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Original Article

Developmental study on ovary of human foetuses

Ambath D. Momin[a,*], Naranbabu Singh[b], Y.I. Singh[b]

[a] Department of Anatomy, North Eastern Indira Ghandhi Regional Institute of Health and Medical Sciences, Shillong, India
[b] Department of Anatomy, Regional Institute of Medical Sciences, Manipur, India

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ABSTRACT

Introduction: The primary gonadal blastema within the genital ridge is formed by two types of somatic cells; cells segregated from the mesonephros and cells of the proliferating coelomic epithelium. The main objectives of the study are to observe the sequential changes of the germ cells and other somatic cells at different gestational ages of the foetal ovary and to look for any variations in its histogenesis.
Method: 52 numbers of human foetuses of different gestational ages were dissected and ovaries were removed. To study the cytoarchitecture, the specimens were fixed in neutral buffered formalin and were subjected to standard histological processing and routine haematoxylin and eosin staining as well as masson's trichrome stain.
Results: Maximum numbers of clusters of oogonium were found at 20th week of gestation, thereafter it gradually decreased in number till 40th week. Primordial follicles are formed as early as 14th week and it gradually increased as the age advanced. Primary follicle appeared at 24th week. Both antral and growing follicles were encountered from 38th week onwards. Interstitial cells were seen from 14th week, reaching peak at 22nd–24th week and very few of them were still seen at 40th week. Surface of the ovary is lined by simple cuboidal epithelium right from 14th week. Invaginating surface epithelial cells were also seen till 25th week.
Discussion: The detailed knowledge of the development of ovary may be helpful to the clinicians, particularly obstretrician and endocrinologist.

1. Introduction

Genital system and urinary system both developed from common mesodermal ridge along the posterior wall of the abdominal cavity, and initially, the excretory duct of both the system enter a common cavity, the cloaca. Gametes are derived from primordial germ cells that are formed in epiblast during the second week of gestation and that move to the wall of yolk sac. During the fourth week of gestation, these cells begins to migrate from the yolk sac towards the developing gonads, where they arrived by the end of fifth week of gestation.[1] The primary gonadal blastema within the genital ridge is formed by two types of somatic cells; cells segregated from the mesonephros and cells of the proliferating coelomic epithelium.[2] Upon arrival at the genital ridge, the PGCs, now called oogonia, intermingle with the somatic cells that will support their subsequent development. These pregranulosa cells arise from epithelial cells that invaginate into the genital ridge.[3] The primordial germ cells pass to the female gonad in the late somite stages. At 5 weeks of ovulational age the gonadal ridge was recognized as a small bulged on the dorsal coelomic wall, lateral to the aorta and medial to the mesonephric duct.[4] Up to the seventh week the ambisexual gonad possesses no sexually differentiating features.[5]

Fingerlike epithelial cord- the gonadal cord soon grows into the underlying mesenchyme. The indifferent gonad now consists of an external cortex and an internal medulla. In embryos with an XX sex chromosome complex, the cortex differentiates further and the medulla regresses. Gonadal cords do not become prominent, but they extend into the medulla and form a rudimentary rete ovarii. This structure and the gonadal cords normally degenerate. Secondary cortical cords extend from the surface epithelium of the developing ovary into the underlying mesenchyme during the early fetal period. As the cortical cords increases in size, primordial germ cells are incorporated in them.[6] These cells undergo mitotic division, by the end of the 3rd month, they are arrange in cluster surrounded by a layer of flat epithelial cells.[1] Maximum mitotic activity in the oogonia occur on 11th–16th weeks of gestation.[7] By 5th month, the total number of germ cells in the ovary reaches

* Corresponding author at: C/O: shri. W.G. Momin, Dr. Ambath D. Momin, Kench's Trace Opposite Police Beat House, Shillong East Khasi Hills, Meghalaya, India.
E-mail address: drambath2000@gmail.com (A.D. Momin).

