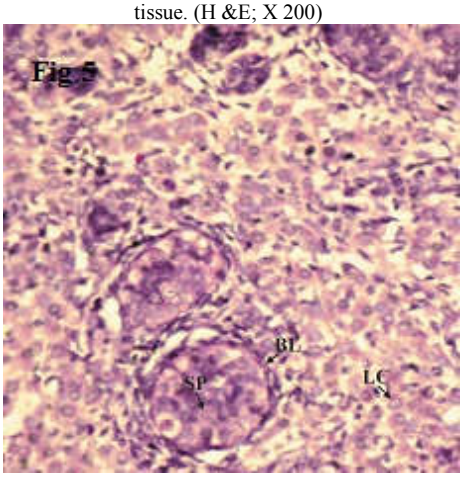

Figure 5 PAS positive reaction inside the testicular cord, basal lamina (BL) and some Leydig cells (LC) at 12weeks. (PAS; X 400)

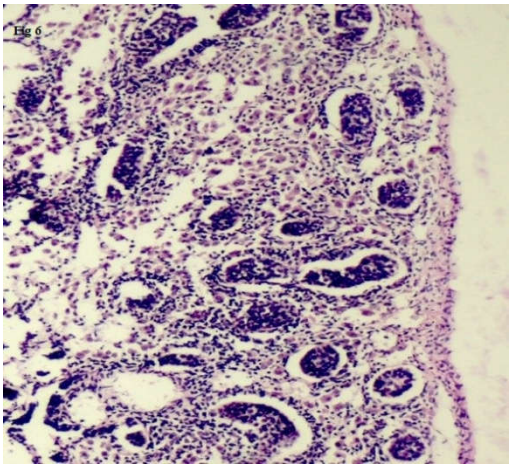

Figure 6 Basal lamina and Leydig cells at 14weeks of gestation. (PAS; X 400)

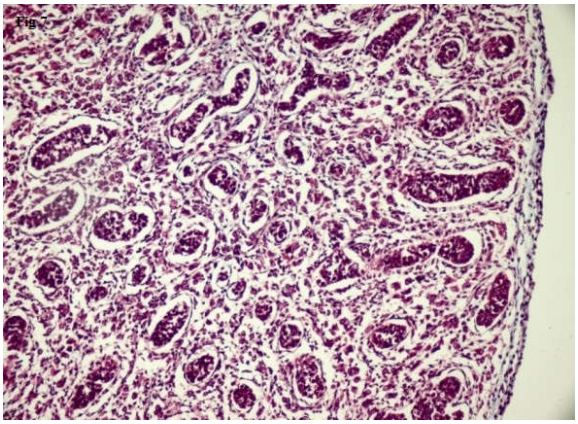

Figure 7 Collagen fibres of tunica albuginea and in peritubular tissue at 14weeks. (Masson's Trichome; X 200)

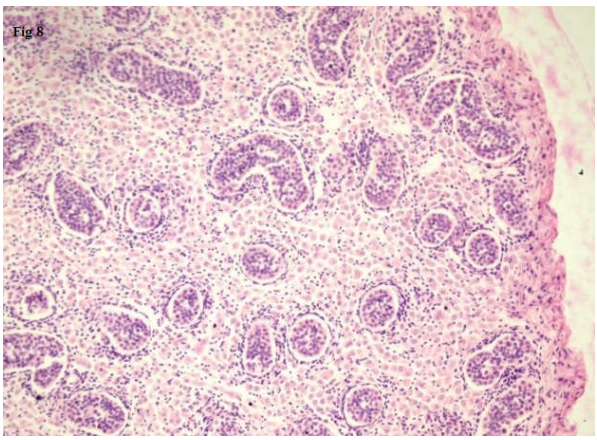

Figure 8 Testicular cords showing C shape to round shape , tunica albuginea and Leydig cells at 17weeks. (H &E; X 200)

