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Research Article

PRENATAL HISTOGENESIS OF HUMAN FETAL TESTIS

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ABSTRACT
One of the defining characteristic features of the living beings is their ability to continue the progeny which is emphatically the function of the testis with an additional course of endocrine function in human species. The present prenatal study of histogenesis of human foetal testis is carried out to know the occurrence of various cell populations like germ cells, leydig cells, sertoli cells and peritubular myoid cells at different gestational periods. The testicular cords with in the mesenchymal tissue between 10wk to 12wks of gestation. Differentiation of leydig cells is well appreciated by 16wks and become more prominent in 18wks.Lobulation is distinct with seminiferous tubules by the end of 24-wks of gestation. Differentiation of sertoli cell precursors and prespermatogonial cells are well marked during 28wk of gestation. Tunica albuginea and the organization of tubules into lobules are observed by 28 wks of gestation.lobules of testis and seminiferous tubules are prominent between 30 to 34 wks of gestation.

Key Words: *Leydig Cells, Seminiferous Tubules, Sertoli Cells, Testis, Tunica Albugenia*

INTRODUCTION
The Latin word "testis" means witness, was used in the firmly established legal principle "testis unus" meaning one witness, .One of the defining characteristic feature of the living beings is their ability to continue the progeny which is emphatically the function of the testis with an additional course of endocrine function in human species. The orchestrated opera of the descent of the testis and the development of seminiferous tubules, leydig cells, the differentiation of the interstitium of the testis represent the morphological and histological maneuvers during the development of testis respectively, which were studied in detail in lower species as rats by In-Shik Kim, Yang,(1999), in pig foetuses by Vorstenbosch,(1987), the studies on histogenesis of human foetal testis were relatively lesser in quantum as by Fukuda and Hedinger, (1975). In view of the direct relation of the degree of descent of the testis (undescended testis) to the development of carcinoma; and also due to the limited research pursued in the foetuses of the southern part of India the present work of histogenesis of testis of human foetuses is taken up to know the occurrence of various cell populations like germ cells, leydig cells, sertoli cells and peritubular myoid cells at different gestational periods and compare the data obtained with the findings of earlier authors.

MATERIALS AND METHODS
The present prenatal study of histogenesis of human foetal testis is carried out in the department of Anatomy, Maharajah's Institute of Medical Sciences, Nellimarla. 50 aborted, unclaimed foetuses obtained from local private and government hospitals, observing all formalities from the parents strictly, with no congenital anomalies were selected for the study. The age of the foetuses ranged from 10 weeks to 40 weeks of gestation and is judged by the crown rump length as per Mossman and Boyd method.
The study material of the human foetuses is categorized into three groups:
1st group - up to 12 weeks of gestation.
2nd group - from 13 to 24 weeks of gestation.
3rd group - from 25 to 40 weeks of gestation.
male foetuses are preserved with the injection of 10% formalin through umbilical vessels. The foetuses are dissected by a median abdominal incision and an incision extending from xiphisternum laterally along the coastal margin and and also scrotal sacs to procure the testes.
Histological study of human foetal testis:

5µ thickness of testis tissue of 10 wks,12 wks, 14wks, 16 wks, 18 wks, 20 wks, 22 wks, 24 wks,26 wks, 28 wks,30 wks, 32 weeks,34 wks, 36 wks,38 wks and 40 wks of gestation are cleared in xylene and passed through descending grades (i.e.,) absolute, 90%, 80%, 70% and 50%., water and are stained using hematoxylin and eosin and mounted using DPX.

