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Histogenesis and organogenesis of the gonad in human embryos*

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INTRODUCTION

Recently, renewed attention has been focused upon the origin of the epithelial cells which constitute the seminiferous tubules and follicular cells, gonadal development, and interspecific differences.

Although the morphogenesis of the embryonic and fetal gonad has been investigated in various mammalian species, the subject remains controversial. With respect to the origin of the epithelial component in the gonad, Witschi (1951) claimed that the major constituent cells of the testis and ovary are of mesenchymal and coelomic epithelial origin, respectively. On the other hand, many investigators have believed that the constituent cells of the testis and ovary have a common origin, which various authors have cited as being the coelomic epithelium (Allen, 1904; Whitehead, 1904; Torry, 1945; Yoshinaga, Hess, Hendrickx & Zamboni, 1988), the mesenchymal cells (Fishel, 1930; Jirasek, 1971; Dang & Fouquet, 1979; Fouquet & Dang, 1980), both the coelomic epithelial and the mesenchymal cells (Gruenwald, 1942; Pinkerton, McKay, Adams & Hertig, 1961; Pelliniemi, 1976), the mesonephros (Byskov & Lintern-Moore, 1973; Upadhyay, Luciani & Zamboni, 1979; Zamboni, Bezard & Mauleon, 1979; Satoh, 1985), or both the coelomic epithelium and mesonephros (Wartenberg, 1982). Furthermore, Yoshinaga *et al.* (1988) insisted upon the existence of interspecific differences, emphasizing that in primates the somatic cells of the gonadal blastema derive from the coelomic mesothelium, in contrast to other mammals, such as ruminants and rodents, where they originate from the mesonephros.

Elucidation of gonadal morphogenesis requires precise observation of the entire gonad, preferably within a single viewing field, and this can only be accomplished by preparation of complete serial semithin sections of the entire gonad using a high-resolution resin embedding method. This must be supplemented by electron microscopy when still greater resolution is required, and these observations must be continued throughout the developmental period under investigation. Electron microscopy alone does not permit overall serial observation of the entire gonad, while the resolution of the conventional paraffin-embedding method of light microscopy is inadequate.

One possible reason for the above-mentioned controversy surrounding gonadal morphogenesis is the fact that many investigations into this phenomenon have failed to satisfy these stringent observational conditions. The present investigation was conducted with the purpose of elucidating the formation of human gonads, the origins

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Table 1. *Ovulational ages of embryos and fetuses*

No.	Estimated ovulational age	Methods for estimation
1	5w4d*	MC+LMP, USG (GS)
2	5w6d*	MC+LMP, USG (GS)
3	6w0d*	MC+LMP, USG (GS)
4	6w1d*	MC+LMP, USG (GS), CRL
5	6w2d*	MC+LMP, USG (GS), BBT
6	6w2d*	MC+LMP, USG (GS), CRL
7	6w3d*	MC+LMP, USG (GS)
8	6w3d	MC+LMP, USG (GS)
9	6w4d	MC+LMP, USG (GS)
10	6w4d*	MC+LMP, USG (GS), CRL
11	7w0d*	MC+LMP, USG (GS)
12	7w4d*	MC+LMP, USG (GS), CRL, BBT
13	10w1d	MC+LMP, USG (CRL), CRL
14	11w1d	MC+LMP, USG (CRL)
15	13w2d	MC+LMP, USG (CRL), CRL
16	13w5d	MC+LMP, USG (CRL), BBT
17	18w2d	MC+LMP, USG (BPD), CRL
18	18w4d	MC+LMP, USG (BPD), CRL

* Serial sections of entire gonad; MC+LMP, menstrual cycle and last menstrual period; USG, ultrasonography; (GS), gestational sac; (CRL), crown–rump length; (BPD), biparietal diameter; CRL, direct measurement of crown–rump length; BBT, basal body temperature; 0w0d, estimated ovulational day.

of the cells involved, and the interspecific differences between humans and rats with respect to gonadal development. This study was performed by histological examination of serial sections of entire gonads and supplementary ultrastructural observations in human embryos, using techniques which satisfy the methodological conditions stated above.

MATERIALS AND METHODS

Gonads of human embryos and fetuses in normal pregnancies were used in the present investigation. Ovulational age was estimated from the menstrual cycle and the last menstrual period, the basal body temperature, or the crown–rump length of embryos and fetuses measured either by ultrasonic tomography or directly. A total of 18 gonads of ovulational age 5 to 18 weeks were examined (see Table 1). Each gonad was excised and fixed by immersion in a 2·5% glutaraldehyde–4% paraformaldehyde fixative in 0·1 M cacodylate buffer (pH 7·4). This was followed by washing with the same buffer, dehydration, and postfixation with 1% osmium tetroxide for 1 h. After dehydration with an ethanol series, the specimens were embedded in Epon 812. Almost complete serial semithin sections were prepared from each specimen, including almost complete longitudinal semithin sections of the entire body of each gonad of 10 embryos (Table 1). The 87000 sections so prepared were stained with toluidine blue and examined under a light microscope. Ultrathin sections corresponding to various selected light microscopic sections were also prepared, stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined with a JEOL-100CX electron microscope. The embryos and fetuses from which the specimens originated were obtained from cases of therapeutic abortion, in conformity with the guidelines of the Japan Obstetric and Gynecological Society (*Journal of Obstetrics and Gynecology of Japan*, **41**, 23, 1989).

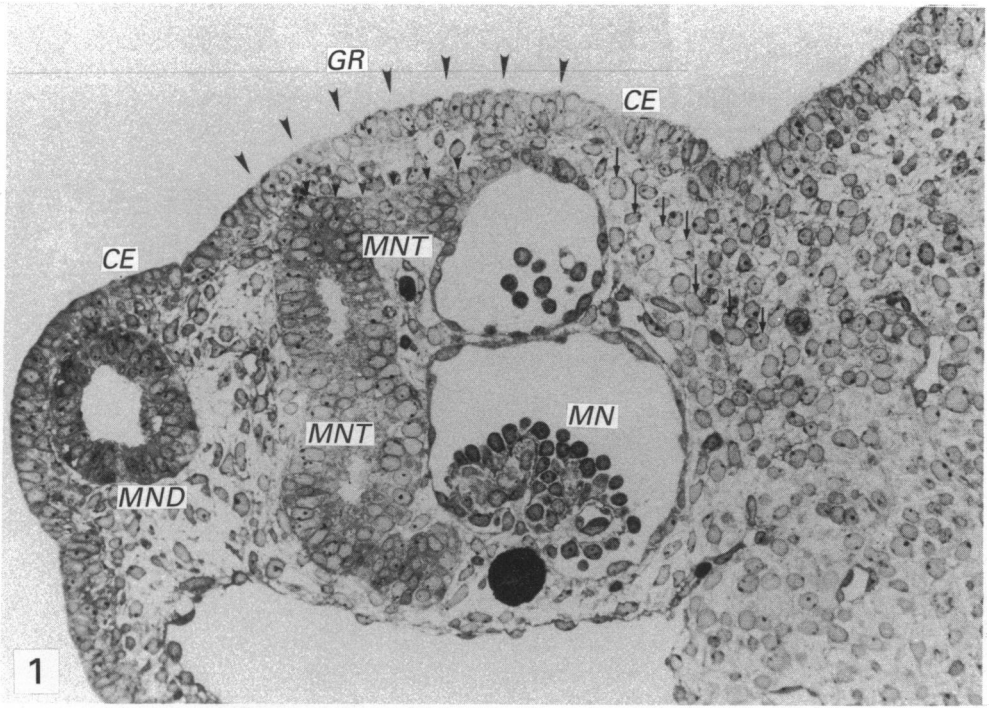


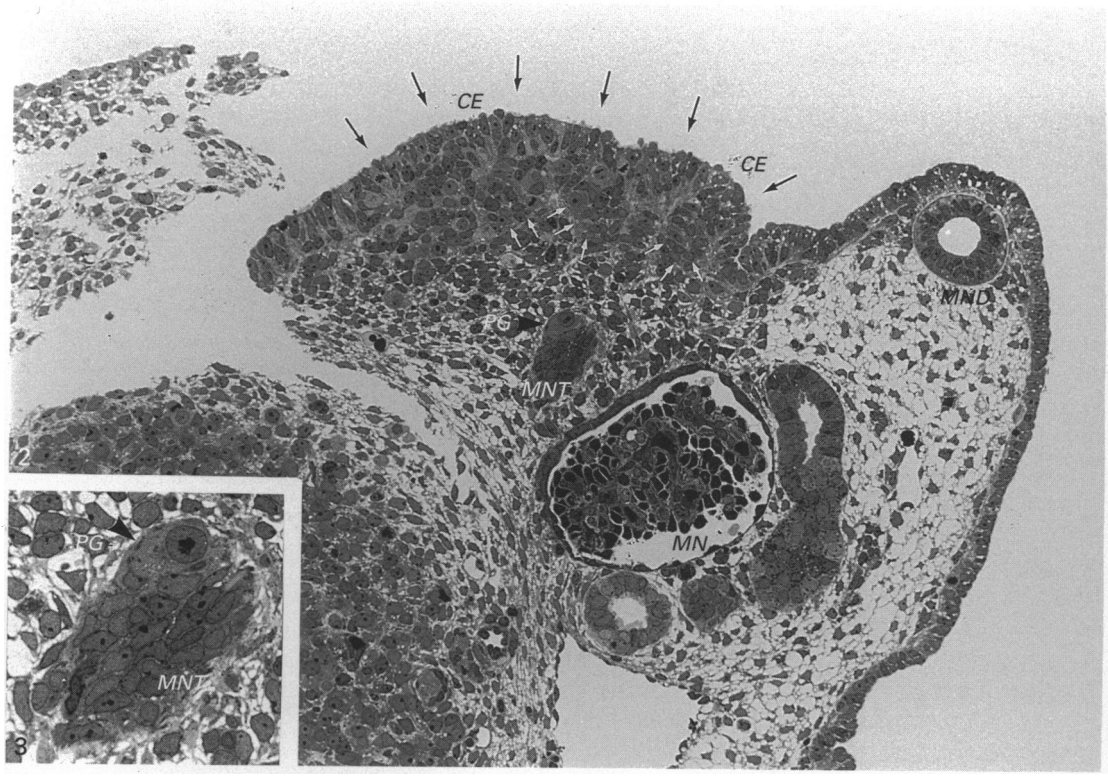
Fig. 1. Human gonad at 5 weeks of ovulational age. The gonadal ridge (large arrowheads) is recognized as a small bulge. The mesonephric tubule (small arrowheads) lies below the coelomic epithelium. Cells (arrows) in the interstitium exhibit characteristics of mesenchymal, not epithelial, cells. ×460.