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maximum, estimated at 7th million. Then apoptosis of the cells begins and many oogonia and primary oocytes degenerate and become atretic. By 7thmonth majority of the oogonia have become degenerated except for a few near the surface. All the surviving primary oocytes have entered prophase of meiosis I, and most of them are individually surrounded by a layer of flat follicular epithelial cells and becomes primordial follicle.[1] 1,000,000 primary oocytes remain at birth, 40,000 by puberty, and only 400 are ovulated during reproductive life.[5] Absence of SRY established the development of female.[1] WT1 can activate the Dax-1 promoter and *Dax*-1is required for ovary development.[3]

2. Materials and methods

The materials studied consisted of 52 numbers of normal human female fetuses of different gestational ages starting from 14th week to 40 week, products of terminated pregnancy under MTP Act of India, 1971 and stillbirths. These fetuses were collected from Obstetrics and Gynecology, RIMS Hospital, Imphal with permission of concerned authorities & parents, and the study was consented by ethical committee. Only those fetuses which were free from any gross anatomical abnormality were selected for the present study. The age of the fetuses was calculated from the obstetrical history, crown rump length (CRL) and gross features. The fetuses were preserved in 10% formalin for 10 days and then dissected. To study the cytoarchitecture, the specimens were fixed in neutral buffered formalin and were subjected to standard histological processing and routine haematoxylin and eosin (H&E) staining as well as Masson Trichrome stains. Slides were examined under trinocular research microscope at different magnification and photographs were taken. The finding of the present study was compared and discussed with the findings of the available literatures.

3. Results

The collected specimens were categorized in five groups: Group I: 14th–20th week, Group II: 21st–25th week, Group III: 26th–30th week, Group IV: 31st–35th week, and group V: 36th–40th week.

3.1. Group I: 14th–20th week

At 14th week the ovary shows outer cortex and inner medulla. Surface of the cortex is lined by single layer of cuboidal cells (Fig. 1). Cortex shows clusters of different size of oogonia. These clusters of oogonia are surrounded by a fibroblast like cells called stromal cells. Oogonia are identified by its comparatively larger size, vesicular nucleus and foamy cytoplasm. Stromal cells are spindle in shape with an oval nucleus and arrange in cords. Scattered numbers of cells with rounded nuclei and lightly stained cytoplasm due to present of fat droplets (interstitial cells) are also observed among the stromal cells (Fig. 1). Invagination of surface epithelial cells into the interior of developing ovarian cortex also seen. Many degenerating oogonia are also observed in the cortex; they are identified by degenerative changes in their nucleus i.e. pyknotic, karyolysis, and karyorrhexis. Some primary oocytes are individually surrounded by very thin flattened epithelial cells, thus forming the primordial follicles. Some of the oocytes of primordial follicles shows degenerative changes as indicated by fragmentation of nuclei. Some dividing oogonia are also encountered at this week which is identified by two separate nuclei; however cytoplasm surrounding each nucleus is in continuity with each other. Cortex also shows scattered number of fine capillaries penetrating from the blood vessels present in the medulla in which some red blood cells can be detected. Deep to cortex there is a fibrovascular layer of connective tissue called medulla. There is no distinct boundary between cortex and medulla. It shows numerous thin walled blood vessels around which there is abundant collection of fibrous tissues (Fig. 1). Medulla is continuous with mesovarium. Collagen fibers can be demonstrated by Masson's Trichrome stained as early as 14th week. At 16th week clusters of oogonium are increased as compared to the previous week. The numbers of interstitial cells surrounding the clusters are comparatively more as compared to previous week. At 18th week many well formed primordial follicles are seen at corticomedullary junction. Numerous thin walled blood vessels and plenty of elongated cells are visible in the medulla (Fig. 2). At 20th week maximum clusters of oogonium were detected.

3.2. Group II: 21st–25th week

Number of clusters of oogonium is comparatively less as compare to the previous groups whereas the number of primordial follicle is increased marginally. At 24th week flattened follicular cells of some follicle started to grow and become cuboidal cells; hence forming primary follicle. Plenty of interstitial cells are still present among the stromal cells. Invaginating cells from surface epithelial cells are also still observed in some areas. Cortex and medulla is well demarcated in this group (Fig. 2).