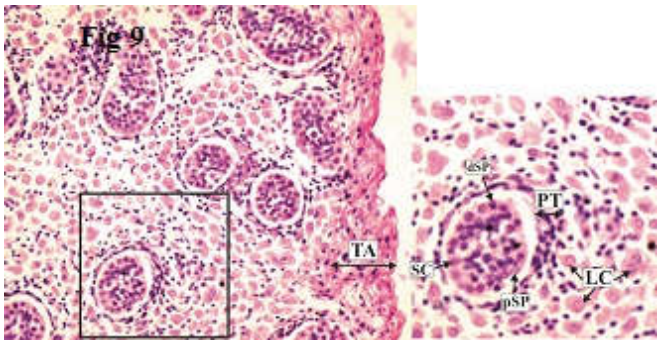

Figure 9 Testicular cord with dark and pale spermatogonia (dSP, pSP), sertoli cells (SC) surrounded by 3-4 layers of peritubular tissue (PT) with prominent Leydig cells (LC) at 17weeks. (H& E; X 400)

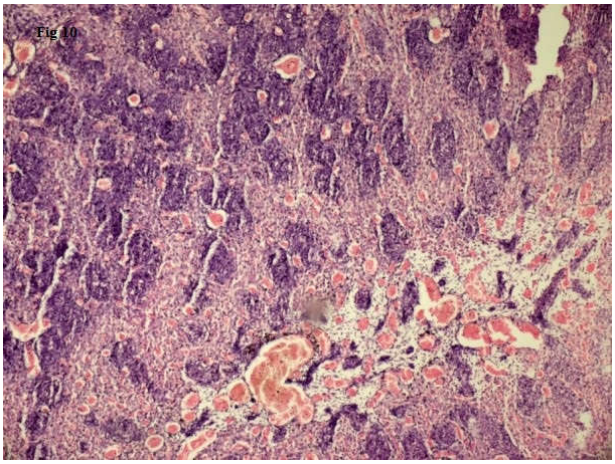

Figure 10 Interstitium showing increased vascularity at 18weeks. (H &E; X 40)

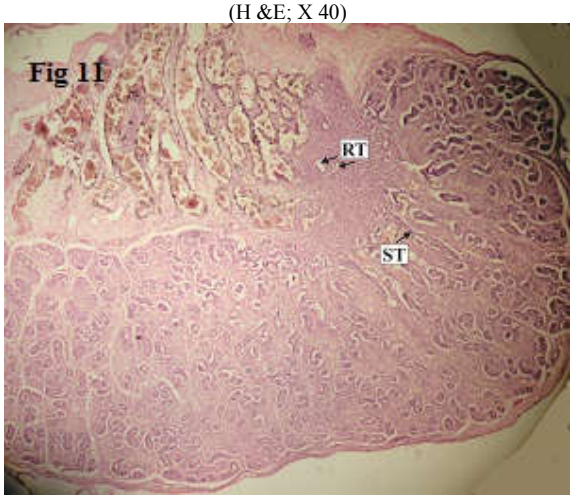

Figure 11 Mediastinum shifted posteriorly with straight tubules (ST) and rete testis (RT) at 19-20weeks. (H& E; X 40)

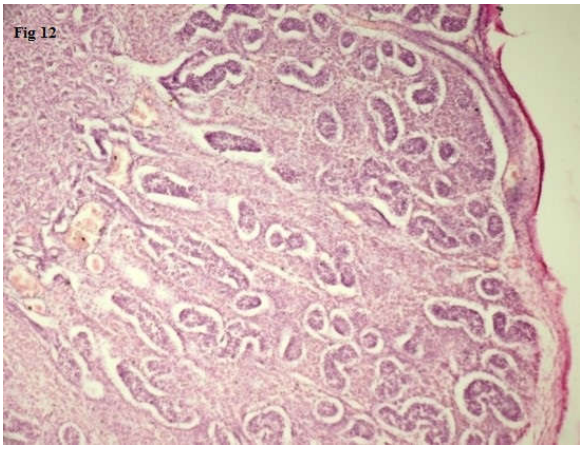

Figure 12 Connective tissue septae forming lobules and straight tubules connecting the testicular cords with mediastinum at 19-20weeks. (H &E; X 100)