Abbreviations for this and subsequent figures: *BL*, basal lamina; *CE*, coelomic epithelium; *DE*, desmosome or desmosome-like structure; *FSC*, folliculogenous sex cord; *GR*, gonadal ridge; *MD*, medulla; *MNT*, mesonephric tubule; *MN*, mesonephric glomerulus; *MND*, mesonephric duct; *OO*, oogonia' *PG*, primordial germ cell; *PSC*, primordial sex cord; *SFC*, seminiferous sex cord.

RESULTS

Gonads at 5 weeks of ovulational age

The gonadal ridge was recognized as a small bulge on the dorsal coelomic wall, lateral to the aorta and medial to the mesonephric duct (Fig. 1). During the latter part of the fifth week of ovulational age, concomitant with the migration of primordial germ cells into the gonadal ridge, the coelomic epithelium proliferated and became thickly stratified, forming a moderate gonadal protrusion (Fig. 2). The stratified coelomic epithelium developed into short pillars, thus forming cord-like structures, the so-called 'primary sex cords'. The mesonephric tubules and glomeruli were situated directly below the coelomic epithelium. None of the coelomic epithelial cells were contiguous with the mesonephros in serial sections of the whole gonad (Figs 1, 2). Some of the primordial germ cells which had migrated into the gonadal ridge were situated in contact with the mesonephric tubules (Fig. 3). No basal lamina was observed at the sites of contact between the primordial germ cells and mesonephric cells.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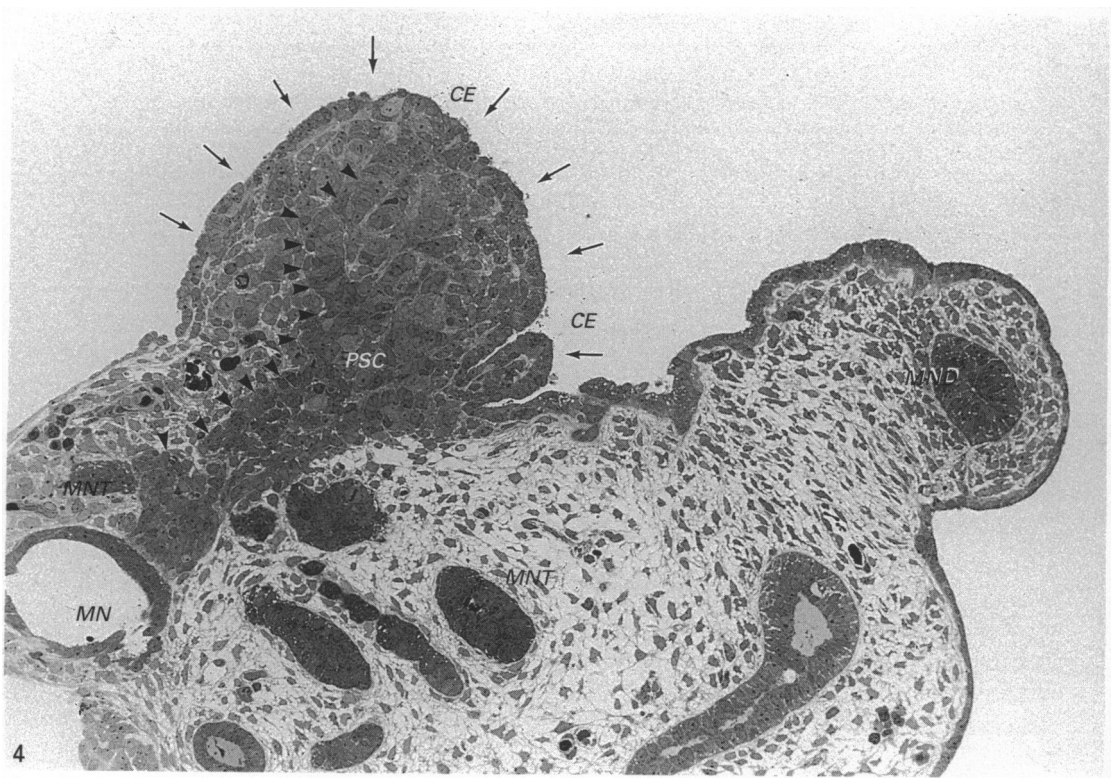
CE
CE
PSC
MND
MNT
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MN
4

Gonads early in the sixth week of ovulational age

Concomitant with the more prominent protrusion of the gonad into the coelomic cavity (Fig. 4), cells which had emerged from the mesonephros located near the superior portion of the gonad and proliferated, formed cord-like structures which will be referred to as 'primordial sex cords' in the present paper. These primordial sex cords were branched from the dorsal (basal) towards the ventral (peripheral) direction and were incorporated into the gonad (Figs 4, 5). In serial sections of entire gonads, each primordial sex cord was contiguous with the mesonephros (Fig. 6), but they displayed no contiguity with the coelomic epithelium, although they did contact or come into close proximity with the coelomic epithelium in some places. At this stage, the stratified and cord-like structures of the coelomic epithelium were stretched and flattened into one to several layers (Figs 4, 5). The coelomic epithelium showed no ramification at this stage, that is, the primary sex cords had not participated in the formation of the primordial sex cords. The cells of the primordial sex cords were stained less intensely by toluidine blue than were the mesonephric cells. Migration of the primordial germ cells into the primordial sex cords was also observed. Basal lamina was observed around the primordial sex cords, clearly in the dorsal (basal) area (Fig. 7), but gradually becoming less distinct with greater proximity to the coelomic epithelium (Figs 8, 9). Vesicles containing basal lamina-like material were seen in the cells of the primordial sex cords. Desmosome or desmosome-like structures (focal cell junctions) were also noted between the cells of the primordial sex cords in various locations, including the areas in which the basal lamina of the cords was disrupted (Figs 7, 8, 9). The cells of the primordial sex cords were epithelial, not mesenchymal.

Gonads (testes) in the middle part of the sixth week of ovulational age

Primordial sex cords had proliferated and branched towards the side of the coelomic epithelium (Fig. 10). These cords were surrounded by a prominent and well-developed basal lamina (Fig. 11). Seminiferous sex cords had been formed by these primordial sex cords. The transverse section of the testis appeared as a circle, and the coelomic epithelium was flattened into one to three layers. Although primordial germ cells were sporadically distributed directly below the coelomic epithelium, no marked proliferation was noted in these sites (Fig. 10). The seminiferous sex cords in the basal part of the testis were stretched owing to rapid gonadal growth.

Figs 2 and 3. Human gonad at late week in the fifth week of ovulational age. Stratification of the coelomic epithelium into several layers, displaying pillar-like structures (white arrows), i.e., the primary sex cords, is noted. The mesonephric tubule lies below the coelomic epithelium, and a primordial germ cell (arrowhead) exhibits contact with a cord-like structure of mesonephric origin. The basal lamina is not seen around the germ cell. Fig. 2, ×260; Fig. 3, ×680.

Figs 4 and 5. Human gonad early in the sixth week of ovulational age. The protrusion of the gonad into the coelomic cavity is more pronounced (compare Fig. 4 with Fig. 2). Cells emerge from the mesonephros and form cord-like structures, i.e., the primordial sex cords. Primordial sex cords (arrowheads) have been incorporated into the gonad, branching from the basal portion of the gonad towards the coelomic epithelium, and displaying no continuity with the coelomic epithelium. The coelomic epithelium is stretched into one to several layers (arrow, Figs 4, 5). Primordial germ cells are seen between the newly formed primordial sex cords. Fig. 4, ×260; Fig. 5, ×510.