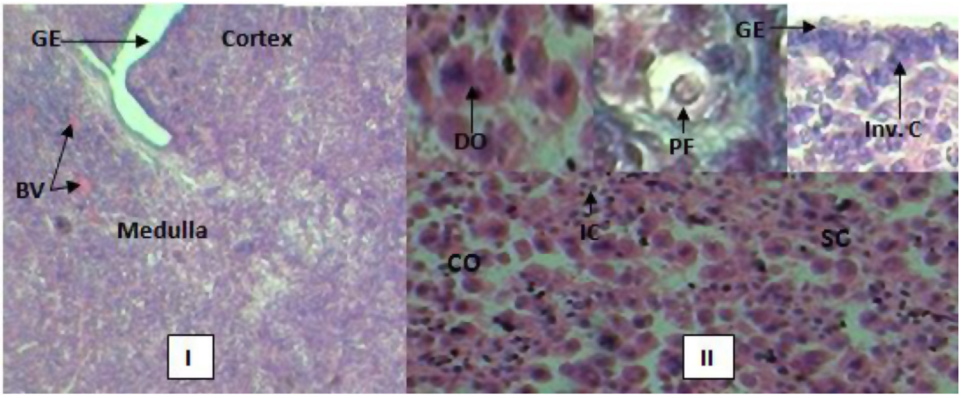

Fig. 1. Ovary of 14th week old foetus stained with haematoxyllin & eosin (H&E) stain. I = Low magnification showing outer cortex(C), inner medulla (M), germinal epithelium (GE) & blood vessels (BV). II = Magnified view showing dividing oogonium(DO), developing primordial follicle (PF), Invaginating cells (Inv. C) from surface epithelium, clusters of oogonium (CO), Stromal cells (SC) and interstitial cells (IC).

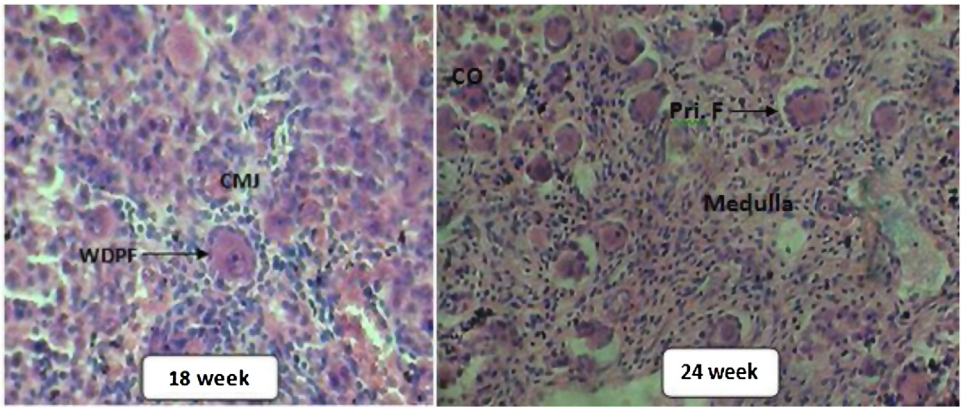

Fig. 2. Showing well developed primordial follicle (WDPF) in the ovary of 18th week old foetus at corticomedullary junction (CMJ). Another photomicrograph showing ovary of 24th week old foetus with primary follicle (Pri.F) at corticomedullary junction. Plenty of clusters of oogonium (CO) seen in the cortical area. (H&E Stain).

3.3. Group III: 26th–30th week

Clusters of oogonium are still seen but their number has decreased as compared to previous age group. Oogonia of different sizes and many degenerating oogonia and oocytes along with small blood vessels are also observed in the cortex. Plenty of primordial follicles have appeared in the corticomedullary junction. Large amount of collagen fibers is observed extending from the medulla up to the deeper part of the cortex. At 30th week just below the surface epithelium; developing tunica albugenia is detected (Fig. 3) .

3.4. Group IV: 31st–5th week

There is no change in lining epithelium. Many primordial follicles and some primary follicles are observed in the cortex. Medulla composed of numerous thin wall blood vessels and plenty of elongated fibroblast like cells. Plenty of collagen fibers are observed both in cortex and medulla.