Observations

Microscopic observations

Group I- up to 12wks:

Panaromic view of testis showing seminiferous tubules as short cords and straight solid cords, which are very few in number. Clusters of cells are observed in the center of the seminiferous cords. Germ cells and sustentacular cells could not be distinguished with each other. Interstitial tissue is more when compared to seminiferous cords at 10 wks gestation (Figure 1).During 12 wks of gestation the length of the solid seminiferous tubules increased along with peritubular interstitial tissue. The primordial germ cells with in the seminiferous cords are not yet distinct (Figure 2)

Group II -13 to 24wks:

At 16 wks.gestation, It is observed that the solid seminiferous tubules started vacuolation and are coiled, rete testis and tunica vasculosa are also observed (Figure 3). Leydig cells are more in number and are prominent at this gestation (Figure 4).

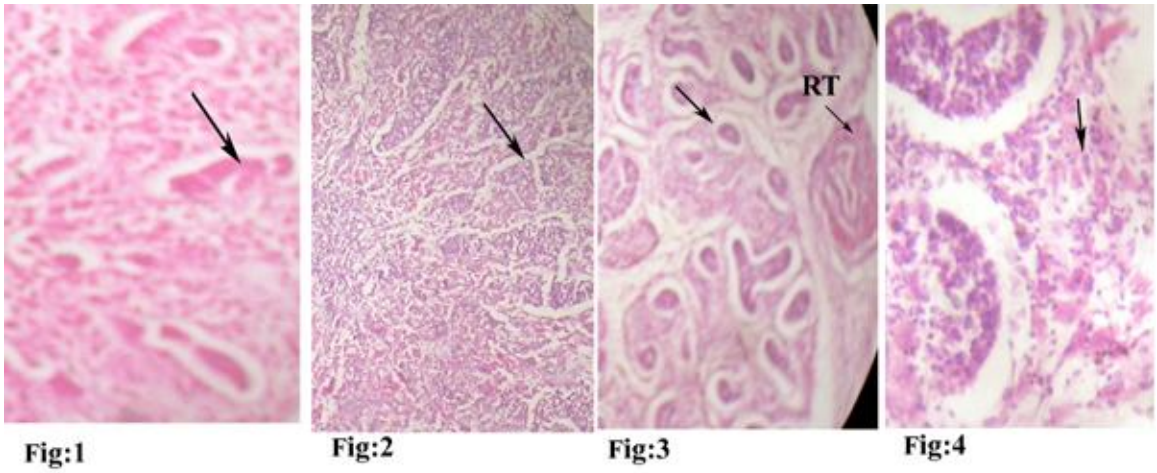

Figure 1: Showing testicular cords at 10th week of gestation, H&E x 100
Figure 2: Showing solid seminiferous tubules at 12th week of gestation, H&E x100
Figure 3: Showing rete testis (RT) and solid seminiferous tubules at 16th week of gestation,H&E x100
Figure4: Showing Leydig cells within the interstitium at 16th week of gestation, H&E x 400

Seminiferous tubules increased in number and are present between the septae forming lobules. The peritubular mesenchymal tissue appeared to be stratified by the presence of increased number of Leydig cells at 22 wks.gestation (Figure 5).At 24wks.gestation; Lobulation of the testis is very much distinct with the presence of tight-coiled seminiferous tubules. (Figure 6). Leydig cells were the most striking and numerous at this gestation. Sertoli cell precursors and prespermatogonial cells are differentiated (Figure 7). Nucleus of sertoli cells are round and pale when compare with the nuclei of prespermatogonial cells which are round and darker.

Group III -25 to 40wks.
The prespermatogenic cells are gradually increasing in number with in the lumen of seminiferous tubules. Vasculature became more prominent along with organization of the tubules with in the lobules. Tunica albugenia is well marked 28wks.gestation (Figure 8).