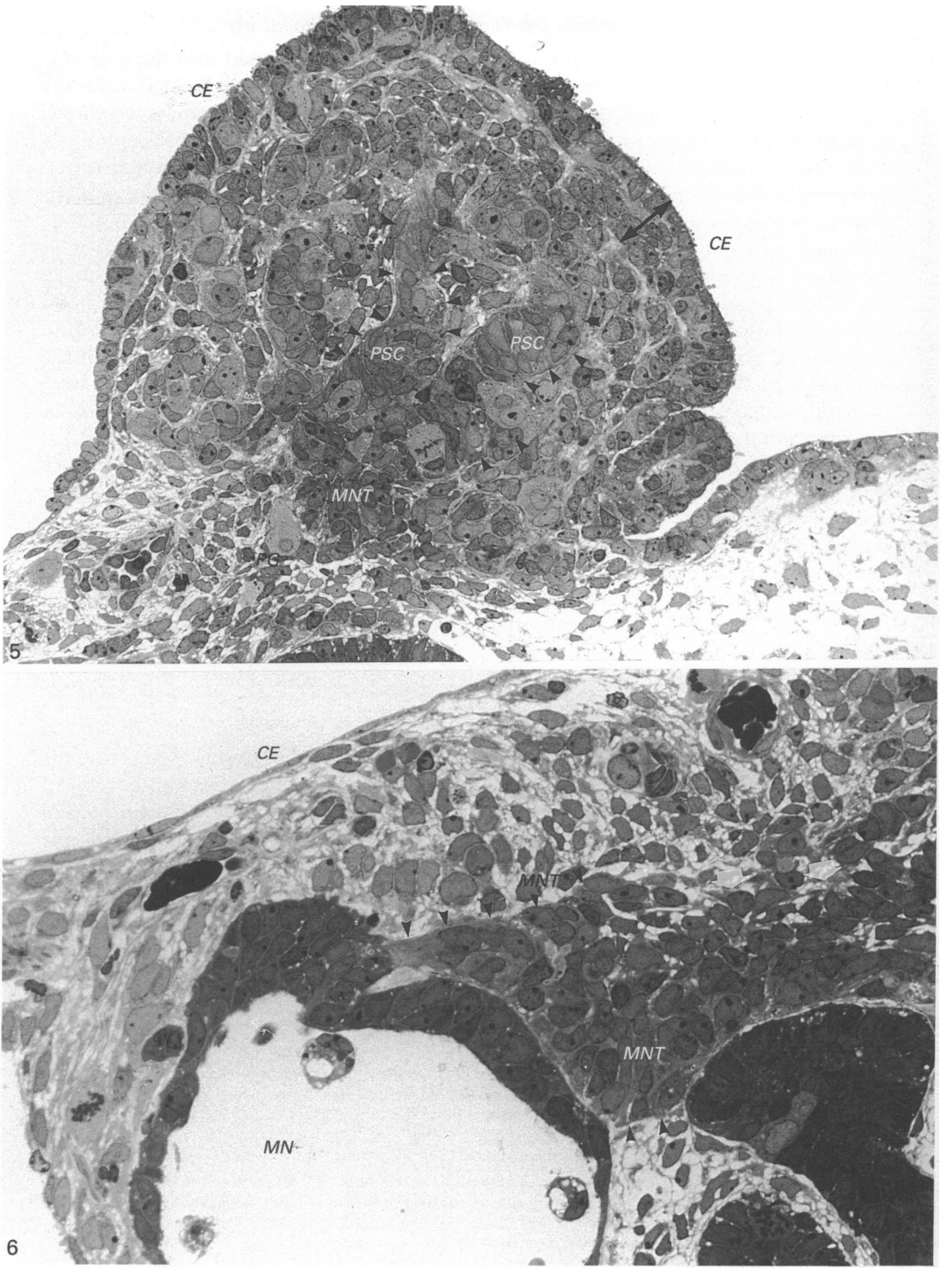
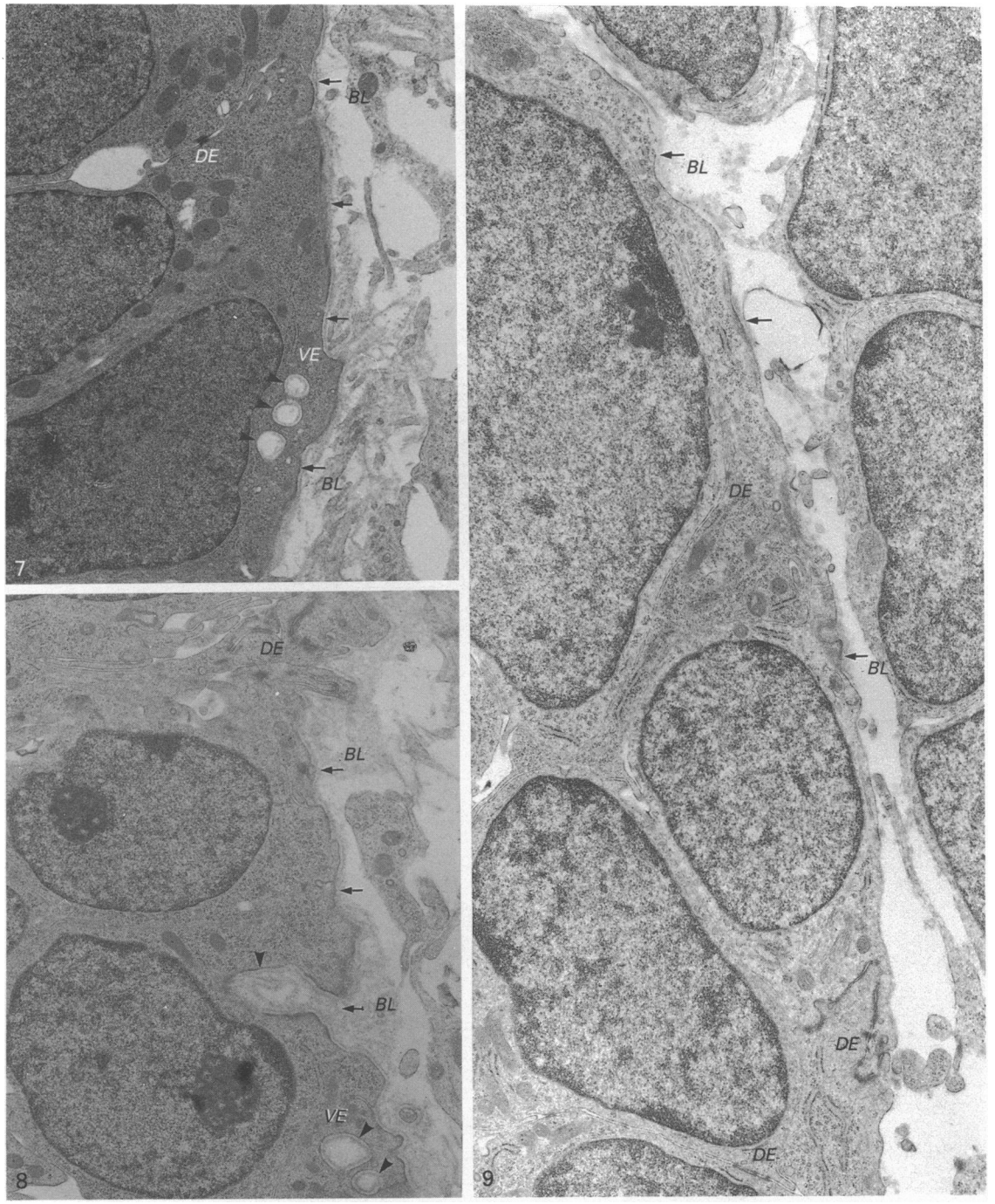
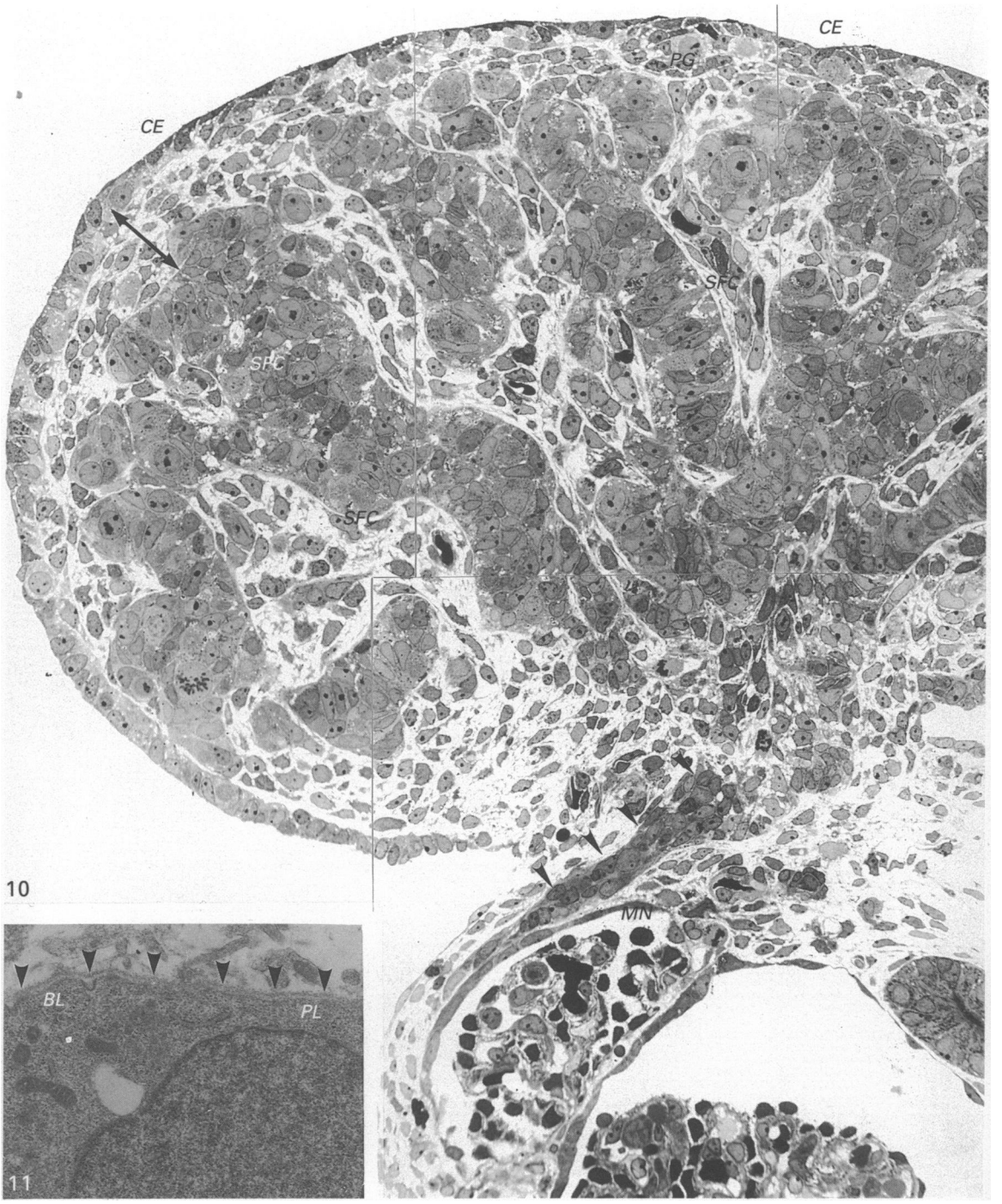


Fig. 6. Human gonad at early in the sixth week of ovulational age. The primordial sex cords are contiguous with the mesonephros (arrowheads). ×650.

For legend to Fig. 5 see previous page.



Figs 7, 8 and 9. Human gonad at early in the sixth week of ovulational age. A basal lamina (arrows) is observed around the cell cords. This basal lamina is evident in the basal areas of the gonad (Fig. 7), but gradually becomes indistinct in the direction of the coelomic epithelium (Figs 8, 9). The primordial sex cord cells possess vesicles containing basal lamina-like material (arrowheads, Figs 7, 8). Cell junctions are seen between these cord cells. Fig. 7, ×7800; Fig. 8, ×9500; Fig. 9, ×10600.



Figs 10 and 11. Human male gonad in the middle of the sixth week of ovulational age. Primordial sex cords in the testis are surrounded by a well-developed basal lamina (Fig. 11), from which seminiferous cords (tubules) have formed. The coelomic epithelium has extended into one to three layers. Seminiferous sex cords are not contiguous with the coelomic epithelium (arrow). Primordial sex cords in the basal portion of the testis appear stretched (arrowheads). Fig. 10, ×480; Fig. 11, ×11400.