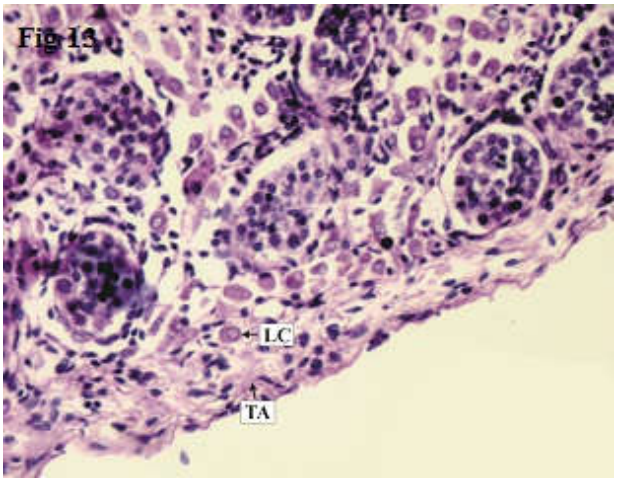

Figure 13 Leydig cells (LC) and basal lamina of testicular cord at 17weeks of gestation. (PAS; X 400)

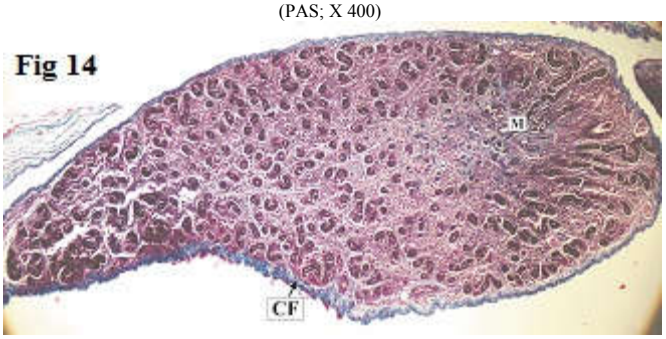

Figure 14 Well defined collagen fibres and posteriorly placed mediastinum at 17weeks. (Masson's Trichrome; X 40)

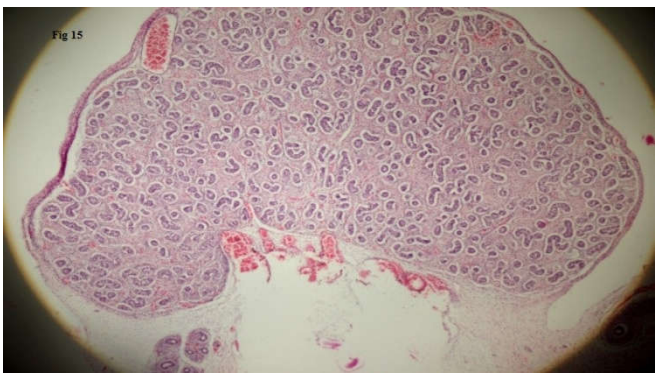

Figure 15 Numerous testicular cords and vascularity at 21weeks of gestation. (H& E; X 40)

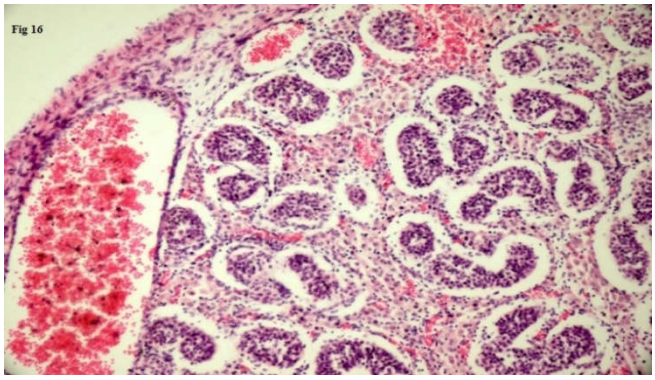

Figure 16 Tunica vasculosa and blood vessels in the interstitium at 21weeks. (H &E; X 200)