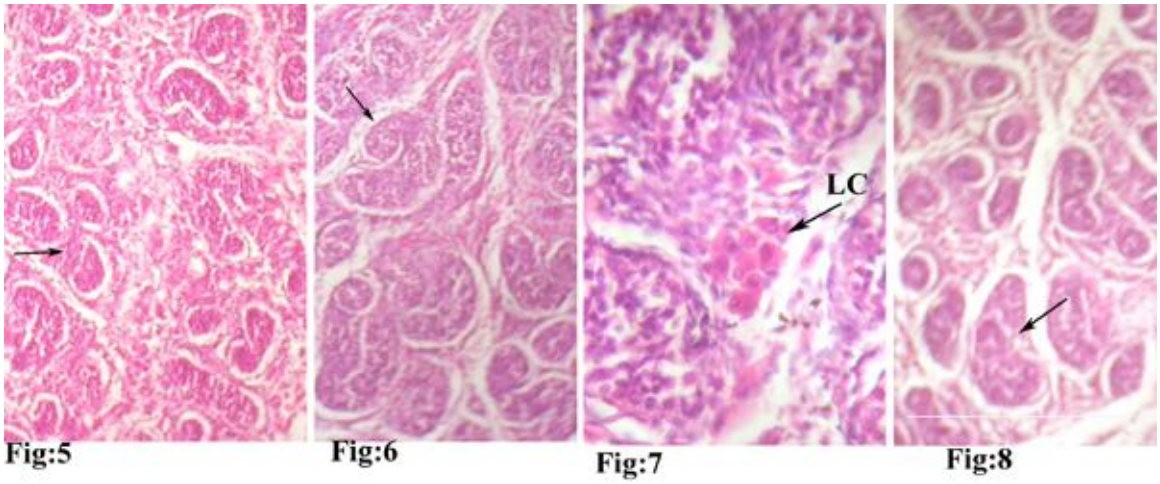

Figure 5: Showing stratification of seminiferous tubules and Leydig cells in the peritubular mesenchyme at 22nd week of gestation, H&E x100
Figure 6: Showing coiled seminiferous tubules at 24th week of gestation, H&E x 100
Figure 7: Showing differentiation of sertoli cells and prespermatogonial cells with prominent Leydig cells (LC)in the interstitium at 24th week of gestation, H&E x 400
Figure 8: Showing prespermatogonic cells at 28th week of gestation , H&E x 100

At 34wks.gestation, Tunica vaginalis, Tunica albugenia, Tunica vasculosa, lobules of the testis and seminiferous tubules are markedly prominent (Figure 9). The lumen of the seminiferous tubule is not clear as it is containing highly proliferating spermatogonial cells (Figure 10).

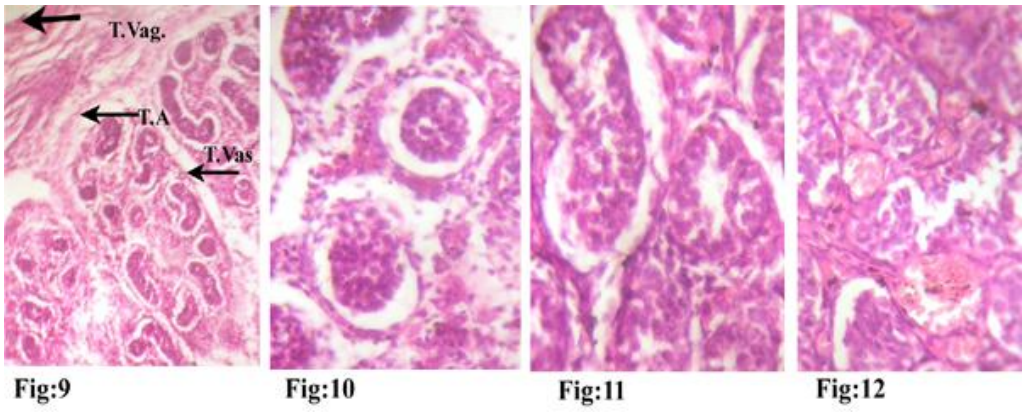

Figure 9: Showing tunica vaginalis,Tunica albugenia, Tunica Vasculosa and seminiferous tubules at 34th week of gestation , H&E x 100
Figure 10: Showing proliferating spermatogonial cells at 34th week of gestation, H&E x 400
Figure 11: Showing vacuolation of seminiferous tubules at 36th week of gestation, H&E x 100
Figure 12: Showing vasculature and tightly coiled seminiferous tubules at 40th week of gestation, H&E x 400