3.5. Group V: 36th–40th week

At 36th week well developed tunica albugenia is seen. At 38th week some multilayered growing follicles are also encountered adjacent to medulla near the mesovarium. These types of multilayered follicles are surrounded by a thickened layered of stromal cells; thus forming a theca folliculi. Some of these types of follicles are in the stage of degeneration. In some follicle, all the parts of the antral follicle i.e. membrana granulosa with cumulus oophorus, zona pellucida, theca interna and externa were observed. In the membrana granulosa comparatively large cells with foamy cytoplasm surrounded by smaller cells are also observed. Developing zona pellucida is well seen in multilayered growing follicle (Fig. 4). At 40th week very few clusters of oogonium are still observed in the cortex and at corticomedullary junction. Some primordial follicles are seen among the clusters of oogonium near the surface. Cortical area shows numerous growing follicles of different size and shape. Scattered numbers of interstitial cells are still observed. Some oocytes with double nuclei are also encountered (Fig. 5). The commonest type of follicle is the primordial. Other types of follicle encountered are the primary, growing and antral follicles. Growing follicles are observed in the inner portion of the cortex near the mesovarium.

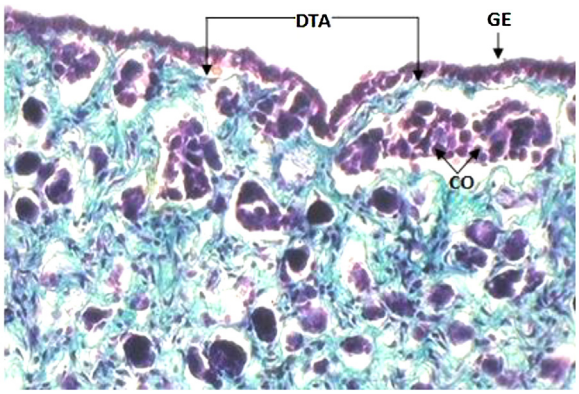

Fig 3. Masson's trichrome stain of ovary at 30th week old foetus showing developing tunica albugenia (DTA) just below the Germinal epithelium (GE). Deep to tunica albugenia few clusters of oogonium (CO) can also be seen.

4. Discussion

The oogonia enter the first meiotic division by the beginning of the third month but stop at the diplotene stage. These cells are the primary oocytes and they become surrounded by the flattened cells called follicular cells.[8] Oogonia initiate the first meiotic division by 3rd and 5th months of fetal life, thereby becoming primary oocytes. However the primary oocytes then quickly enter a state of meiotic arrest that persists until after puberty. By 5th month, the number of primordial follicles in the ovaries peaks at about 7 million.[9] According to Sadler TW,[1] primordial germ cells differentiated into oogonia and undergo a number of mitotic divisions by the end of the third month. However Moore KL, Persaud TVN,[6] state that at approximately 16th weeks the cortical cords breaks up into isolated cell clusters and primordial follicles are also appeared in this week. In our study we have witness the presence of primordial follicle as early as 14 week which is the youngest foetus of the present study.

According to Byskov A.G et al,[10] at 32nd week post conceptional ovaries, many oogonia of the medulla and inner part of the cortex have entered meiosis, but a thin cortical rim of oogonia are still present. By the time of birth, only a few oogonia are still present. In the present study we encountered very few clusters of oogonium in the cortex even at 40th week thereby confirms its presence at birth as stated by Byskov A.G et al,[10] (Fig. 5). Maximum mitotic activity in the oogonium occurs on the 11th–16th weeks of gestation,[6] the germ cells in the ovaries of the foetuses from 150 to 160 mm (Crown rump length) have more active stages of mitosis and meiosis than do the ovaries of younger or older foetuses,[11] We have encountered large number of clusters of oogonium at 16 week to 20 week along with some dividing oogonia which indicates that