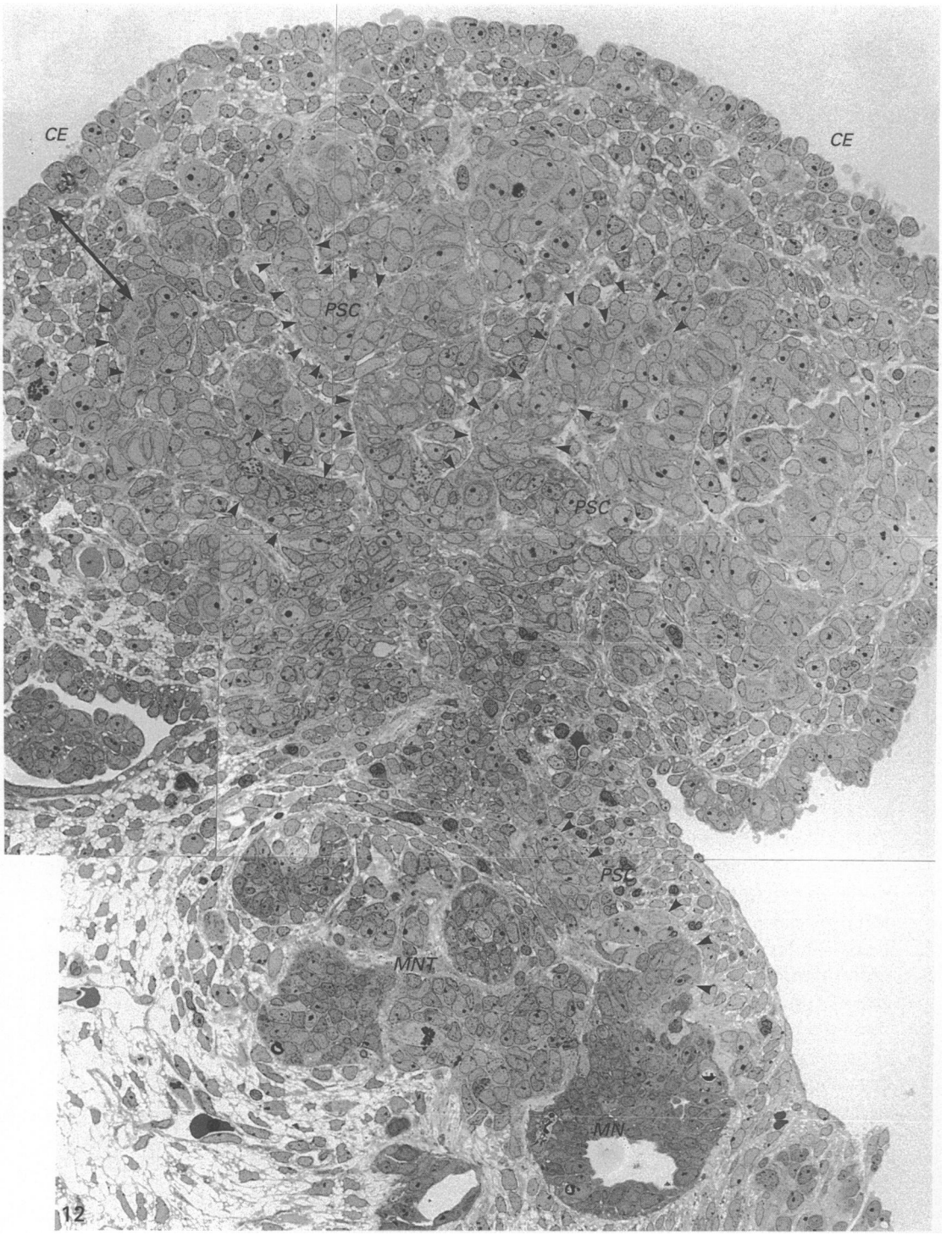
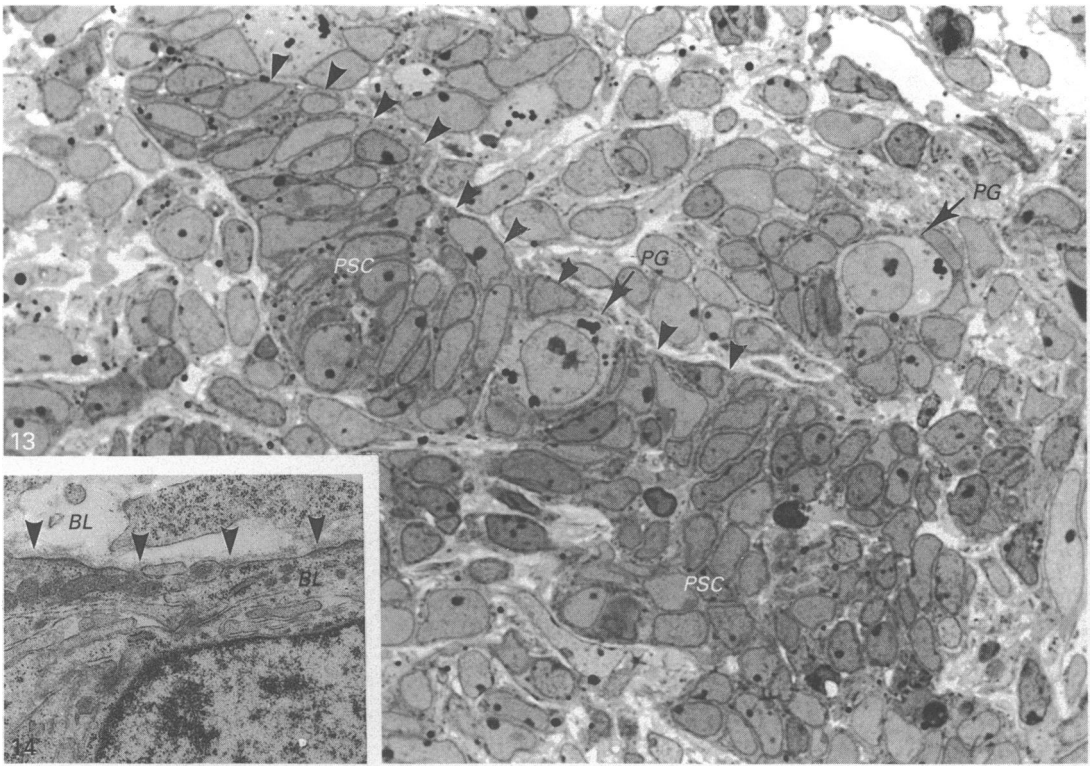


Fig. 12. Human female gonad in the middle of the sixth week of ovulational age. The ovary is also composed of the primordial sex cords (arrowheads). These cords branch from the basal to the peripheral area of the ovary, and are contiguous with the mesonephros (lower arrowheads). In contrast, these cords display no continuity with the coelomic epithelium (arrow). The distribution of the primordial sex cords is very similar to that of the testis (see Fig. 10). ×480.



Figs 13 and 14. Human female gonad in the middle of the sixth week of ovulational age. A basal lamina (arrowheads) is observed around the cell cords, but is less well developed than that in the testis. Fig. 13, ×1000; Fig. 14, ×11100.

Gonads (ovaries) in the middle part of the sixth week of ovulational age

The distribution of the primordial sex cords in the ovary at this stage was very similar to that observed in the testis (Figs 10, 12). The cells of the primordial sex cords in the ovary were stained to the same intensity as those of the primordial sex cords at the previous stage, but less intensely than the cells of the seminiferous sex cords in the testis. Differences between the ovary and the testis were evident. In serial sections of entire gonads, each primordial sex cord was extended and branched from the basal to the peripheral region of the ovary. They were contiguous with the mesonephros, but displayed no contiguity with the coelomic epithelium, which was flattened into one to three layers. Basal lamina was observed around the primordial sex cords, but displayed poorer development and preservation than in the testis (Figs 13, 14). Microvilli and desmosomes or desmosome-like structures were also present between the primordial sex cords.

Gonads (ovaries) at the seventh week of ovulational age

The proliferation of the germ cells in the distal portion of the primordial sex cords was pronounced, and overall enlargement of these cords was observed (Fig. 15). The primordial sex cords were still contiguous with the mesonephros and those situated in

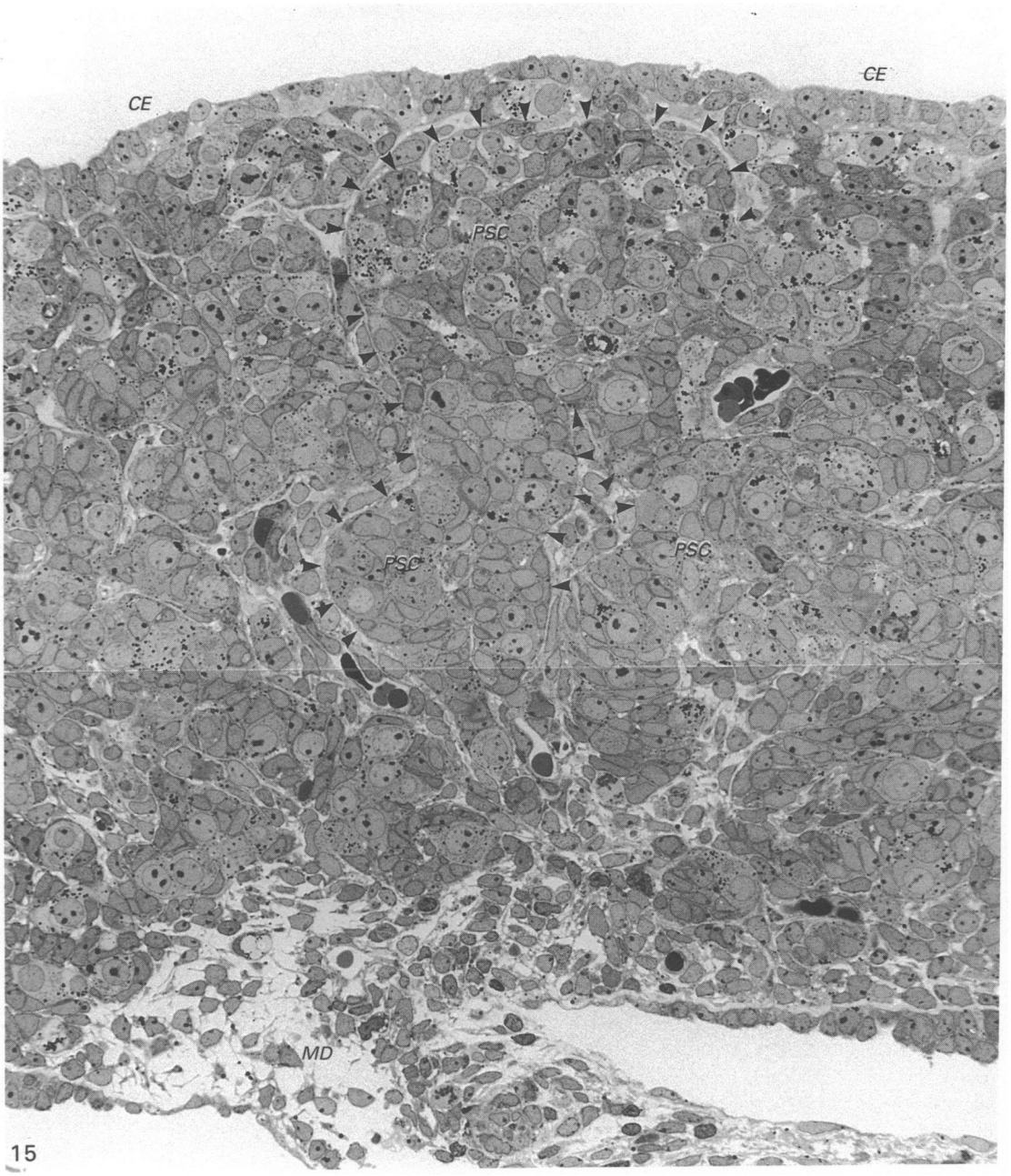


Fig. 15. Human female gonad at 7 weeks of ovulational age. The enlarged primordial sex cords (arrowheads) occupy the greater portion of the gonad. × 720.

the basal portion of the ovary were stretched (Fig. 16). The cells of the peripheral portions of the enlarged primordial sex cords were stained more intensely than those of the basal portions. The coelomic epithelium had stretched, due to enlargement of the primordial sex cords, and appeared to form almost a single layer.