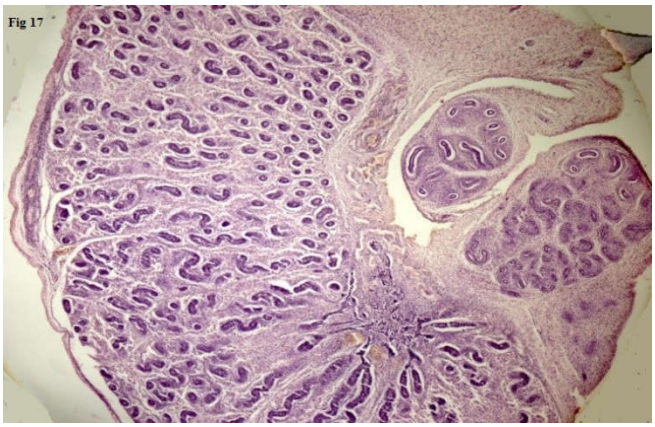

Figure 17 Prominent testicular cords with less number of Leydig cells at 23weeks of gestation. (H& E; X 40**)**

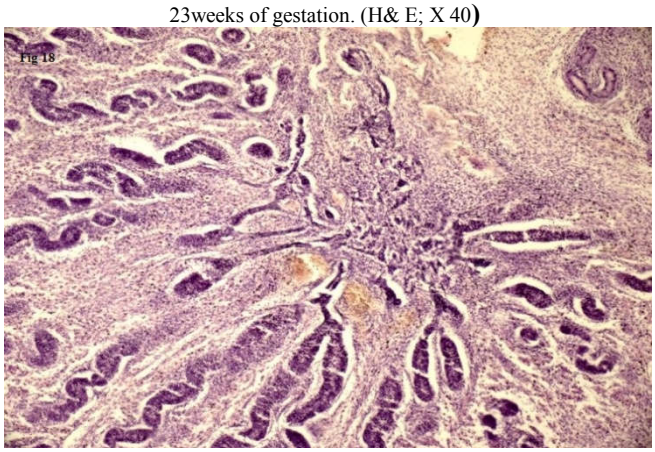

Figure 18 Testicular cords, straight tubules and rete testis at 23weeks. (H &E; X 100)

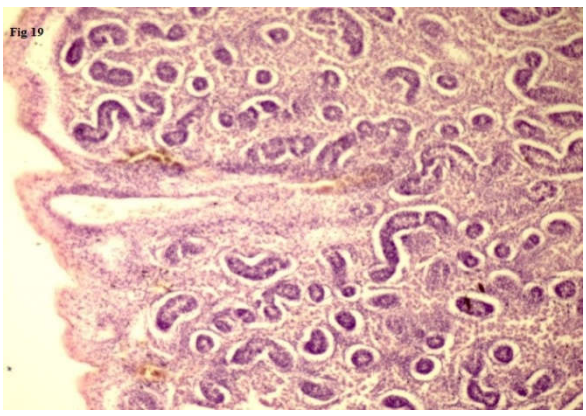

Figure 19 Connective tissue septa containing blood vessels and testicular cords at 23weeks. (H &E; X 100)

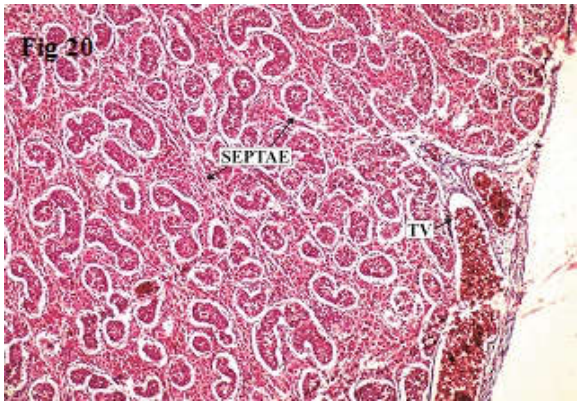

Figure 20 Tunica vasculosa (TV) and septae made up of collagen fibres at 21weeks of gestation. (Masson's trichrome; X100)

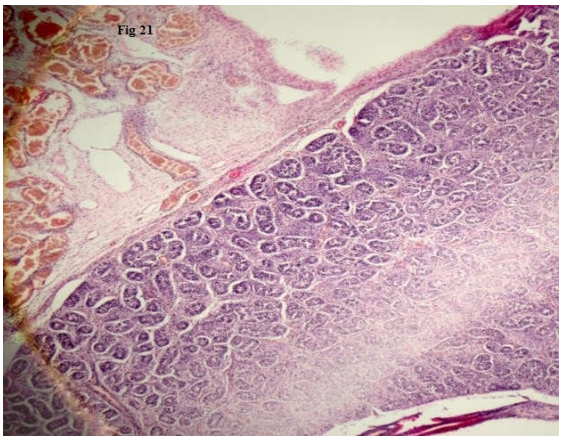

Figure 21 Numerous testicular cords and rete testis with efferent ductules at 26weeks of gestation. (H& E; X 40)