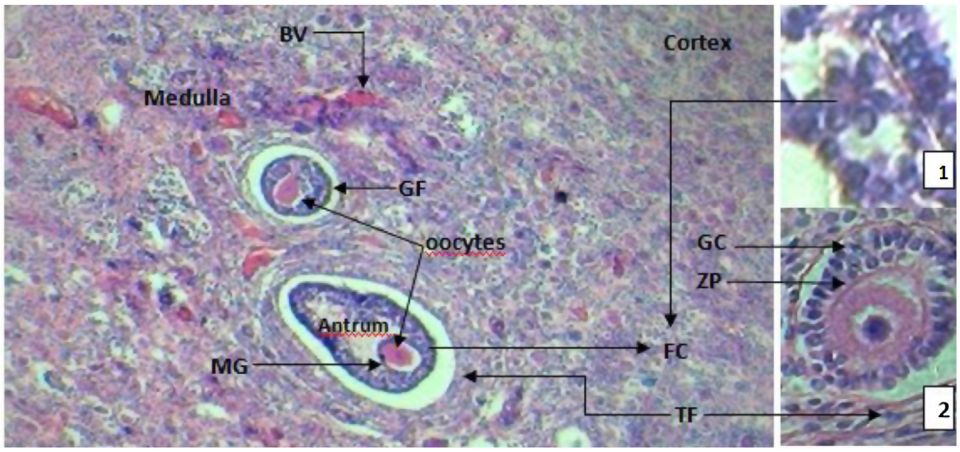

Fig. 4. Cytoarchitecture of 38th week foetus stained with H&E showing growing follicle (GF) and antral follicle (AF) at corticomedullary junction (CMJ). Very prominent blood vessels (BV) are also seen in medulla. Fully formed antrum and membrana granulose (MG) cells surrounding the oocytes are also observed. Theca folliculi (FC) surrounds the antral and growing follicle (GF). 1 = Magnified view of foamy cell (FC) surrounded by other smaller cells in membrana granulose. 2 = Magnified view of growing follicle (GF) showing developing zona pellucid (ZP) & multilaminar granulose cells (GC).

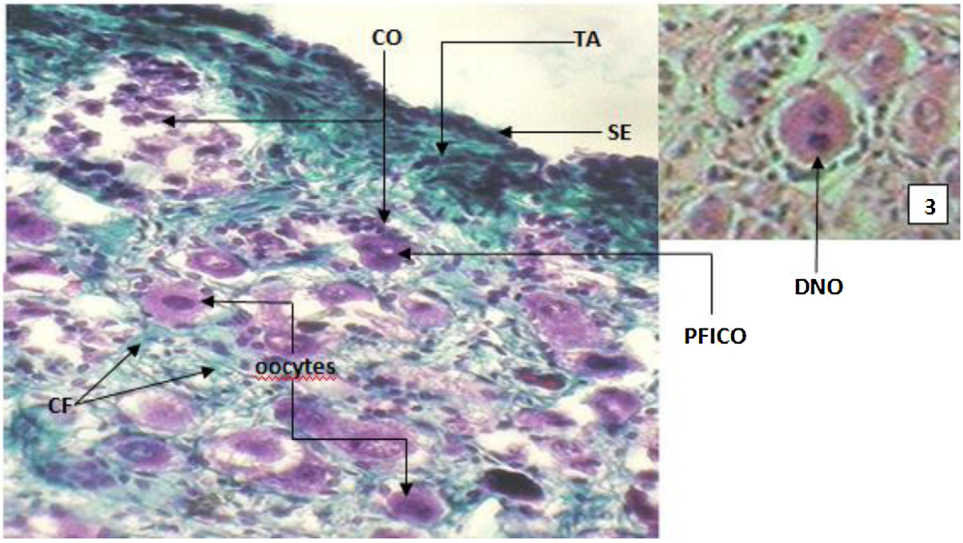

Fig. 5. Masson's trichrome stain at 40th week showing plenty collagen fibers (CF), well formed tunica albugenia (TA), and clusters of oogonium(CO) near the surface epithelium (SE). Some primordial follicles are also seen among the cluster of oogonium(PFICO). 3 = Magnified view of double nuclei of oocytes (DNO) stained with H&E.

maximum mitotic activity is going on at these week. Dividing oogonia are detected right from 14th week up to 40th week, however their numbers are very less after 30th week.