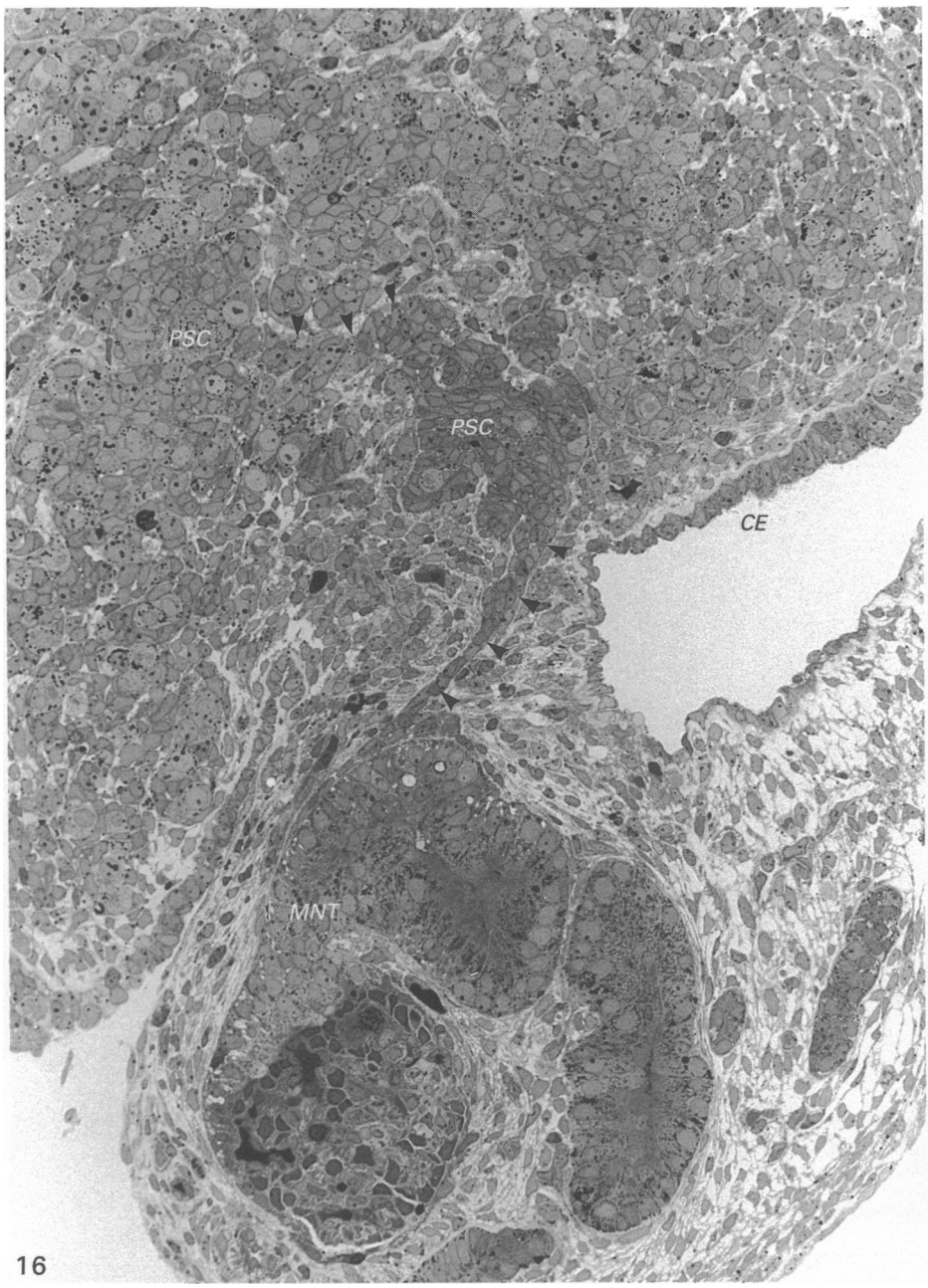
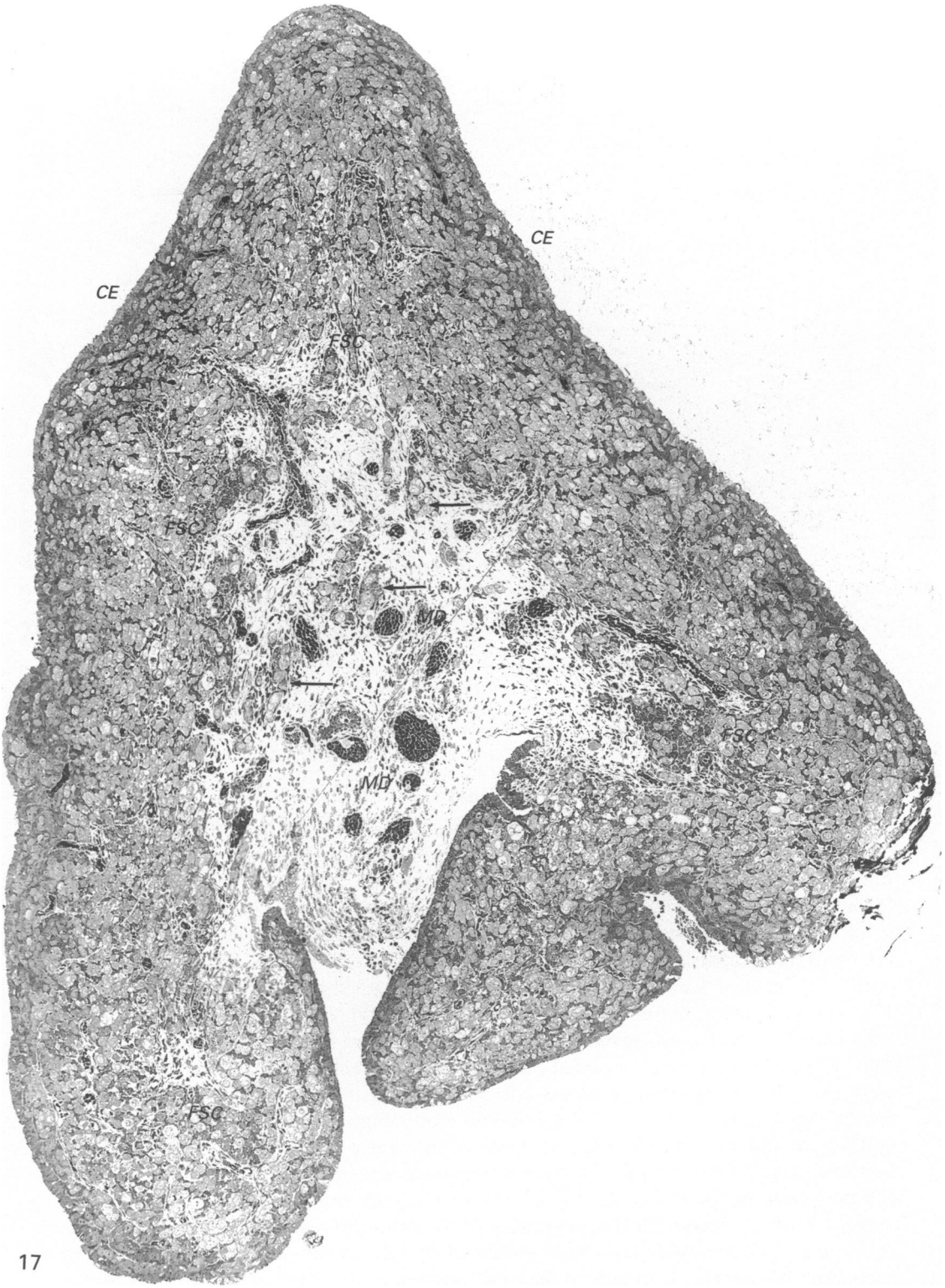


Fig. 16. Human female gonad at 7 weeks of ovulational age. The primordial sex cords (lower arrowheads) are contiguous with the mesonephros, and those lying in the basal portion of the ovary appear stretched. ×400.

Fig. 17. Human female gonad at 13 weeks of ovulational age. Marked proliferation of primordial germ cells, enlargement of the primordial sex cords and laterally oriented enlargement of the ovary is observed. At this stage, interstitial tissue, the so-called medulla, has been formed in the basal portion of the ovary, and the primordial sex cords display relocation or migration concomitant with the formation of 'folliculogenous sex cords', while the primordial sex cords previously present in the base of the ovary (arrows) have split into clusters. ×120.