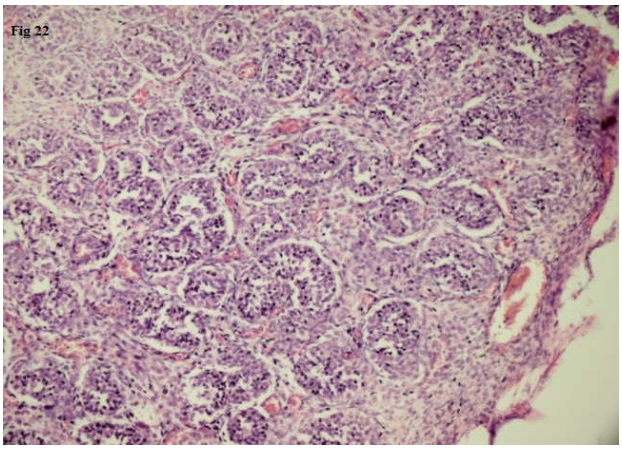

Figure 22 Testicular cords showing central degeneration of cells with small lumen and blood vessels in the interstitium. (H &E; X 400)

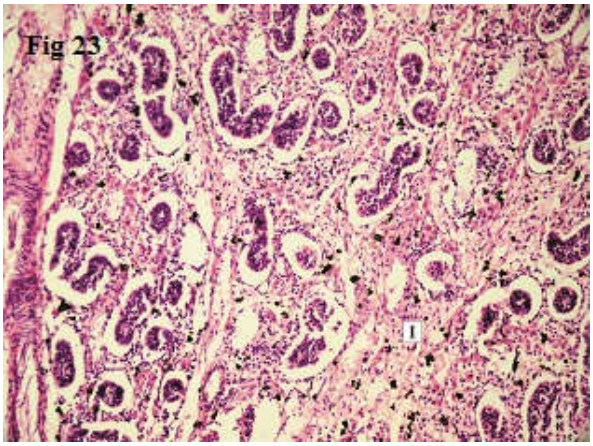

Figure 23 Involution of Leydig cells with undifferentiated fires and fibroblasts in the interstitium at 28weeks. (H& E; X 400)

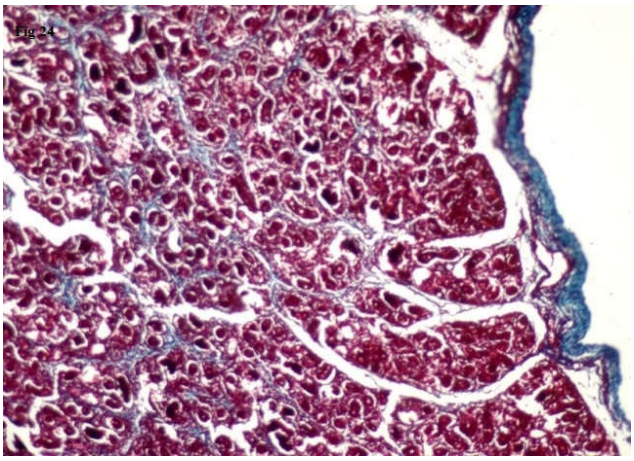

Figure 24 Bluish collagen fibres – Capsule and septae at 25weeks. (Masson's Trichome; X 100)

DISCUSSION

The histogenesis of testis has been the subject of interest for large number of researchers. The development of testicular cords, sertoli cells and Leydig cells have been studied by various authors [12,13,14,15,16,17]

In the present study, under light microscopy, capsule was identifiable and measured 17.32 micron at 12-16weeks of gestation and thickness increased to 141.74 micron at 24-28weeks. The capsule was initially thin and as the gestational age advanced it was thicker with increase in number of blood vessels. The outer capsule was composed of spindle shaped fibroblast like cells and densely packed collagen fibres. Whereas inner layer composed of loosely packed connective tissue with blood vessels. None of the authors have done micrometry on human fetal testis; therefore no study was available for comparison with the present study.