70% of the germ cells are lose by apoptosis. TGF-beta1 and −beta2 and Fas ligand have been shown to induce the death of oogonia, and may do so by modulation of the activity of the Bcl-2 family of mitochondrial genes.[3] Germ cells are lost in the greatest numbers immediately prior to and during the process of primordial follicle formation, which occurs during embryonic and early postnatal development.[12] 7,000,000 primary oocytes estimated to be present at the fifth month of gestation, 1,000,000 remain at birth, 40,000 by puberty, and only 400 are ovulated during reproductive life.[5] As the number of primary oocytes decreases from 7,000,000 at fifth month to 1,000,000 at birth, the degeneration process is going on in all the stage of fetal ovarian development. In the present study degenerating oogonia and oocytes are seen from14th week, but their maximum numbers are seen during 21st–25th week and then gradually decreased till 40th week.

Moore SL et al,[13] reported that antral follicles were present in 60% of the newborn ovaries. Whereas Grbesa D et al,[14] stated that at 22nd–28th week, middle part of the cortex contain both primordial and primary follicles and inner part of cortex shows both unilaminar and multilamilar primary follicles and two antral follicle surrounded by theca folliculi. At 37th week numerous primary follicle, few preantral and 9 antral follicles were seen in the corticomedullary junction. Sforza C et al,[15] also reported both secondary and antral follicles in newborn ovary. In the present study we encountered primary follicle from 24th week onwards and at 38th week we also observed antral follicle at cortico-medullary junction.

Kurilo LF,[7] stated that connective tissue elements of the tunica albugenia appear on 27th week and by the 40th week the ovarian tunica albugenia is seen distinctly whereas according to Arey LB,[16] tunica albugenia appeared at 7 month of intrauterine life. In present study we observed a thin layer of connective tissue in the form of tunica albugenia at 30 week and it can be seen distinctly from 36th week onwards. Hence the finding of the present study is

more or less similar with the findings of the above mention authors.

Moore KL, Parsaud TVN,[6] stated that secondary cortical cords extend from the surface epithelium of the developing ovary into the underlying mesenchyme during the early fetal period. In our study we also encountered distinct invaginating surface epithelial cells as early as from 14th week and they are seen up to 25th week. These cells are continued with the cells surrounding the clusters of oogonium. According to Papadaki L and Beilby JOW,[17] the entire surface of the ovary of 24th week foetus was covered by single layer of cuboidal epithelium. Auersperg N et al,[18] mentioned that at about 10th weeks of development and continuing to 5th month of human gestational, ovarian surface epithelium changes from flat to cuboidal simple epithelium. In the present study we saw that the entire surface of the ovary is lined by simple cuboidal epithelium as early as from 14th week till term foetuses.

The connective tissue penetrating into the inner half of the cortex, has large, round or ellipsoid cells with round nuclei and abundant cytoplasm called interstitial cells were identified at 15th weeks, and they are most abundant at 18 weeks of gestation. They are still seen in 21st week and their numbers gradually decreases thereafter and they are occasionally seen even at 31 weeks but rarely at 40th week.[19] Whereas, O' Rahilly R, Muller F[20] stated that steroid producing interstitial cells can be detected in human ovary during the first half of the second trimester. In the present study interstitial cell is observed from 14th week, its number is found to be increased gradually with maximum number at 22nd–24th week. Thereafter, its number is gradually decreased significantly from 26th week but still very few of them are still seen even at 40th week. The finding of the present study is inconformity with finding of Konishi I et al.[19]

5. Conclusion

In the present study we have found that the maximum numbers of oogonia are found at 20th week, thereafter it gradually decreased but still few are seen even at term foetuses. Primordial follicles are formed from as early as 14th week and their number gradually increased as the age advanced. Primary follicles are observed from 24th week onwards whereas antral follicle at 38th week at corticomedullary junction. Interstitial cells are observed from 14th week with maximum number reaching at 22nd–24th week, then their number gradually decreased but very few are still seen even at 40 week foetuses. The detailed knowledge of the development of ovary may be helpful to the clinician, particularly obstetricians and endocrinologist.

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