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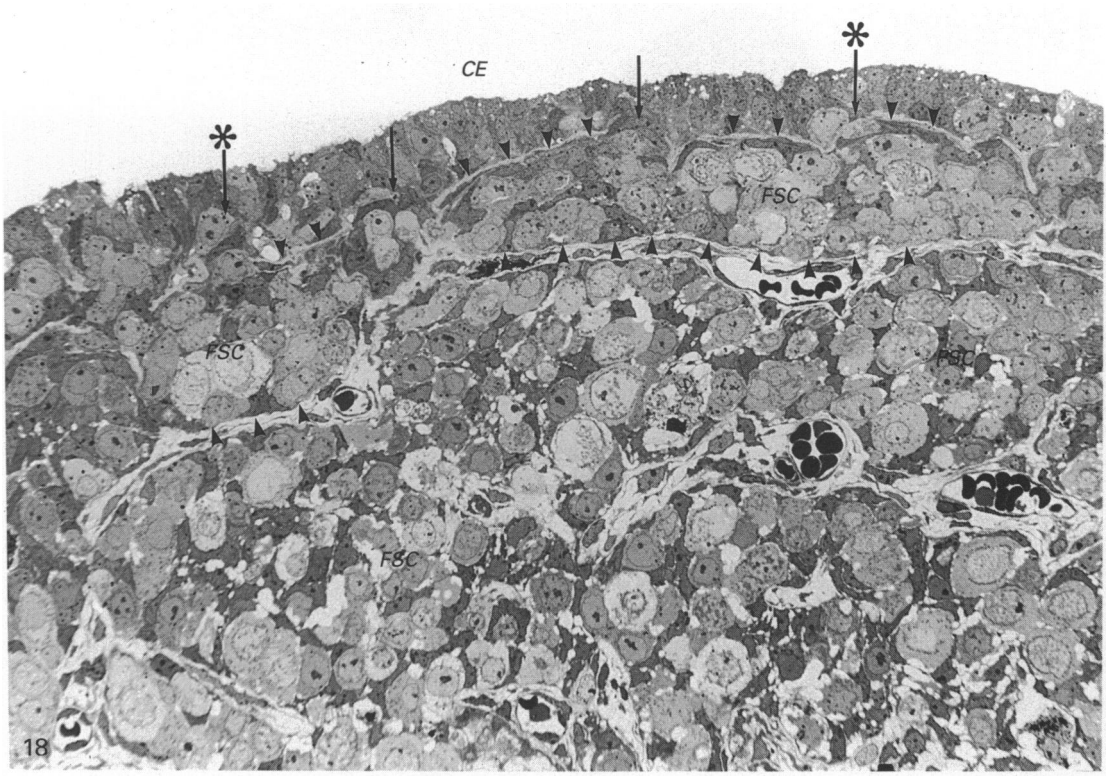
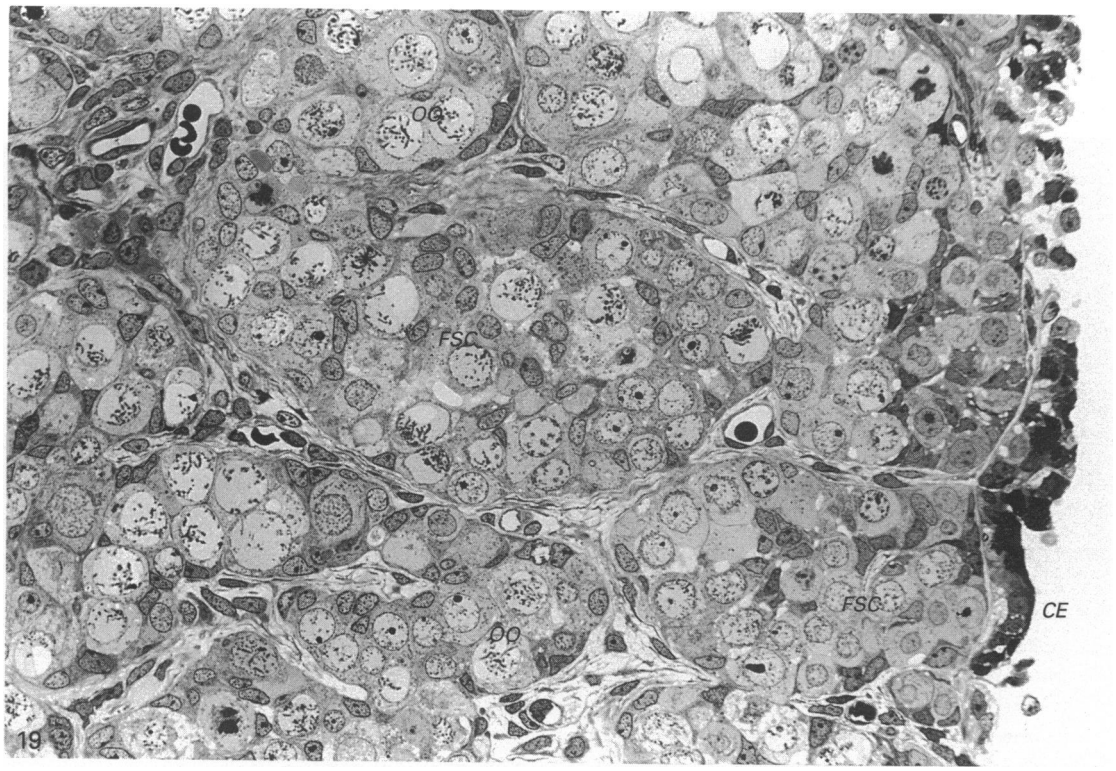


Fig. 18. Human female gonad at 13 weeks of ovulational age. The folliculogenous sex cords (arrowheads) run below and parallel to the coelomic epithelium and are flatter than the cords at the centre of the ovary. The coelomic epithelial cells display contact with the cell cords via primordial germ cells (*) at certain sites devoid of a basal lamina (arrows). × 510.

Gonads (ovaries) at 13 weeks of ovulational age

Owing to vigorous proliferation of the primordial germ cells, the enlargement of the primordial sex cords was pronounced and laterally-oriented growth of the ovary was observed in horizontal sections (Fig. 17). In relation to these phenomena, newly-formed interstitial tissue had appeared in the basal portion of the ovary, and the primordial sex cords had undergone displacement towards the peripheral regions of the ovary to form structures which will hereafter be referred to as 'folliculogenous sex cords'. Transverse sections of the ovary presented a mushroom-like appearance (Fig. 17). The primordial sex cords previously present in this basal area of the ovary had split and fragmented into islands or clusters (Fig. 17) and formed the 'rete ovarii'. The cells of the folliculogenous sex cord were more intensely stained by toluidine blue than were the cells of the primordial sex cords at previous stages of development. Many folliculogenous sex cords near the coelomic epithelium ran parallel to the coelomic epithelium and were flatter than the cords at the centre of the ovary (Fig. 18). Serial sections revealed branching of the folliculogenous sex cords from the centre towards the periphery of the ovary, as was observed in the primordial sex cords at previous stages. No degeneration or disappearance of the primordial sex cords was observed at this stage, which would have been indicated by the accumulation of cell debris or secondary lysosomes, especially in the basal portion of the ovary, or so-called medulla. The coelomic epithelium was almost completely surrounded by basal lamina. No



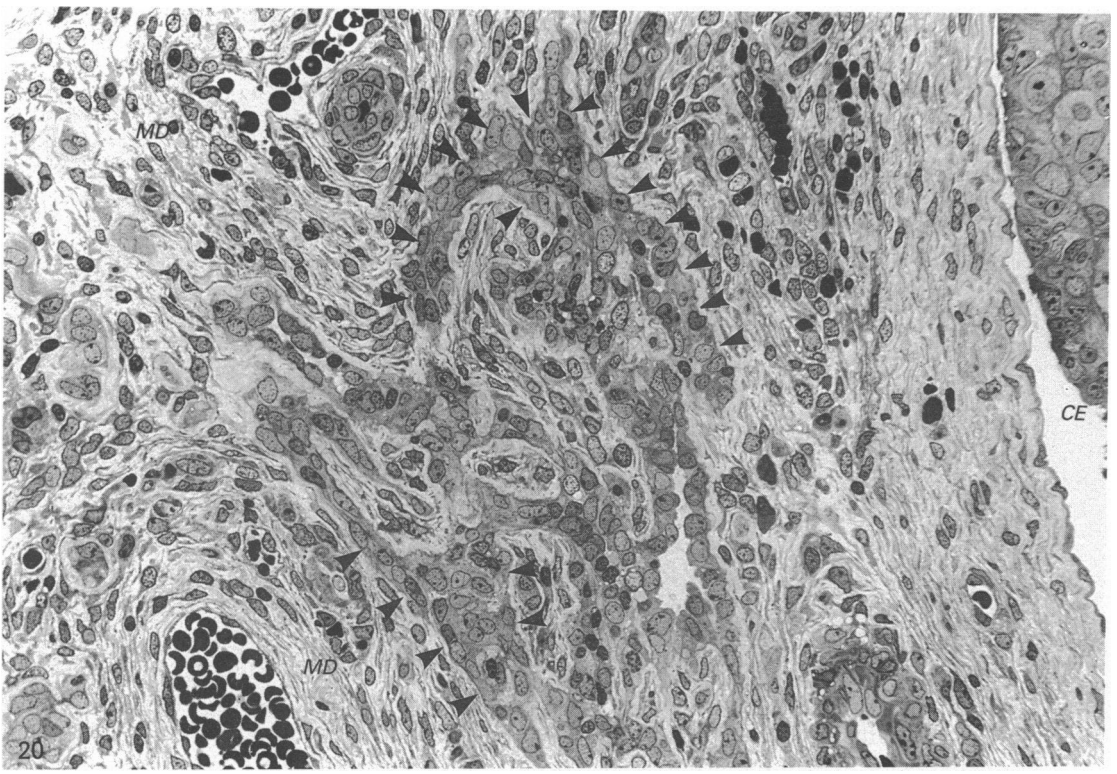


Fig. 19. Human female gonad at 18 weeks of ovulational age. Germ cells and oogonia display marked proliferation. Coelomic epithelial cells appear in one to three layers. × 500.

Fig. 20. Human female gonad at 18 weeks of ovulational age. Primordial sex cord cells in the basal portion of the ovary assume the form of islands or clusters (arrowheads). × 480.

proliferation of coelomic epithelial cells into the subepithelial region of the ovary was seen. Sites of contact between the folliculogenous sex cords and the coelomic epithelium, via the primordial germ cells, were also observed (Fig. 18); however, these sites were small and limited in number.

Gonads (ovaries) at 18 weeks of ovulational age

Many vigorously proliferating germ cells and oogonia were noted in the folliculogenous sex cords (Fig. 19). The adjacent interstitium was compressed, due to the enlargement of the folliculogenous sex cords. Clusters of previous primordial sex cords, the 'rete ovarii', were present in the basal portion of the ovary (Fig. 20).

DISCUSSION

One of the features of the present study was the preparation and observation of almost complete horizontal serial semithin sections of entire gonads from 10 embryos at early and closely sequential stages of gonadal development. It has revealed that the principal constituent cells of the gonads are derived from the mesonephros and that the coelomic epithelium is not involved in the formation of this principal component at any stage of development (Figs 21, 22).

In the present paper, the sex cords emerging from the mesonephros concurrently with the prominent protrusion of the gonad are referred to as the 'primordial sex cords', while the sex cords formed in the ovary by enlargement and displacement to the peripheral regions of the gonad of the primordial sex cords have been termed the 'folliculogenous sex cords'. The reasons for this nomenclature are given in the final two paragraphs of the Discussion.

Origin of the primordial sex cords

The primordial sex cords are of mesonephric origin and are not identical to the primary sex cords, which are formed by cord-like arrangements of stratified coelomic epithelium, as is substantiated by the following observations made on nearly complete serial sections of entire gonads, as well as on ultrathin sections of selected specimens.

1. In the indifferent stage, each primordial sex cord is contiguous with the mesonephros, while the basal lamina of the primordial sex cords is also contiguous with that of the mesonephros. The primordial sex cords branch from the mesonephros towards the coelomic epithelium. The cells of the primordial sex cords possess epithelial and not mesenchymal characteristics.

2. No anatomical continuity is observed between the cells of the primordial sex cords and those derived from the coelomic epithelium. Although proliferation of the coelomic epithelial cells is observed in the vicinity of the primordial germ cells (discussed below) where pillar-like structures, i.e., primary sex cords, are formed, these pillar-like structures do not develop into branched structures, and do not show contact with the mesonephros subsequent to deep penetration into the gonad or association with the primordial sex cords.