Mantraratnam *et al* [1] studied 50 normal fetuses from 10-40 weeks of gestation and observed that upto 12weeks few numbers of straight solid seminiferous cords were present, germ cells and sertoli cells could not be distinguished from each other. The primordial germ cells within the seminiferous cords were not distinct. Similar findings were also observed in present study. At 16weeks the solid seminiferous cords, rete testis and tunica vasculosa were observed. Leydig cells were more prominent at this gestation. As the age advanced loubulation of testis was much distinct with presence of tightly coiled seminiferous tubules. At24weeksLeydig cells were more numerous and sertoli cell precursors and pre spermatogonial cells were differentiated. From 34weeks onwards tunica vaginalis, tunica albuginea, tunica vasculosa, lobules of testis and seminiferous tubules were markedly prominent. The lumen of the seminiferous tubules was not clear due to highly proliferating spermatogonial cells. By the end of 40 weeks the fetal testis had not attained the cytoarchitecture of adult testis suggesting testicular differentiation and growth postnatally.

Wahengbam *et al* [5] attributed that from 13weeks onwards, testis was covered by fibrous tunica albuginea that formed the thickened mediastinum testis posteriorly and the tunica vasculosa was visible at 17weeks. The early sign of tubular organisation were visible at 13weeks. Large eosinophilic, polygonal Leydig cells were identifiable deep to tunica albuginea. In the present study it was noticed that mediastinum of testis was present centrally by the end of 16weeks. Gradually the mediastinum shifted posteriorly from 16weeks onwards. The tunica vasculosa were well visible from 20weeks of gestation. The tubular organisation was visible at 12weeks of gestation and Leydig cells were identifiable after the appearance of tunica albuginea.

Wahengbam *et al* [5] observed that from 9-12 weeks the parenchyma of testis was divided into an outer 1/4th of darker zone and inner 3/4th of lighter zone. The outer zone was invaded by radially disposed cords and inner zone had cellular clumps and loose network forming rete testis. From 17weeks of gestation incomplete septae separating the parenchyma testis into different lobules were observed. Before 20weeks the seminiferous tubules were surrounded by only 1-2 layers of peritubular tissue. At 24weeks seminiferous tubules became numerous with better appreciated pale and dark spermatogonia. The Leydig cells reduced in number as well as in size, with the reduction in volume of the interstitial tissue. Before 28weeks the spermatogonic cells were more numerous than the sertoli cells. Similar findings were observed in the present study.

Kurihara *et al* [12] observed that at 90days (13weeks) the testicular cords were identified more clearly than those at 70days (10weeks). In the testicular cords the germ cells and sertoli cells were difficult to discriminate at this period. In the present study similar findingswere observed at 12weeks of gestation. PAS positivity in testicular cords as well aspositive collagen bundles by Masson's trichrome (MT) was observed at 70days. With maturation, both PAS and MT stains of the cords had been weakened. In the present study basal lamina of testicular cords and some cells inside the testicular cords were PAS positive. As the age advanced the collagen fibres in the tunica albuginea and in the interstitium of testis were increased and fibres were well stained with Masson's trichrome.

Fukuda *et al* [13] observed that until 10weeks most of germ cells belonged to gonocyte type, were roughly spherical in shape and contained round and centrally placed nucleus with 1-2 nucleoli. The intermediate cell having round to oval shape slightly eccentric placed nucleus became predominant at around 15weeks along with gonocytes and few fetal spermatogonia. After 22weeks most of germ cells were fetal spermatogonia with almost absence of gonocytes. Upto 13weeks of gestation sertoli cells were stellate in shape and were resting on basal lamina. In older fetuses, the sertoli cells became pyramidal with oval nucleus and cytoplasm was found to be paler than earlier stage.