Leydig cells are decreased in the number by the process of involution. During 36wks.gestation, (Figure 11). The lumen is clearly seen with numerous pale and dark spermatogonial cells. The interstitial tissue has almost disappeared containing sickle shaped fibroblast cells extending all over the septa between the testicular lobules. Vascularization is well developed. The seminiferous tubules are tightly coiled having no space between the adjacent tubules. This shows that the length of the tubules are increasing and becoming regularly coiled by the end of 40 wks (Figure 12). The foetal testis had not yet attained the cytoarchitecture of adult testis suggesting testicular differentiation and growth postnatally.

DISCUSSION

One of the critical events in sexual differentiation occurs in the seventh week. Embryos, which contain the Y chromosome, have a specific gene known as SRY gene. This gene for a testes determining factor determines the path of formation of testes. SRY gene is expressed in the mesenchyme and is expressed immediately. The first step in the development of the testes is the formation of the tunica albuginea, a layer of fibrous connective tissue which separates the sex cords now known as seminiferous cords from the surface epithelium. As such, in the male SRY gene causes the medulla of the developing gonad to be very well developed. Within the tunica albuginea, the seminiferous cords are separated from one another by mesenchyme. This mesenchyme will produce Leydig cells. Leydig cells are important as they produce testosterone, which travels to receptors in the mesonephric duct. This maintains the presence of mesonephric ducts. The seminiferous cords also have an important role in male differentiation. The cords are solid (i.e. no lumen) until puberty but they are made up of a large number of Sertoli cells, a highly proliferative cell which secretes antimullerian hormone. Antimullerian hormone inhibits the paramesonephric ducts, which then degenerate around the ninth week of development. The seminiferous cords remain solid until puberty at which time a lumen develops and they become the seminiferous tubules.

Lauri J. Pelliniemi and Mikko Niemi (1969) summarized in their study on thirteen male human foetuses ranging in crown-rump length from 29 to 212 mm (ages 8–27 weeks). Four developmental phases are distinguished. 1. The predifferentiation phase (below 8 weeks): The interstitium containing only undifferentiated mesenchymal cells. 2. The differentiation phase (8–14 weeks): Leydig cells develop and gradually fill the space between the germ cords. 3. The maturity phase (14–18 weeks): The interstitium occupies more than one half of the total area in the testis sections and is filled with mature foetal Leydig cells and 4. The involution phase (18–40 weeks): Most of the Leydig cells gradually degenerate and disappear. In human, the chronology of the testicular development has been studied by Romain Lambrot et al (2006). The first morphological sign of testicular differentiation is the formation of testicular cords, which has been observed between 6 and 7 wks gestation. Steroid secreting Leydig cells can be seen in the testis at 8 wks gestation. At 14–16 wk gestation there is an increase in the number of Leydig cells. In the present study also, testis containing testicular cords and interstitium containing undifferentiated mesenchymal cells is observed. The interstitium has occupied more than the seminiferous cords upto 16 wks gestation. Leydig cells were observed at 16 weeks gestation and showed an involution by the starting of third trimester. The present observations showed approximately close findings as the above authors.

Javier Codesal et al. (1990) also have found the histological pattern of the testis under light microscope. The number of Leydig cells decreased progressively from the 24th week of gestation up to birth and remained unchanged up to the second month of postnatal life. Brenda L.Waters, Thomas D. and Trainer (1996) described that during foetal life, the tunica albuginea progressively increases in thickness between 29 and 32 weeks and seen as two layers. Beyond 25 to 28 weeks, septa are invariably present. Tubules begin as straight structures and become maximally coiled by 30 weeks. Leydig cells are most numerous between 17 and 19 weeks and decline thereafter. The results obtained showed proximity to the results of the above authors.

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