3. No primordial sex cord-like epithelial cords lacking continuity with both stratified coelomic epithelium and the mesonephros are seen in the gonad, that is, there were no epithelial cords separate from the coelomic epithelium. The coelomic epithelial cells, that is, the primary sex cords, thus do not participate in the formation of the primordial sex cords, seminiferous sex cords, or folliculogenous sex cords.

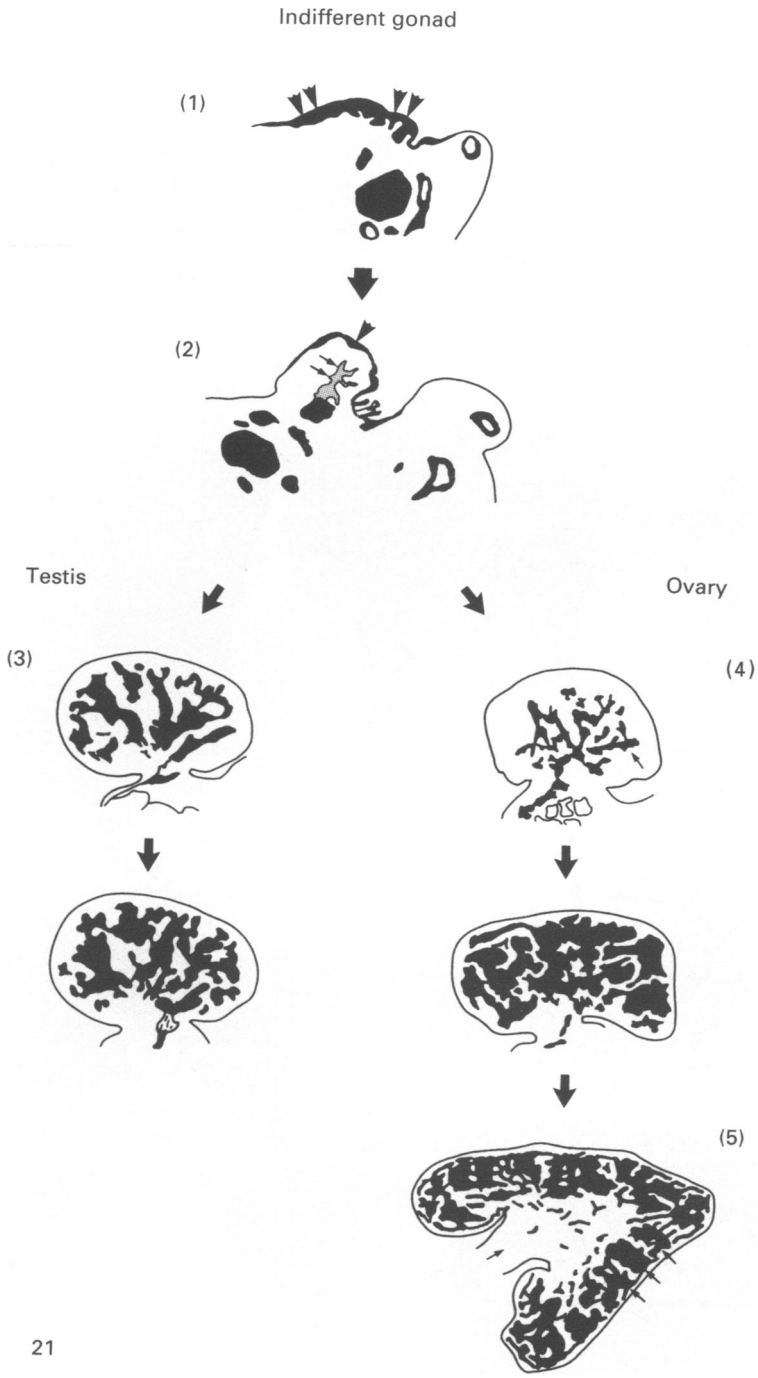


Fig. 21. Morphogenesis of the human gonad, part 1. Formation of the indifferent gonad. (1) The coelomic epithelium becomes thickly stratified and forms the primary sex cords, and a moderate protrusion of the gonad into the coelomic cavity is formed. (2) Cells emerging from the mesonephros are incorporated into the gonad and form primordial sex cords (arrows). A prominent protrusion of the gonad into the coelomic cavity is formed and the coelomic epithelium flattens. Formation of the testis. (3) In the testis, a conspicuous basal lamina is formed around the cords and seminiferous tubules are formed from these cords. Formation of the ovary. (4) The ovary is formed from the primordial sex cords. The distribution of the primordial sex cords is similar to that observed in the testis. (5) The primordial sex cords enlarge and an interstitium, the so-called medulla, emerges. The primordial sex cords relocate or migrate to the peripheral regions of the ovary, forming the folliculogenous sex cords (arrows).

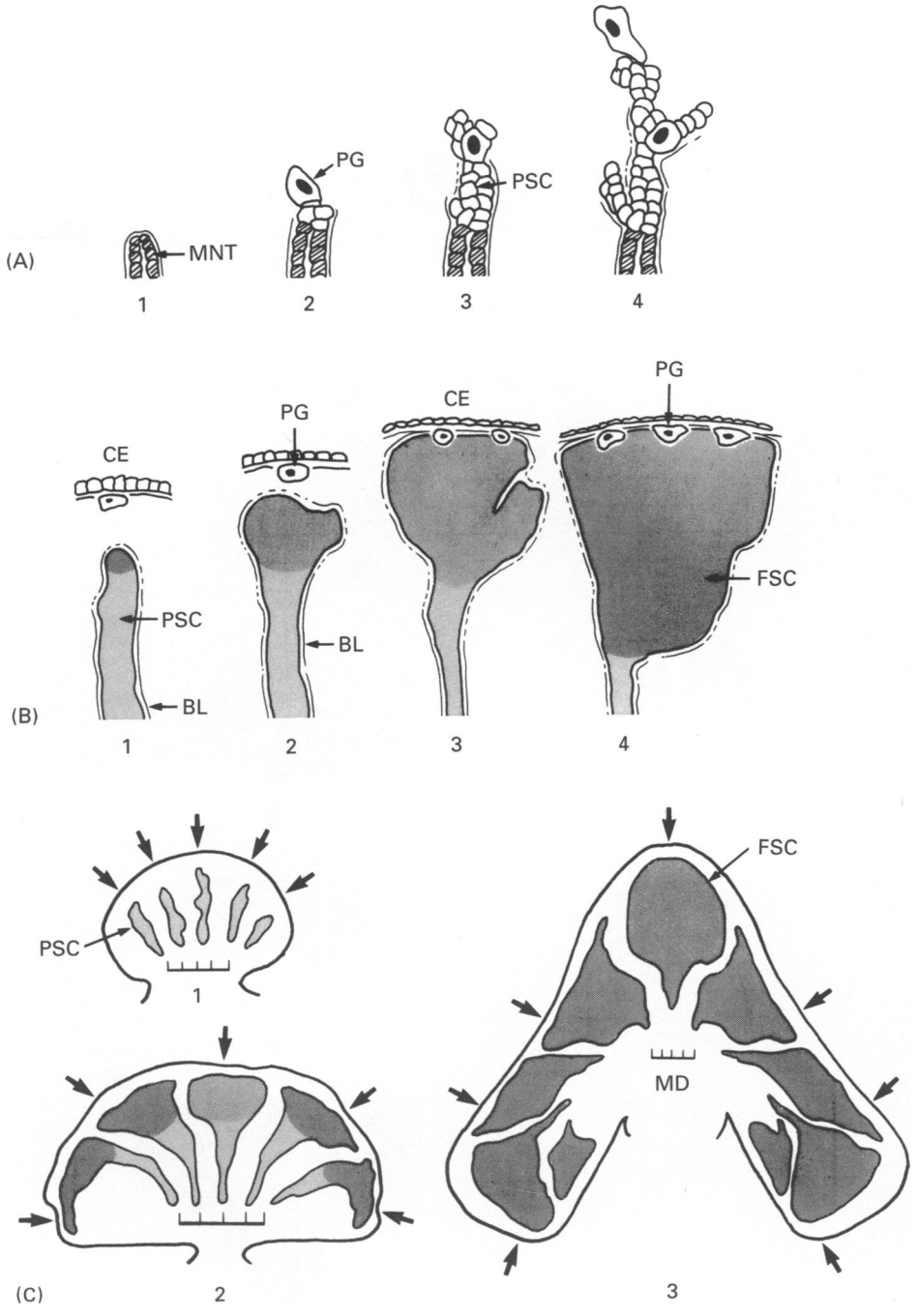


Fig. 22. Morphogenesis of the human gonad, part 2. (A) formation of primordial sex cords from the mesonephros. (B) Formation of folliculogenous sex cords from primordial sex cords. (C) Formation of the ovary.

In the present study, the precise event of primordial sex cord budding from the mesonephros has been observed for the first time. The primordial sex cords are formed by budding of cells of mesonephric origin from sites where the basal lamina of the mesonephros is eliminated, probably by contact with the primordial germ cells (discussed below). Concomitantly with the growth of the primordial sex cords, this basal lamina is gradually formed from the mesonephric side and extends towards the coelomic epithelium at the periphery of the primordial sex cords.

Flattening of the stratified coelomic epithelium

The primary sex cords display no continuity with the mesonephros or primordial sex cords at any stage or at any site, indicating that the stratified coelomic epithelium extends into one or several layers. In fact, as may be seen by comparing Figures 4 and 2, the prominent protrusion of the gonad into the coelomic cavity occurs rapidly, within a matter of half a day. These figures appear to indicate that the stratification and cord-like arrangement of the coelomic epithelium represents a state of preparation for the subsequent prominent protrusion of the gonad which is concomitant with the emergence of the primordial sex cords in contiguity with the mesonephros.

With respect to this phenomenon, it may be conjectured that the coelomic epithelium assumes the form of a single layer at an extremely early stage of gonadogenesis, and that cells of coelomic epithelial origin may display some tendency to develop into a single layer, even if they exhibit a cord-like arrangement. On the other hand, mesonephric cells initially appear in tubular forms, and the primordial sex cords, which are of mesonephric origin, display some characteristics indicating the formation of tubular structures. Thus in the testis the primordial sex cord cells form the seminiferous tubules, while in the ovary follicles rather than tubules are formed, related to the poorer development of the basal lamina and the division of the folliculogenous sex cords into fragments.

Formation of folliculogenous sex cords

The following findings made in the present study contradict the earlier contention that 'secondary' sex cords are formed by continued proliferation of the coelomic epithelium.