In the present study between 11-12weeks, few round to spherical primordial germ cells were found with vesicular nucleus and 2-3 nucleoli towards the periphery. Upto 16weeks large cells which were spherical to oblong in shape, were located close to the basement membrane of developing sex cords. Between 20-24weeks large cells decreased in number and showed degenerative changes in their nucleus. As the age advanced, the small cellsfurther increased in number. Between 12-16weeks,the spermatogonia and sertoli cells could not be distinguished with each other. With the advancing age the irregular shaped sertoli cells were differentiating containing

elongated to pyramidal shape nucleus perpendicular to basal lamina.

Codesal *et al* [14] concluded that involution of fetal Leydig cells began after the 24 weeks. From this age to birth the number of fetal Leydig cells decreased by 60%. From the third month after birth, the number of infantile Leydig cells increased whereas fetal Leydig cells decreased markedly.

Macini *et al* [15] observedtwo cycles in the development of leydig cells; one fetal and other pubertal. In fetal life three types of fetal leydig cells were observed. Upto sixth month of gestation leydig cells increased both in size and number. At this time involution began and reached a peak just before birth. Several weeks after birth degenerating leydig cells had disappeared almost completely.

The present study showed that between 16-20weeks of gestation the number and size of leydig cells was more prominent. Thereafter the leydig cells gradually regressed in size and number.

Prince [18] suggested that the peak period of Leydig cells were at 14-18weeks, referred to as the fetal Leydig cell population. During neonatal phase, 2-3month after birth Leydig cells increased in number and referred as neonatal Leydig cell population. From puberty onwards leydig cells was referred to as adult leydig cell population. Thus the morphological studies support both cell regression and cell degeneration after the fetal and neonatal period. The present study was done in fetal period only and showed the gradual regression in Leydig cells after 20weeks of gestation.

Pelliniemi *et al* [17] noted that below 8weekspredifferentiation phases was noted containing only undifferentiated mesenchymal cell. Between 8-14weeksdifferentiation phase observed in which leydig cells developed and gradually filled the space between the testicular cords. The maturity phase showed more than one half of the total area of testis filled with the mature leydig cells between 14-18weeks. In involution phase most of the leydig cells gradually degenerated and disappeared from 18-40weeks of gestation.

The present study observed that after the appearance of tunica albuginea the leydig cells started to differentiate at 12weeks of gestation. With advancing age, the number and size of Leydig cellsincreased and testicular cords widely separated and almost $2/3^{rd}$ of interstitium was filled with leydig cells at 18weeks. Upto22weeks the Leydig cells were still prominent, after that it started regressing in size and number. Between 24-28weeks, leydig cellsreduced in number and greater amount of fibres, mesenchymal cells, fibroblast cells and blood vessels were seen.

The literature available on the histological changes of human fetal testis is scanty although vast study had been done on animals. In the present work various cell populations were observed at different gestational age groups. All the cells increased in number and size with increase in gestation except the Leydig cells.

SUMMARY AND CONCLUSION

The knowledge of histology of the testis is important in case of undescended testis, cryptorchidism, hypospadias, ectopic testis, inguinal hernias, infertility, and testicular tumors.

The histological changes were observed in both the testis from 12-28weeks of gestation. The capsule was initially thin (17.32 micron), as gestational age advanced it became thicker (141.74 micron), more folded and compacted. The tunica albuginea consisted of outer densely packed collagen fibres and inner loosely arranged connective tissue with blood vessels. With increasing gestational age connective tissue septae invading the parenchyma of testis became deeper and divided the testis into complete/incomplete lobules.

Between 11-12weeks in the parenchyma of testis large number of undifferentiated mesenchymal cells, few primordial germ cells and small blood vessels were observed. At 12 weekthe parenchyma was divided into an outer $1/4^{th}$ of darker zone and inner $3/4^{th}$ of lighter zone and differentiated into testicular cords and interstitium of testis. As age advanced, the testicular cords started coiling with variable shapes i.e. C shaped, horse shoe or reverse question mark shaped and were surrounded by 2-3 layers of peritubular tissue. Initially the sertoli cells were not differentiated from spermatogonia but after 16 weeks the sertoli cells were differentiated. With the advancing fetal age the testicular cords increased and became more crowded.