1. At ovulational age of 13 weeks, many 'secondary' sex cords directly below the coelomic epithelium were flatter than those of the central portion, as though they had been stretched, and ran parallel to the superficial lining of the coelomic epithelium. The basal lamina was observed in almost the entire area under the superficial lining of the coelomic epithelium. These observations do not appear to indicate vigorous proliferation of the coelomic epithelial cells.
2. Serial sections revealed that 'secondary' sex cords assumed a form characterized by a confluence of the branches of their peripheral cords towards the centre of the ovary, similar to that observed in the primordial sex cords. The 'secondary' sex cords did not appear as mere extrusions from the coelomic epithelium.
3. None of the findings concerning the gonad of either sex at any stage revealed degeneration or disappearance of primordial sex cords, which would be indicated by the presence of cell debris or secondary lysosomes.

It seems unreasonable to suppose that primordial sex cords which occupied the greater portion of the gonads during the early stages of ovarian development disappear and that 'secondary' sex cords of the same thickness suddenly emerge from the coelomic epithelium. These considerations support the view that folliculogenous sex cords are formed from primordial sex cords.

Sites of contact between sex cords and coelomic epithelium

The fusion of the coelomic epithelium and primordial sex cords or folliculogenous sex cords occurred at sites where the basal lamina was partially absent. The most likely explanation for this is as follows.

1. Primordial sex cords and enlarged folliculogenous sex cords are distributed directly below, or very close to, the coelomic epithelium in some places.

2. Primordial germ cells possess some capability for dissolving the basal lamina, and the observations of the present study suggested that those situated between the coelomic epithelium and the sex cords dissolve their basement membranes, thereby becoming points of contact in the fusion of these structures. In fact, serial sections revealed primordial or folliculogenous sex cords joined to the coelomic epithelium via the primordial germ cells (Fig. 18). Such dissolution of the basal lamina by the primordial cells has, in fact, been observed in the rat (Satoh, 1985). This phenomenon was described as follows. 'Disappearance of the basal lamina is observed in regions where the germ cells are in contact with or in the close vicinity of the coelomic epithelium and clear cord cells... It is more reasonable to consider that the primordial germ cells partially dissolve the adjacent basal lamina and enter the clear cell cords rather than that they migrate towards defects in the basal lamina. However, this action of the germ cells seems to be of a transient nature.' This conclusion may very possibly also be applicable to human primordial germ cells, although at present this hypothesis is supported only by morphological evidence and requires more detailed confirmation.

The fusion of the coelomic epithelium and folliculogenous sex cords observed in the present study suggests the possibility that cells derived from the coelomic epithelium invade the gonad through the sites which lack basal lamina, thereby forming a portion of the peripheral region of the gonad. However, the regions, if any, in which this occurs are apparently confined to an extremely small fraction of the entire gonad. Moreover, the possibility that the coelomic epithelial cells form the primary follicles appears even less likely for the following reasons. (1) Serial sections revealed that the coelomic epithelium is almost completely surrounded by basal lamina, and the areas devoid of basal lamina are small and limited in number. (2) Primary follicles directly below the coelomic epithelium have not been observed in more advanced stages.

Gonadogenesis in primates and humans

Studies of gonadal development in primates and humans using plastic-embedded semithin sections and ultrathin sections (Dang & Fouquet, 1979; Fouquet & Dang, 1980; Wartenberg, 1982; Yoshinaga *et al.*, 1988) have been few in number as compared with those using conventional paraffin-embedded sections (Allen, 1904; Whitehead, 1904; van Wagenen & Simpson, 1965), and the results of these studies have been controversial.

Owing to the limited resolution possible with paraffin-embedded material, studies employing this method alone may not permit recognition of the primordial sex cords, while clearly revealing the primary sex cords, seminiferous sex cords, and folliculogenous sex cords, which are intensely stained by toluidine blue. This limitation may result in erroneous interpretation of the developmental process, that is, the view that the primary sex cords separate from the coelomic epithelium in the early stages and differentiate into the seminiferous sex cords, and that, in the ovary, the secondary sex cords are formed by the continued proliferation of the coelomic epithelium.

Dang & Fouquet (1979), as well as Fouquet & Dang (1980), stated that the somatic cells of the gonadal blastema of *Macaca fascicularis* are derived from the mesonephric

mesenchyme in contact with the proximal loops of the anterior tubules. However, it is questionable whether the mesenchymal cells differentiate into the epithelial cells in the advanced stages of embryogenesis. According to the findings of the present investigation, the cells of the 'mesonephric mesenchyme' described in the studies were actually the epithelial cells, possessing a basal lamina and cell junctions, which had already been present in, or emerged from, the mesonephros in previous stages. These cells are therefore derivatives of the mesonephros.

Wartenberg (1982) reported that in man the primary gonadal blastema within the genital ridge is formed by two types of somatic cells, namely, cells segregated from the mesonephros and cells of the proliferating coelomic epithelium, and that these two types of cells display a tendency to intermingle. However, according to the results of the present study, the cells referred to were actually mesenchymal (Fig. 1) and no findings indicating that the epithelial cells and mesonephric cells segregate or intermingle were obtained. Wartenberg's study did not include observations at the highly important stage at ovulational age 42–45 days, and failed to describe findings concerning the budding sites of primordial sex cords, as shown in Figures 4 and 5, and complete separation between primordial sex cords and coelomic epithelium, as shown in Figures 4, 5, 10 and 12 of the present paper. It was also reported that in the portion of the cortical region which had developed from superficial epithelium, the mesonephric cells do not completely penetrate the superficial blastemal layer. The reason for this interpretation may be that the observed sites of contact between enlarged folliculogenous sex cords and coelomic epithelium via primordial germ cells (described above) are attributed to invasion of coelomic epithelium into the ovary, or to residual sites of coelomic epithelium.

Recently, Yoshinaga *et al.* (1988) have reported that in the galago, the somatic cells of the gonadal blastema, i.e., the precursors of the testicular and ovarian sustentacular cells, derive from the coelomic epithelium and that the somatic cells are formed by intense proliferation of the coelomic mesothelium. In order to prove the coelomic epithelial origin of the gonad, it would be necessary to explain the processes occurring from the stage at which stratified coelomic epithelium displays no continuity with the mesonephros, as seen in Figure 2 of the present paper, to the stage at which the sex cords anatomically contiguous with the mesonephros are separated from the coelomic epithelium, as seen in Figures 4, 5, 10 and 12 of the present paper. Definite explanations must also be provided to indicate where and how the so-called 'slender cord' (primordial sex cord) is demarcated from the compact cellular mass of the coelomic mesothelial cells at the 'blastema stage' and branches from the mesonephros toward the coelomic epithelium.

More specifically, it is necessary to demonstrate (1) the process of separation of coelomic epithelial cells from the superficial lining; (2) the existence of epithelial cells arranged in a cord-like manner, which have separated from the superficial lining of the coelomic epithelium and are not contiguous with the mesonephros under the superficial lining of the coelomic epithelium (in the case of contact with the mesonephros after separation from the coelomic epithelium) and/or (3) the existence of epithelial cells arranged in a cord-like manner, which are contiguous with both the coelomic epithelium and the mesonephros (in the case of separation from the coelomic epithelium after contact with the mesonephros); and (4) the process of demarcation and branching of the cellular mass or cords. However, no findings indicating these processes could be obtained in the present investigation.

As to interspecific differences, Yoshinaga *et al.* (1988) speculated that differences in patterns and mechanisms of gonadogenesis may be attributable to interspecific

differences with respect to the structural complexity, functionality and stages of differentiation or involution of mesonephroi on the one hand and the time of gonadal development on the other. However, according to the findings of the present and previous investigations (Satoh, 1985), the gonads in both rats and humans are formed from cells derived from the mesonephros and there is no relationship between the stages of differentiation or involution of the mesonephroi and differences in the derivation of gonadal components. The differences in gonadogenesis between the human and the rat are basically as follows. In rat gonads, the primordial sex cords of mesonephric origin in both the testis and the ovary differentiate directly, in situ, into the seminiferous sex cords and folliculogenous sex cords, respectively (Satoh, 1985). On the other hand, in the human ovary, the primordial sex cords relocate or 'migrate' to the peripheral region of the ovary and form the folliculogenous sex cords because of the formation of the interstitium in the basal part of the ovary, hence the continuity between the folliculogenous sex cords and the mesonephros is lost.

The reason for the introduction of the new terminology 'primordial sex cords' and 'folliculogenous sex cords' in this paper for the explanation of a new aspect of the gonadogenesis is as follows. At present, the 'primary sex cords' are generally accepted as consisting of stratified coelomic epithelium when, in fact, at the stage of preparation for flattening of the coelomic epithelium, these structures display a winding pattern and cord-like arrangement, which is the reason for the appellation primary 'sex cords' and the cause for the current confusion concerning the processes of gonadal development. The present investigation demonstrates that the 'primordial' sex cords are not identical with the 'primary' sex cords in regard to structure, location and origin, i.e., the former consist of ramified structures contiguous with the mesonephros while the latter are peduncular structures from the coelomic epithelium, in accordance with the distinction between mesonephric origin and coelomic epithelial origin. Furthermore, the word 'primary' connotes the existence of a corresponding 'secondary' structure, but in fact the present investigation revealed no 'secondary' sex cords formed by continued proliferation of the coelomic epithelium. Thus the application of the term 'primary sex cords' to the structures referred to here as 'primordial sex cords' is inappropriate.

The term 'folligulogenous sex cords' is histologically defined as the cords formed by enlargement and relocation at the peripheral portion of the ovary of the primordial sex cords, not by proliferation of the coelomic epithelium, concomitant with more intense staining by toluidine blue. Histogenetically, this term implies the origin of the follicular cells, similar to the terms 'seminiferous sex cords' and 'seminiferous tubules'.

SUMMARY

The histogenesis and organogenesis of the human gonad in 12 embryos and 6 fetuses of ovulational ages 5 to 18 weeks was investigated by histological and ultrastructural examination, including observation of almost complete serial Epon-embedded sections of entire gonads of 10 embryos. This investigation revealed that the main constituent cells of the gonads are derived from the mesonephros, and that the coelomic epithelium is not involved in the formation of the main component at any stage. With the migration of the primordial germ cells into the gonadal ridge, the coelomic epithelium becomes stratified to form a moderate protrusion of the gonad into the coelomic cavity and the coelomic epithelial cells develop into short pillars which form cord-like structures, the so-called primary sex cords. Shortly afterwards, concomitantly with the development into the subsequent prominent protrusion of the

gonad into the coelomic cavity, cells emerging from the mesonephros are incorporated into the gonad to form 'primordial sex cords'. At this stage, a stratified, pile-like arrangement of coelomic epithelium flattens into monolaminar or oligolaminar structures. In the testis, the 'primordial sex cords' differentiate into seminiferous sex cords by elaborating a surrounding basal lamina. In the ovary, these 'primordial sex cords' become displaced towards the peripheral regions of the gonad by the enlargement of these cords, as well as by the formation of the interstitium, or so-called medulla, at the base of the ovary; they differentiate into 'folliculogenous sex cords' which give rise to follicular cells.

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