The interstitium of testis was comprised of mesenchymal cells, Leydig cells, differentiating fibroblast and capillaries. Upto 20weeks, number of Leydig cells increased, thereafter the number and size of Leydig cells reduced progressively. The vascularity of the interstitium increased with advancing age.
Between 12-16weeks mediastinum of testis was placed centrally with solid rete testis with no lumen. Gradually the mediastinum of testis shifted posteriorly and became smaller. The rete testis became luminised (>16-20weeks) suggesting early degenerative changes and with the advancing of age, rete testis were interconnecting the straight tubules to the efferent ductules.

Reference

1. Mantraratnam PP, Rao BN. 2012. Prenatal histogenesis of human fetal testis. Int J App Basic Med Res.,2(2):112-16.
2. Standring S, Ellis H, Healy JC, Johnson D, Williams A. 2005. Abdomen and pelvis. In: Gray's anatomy- The anatomical basis of clinical practice. 39th ed. Philadelphia: Elesvier Churchill Livingstone,:p1305-17.
3. Dutta AK. 2010. Male Genital System. In: Essentials of Human Anatomy. 9th ed. Kolkata,:p332-78.
4. Khatwa UA, Menon PS.2000. Management of undescended testis. Indian J Pediatr., 67(6):449–54.
5. Wahengbam S., Singh Arunachandra S, Damayanti N. 2011. Development and morphogenesis of testis in humane testis. J. Anat Soc India., 60(2): 160- 67.
6. Hamilton WJ, Mossman HW. 1972. Prenatal development of form and function in the urogenital system. In: Hamilton, Boyd and Mossman's Human embryology. 4th ed. W. Heffer and sons Lt, London, p377-436.

7. Moore KL, Persaud TVN. 2003. The Urogenital system. In: The developing human. 7th ed. USA: Elesvier:p288-327.
8. Satoh M. 1991. Histogenesis and organogenesis of the gonad in human embryos. J Anat., 177:85–107.
9. Farooqui MM, Chandrapal, Archana , Prakash A. 2012. Histological and histochemical studies on the prenatal development of testis in goat (Capra hircus). Int J Marphol., 30(4):1408-21.
10. Gofur MR, khan MZI, Karim MR, Islam MN. 2008. Histomorphology and histochemistry of testis of Indigenous bull of bangladesh. Bangl J Vet Med., 6(1):67-74.
11. Mancini RE, Narbaitz R, Lavieri JC. 1960. Origin and development of the germinative epithelium and Sertoli cells in the human testis: cytological, cytochemical, and quantitative study. Anat Rec., 136:477–89.
12. Kurihara M, Qu N, Cho BH, Kitaoka M, Ogawa Y, Yi S-Q, *et al*. 2010. Histological development of human testicular cords from 70 to 90 days of gestation. Okajimas Folia AnatJpn., 87(3):103–8.
13. Fukuda T, Hedinger C, Groscurth P. 1975. Ultrastructure of developing germ cells in the fetal human testis. Cell Tissue Res., 161(1):55–70.
14. Codesal J, Regadra J, Nistal M, Sejas JR, Poniguna R. 1990. Involution of human fetal leydig cells. An Immunohisochemical, ultrastructral and quantitative study. J Anat., 172:103-14.
15. Mancini RE, Vilar O, Lavieri JC, Andrada JA, Heinrich JJ. 1963. Development of leydig cell in the normal human testis. A cytological, cytochemical and quantitative study. Am J Anat., 112:203-14.
16. Fawcett DW, Burgos MH. 1960. Studies on the fine structure of the mammalian testis. The human interstitial tissue, Am J Anat., 107;245-70.
17. Pelliniemi JL, Niemi M. 1969. Fine structure of human fetal testis. Cell and Tissue Res., 99(4):507-22.
18. Prince FP. 2001. The triphasic nature of Leydig cell development in humans, and comments on nomenclature. J Endocrinol., 168(2):213–6.

How to cite this article:

Amandeep Kaur, Kanchan Kapoor, Mahesh K Sharma and Jessy J P (2020) 'Histological Changes of Human Fetal Testis in Second Trimester', *International Journal of Current Advanced Research,* 09(02), pp. 21152-21162.
DOI: http://dx.doi.org/10.24327/ijcar.2020. 21162.4150

****