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Original Article

Histogenesis of uterus in human fetuses

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ABSTRACT

Introduction: The histological changes during the development of the human uterus throughout the fetal period exhibit varying cellular patterns in the lining epithelium. The present study documents these progressive changes during fetal uterine maturation.
Methods: 70 fetal uteri whose age varies from 14th to 40th gestational weeks without any external anomalies were studied.
Results: At 14 weeks, the uterine lining epithelium is predominantly pseudostratified columnar, the nuclei being arranged at varying levels. However, the lower part of cervix shows epithelium comprising of a mosaic of flat or slightly raised polygonal cells which gradually changes to stratified squamous epithelium with advancing gestation. From 32 weeks onwards, the pseudostratification of uterine epithelium changes to simple columnar epithelium progressively. At 14 weeks, two distinct layers of mesenchymal cells are apparent, elongated cells in abundance at the subserosal layer and sparsely arranged rounded cells towards the lumen. Endometrial glands appear by the 17th week.
Discussion: Except for the lower part of cervix, pseudostratified columnar epithelium lines the fetal uterus. As gestation progresses, pseudostratification gradually changes to simple columnar epithelium. The palmate folds of the epithelium appear by 17 weeks in cervical canal. The smooth muscle bundle appears by 24 weeks of gestation. Undifferentiated mesenchymal cells around paramesonephric duct develop into both smooth muscles and endometrial cells.

1. Introduction

The uterus is the most important reproductive organ present in the pelvis, which develops from a paired paramesonephric duct, originating from coelomic epithelium extending beside the mesonephric ducts. The paired duct begins to fuse from vaginal plate and forms the primordial body of the uterus.[1]

Endometrium is lined by simple columnar epithelium, deep to this there is lamina propria filled with tubular endometrial glands; it penetrates as far as the boundary with the myometrium. The cervical part, except for the lowermost third, is lined by simple columnar epithelium with tubular glands. Its lower third is covered by non-keratinizing stratified squamous epithelium.

Myometrium is a fibromuscular layer forming most of the uterine wall. The most internal layer (stratum mucosum) is composed mostly of longitudinal and some oblique smooth muscle. External to the submucosal layer is the stratum vasculare, a zone rich in blood vessel as well as longitudinal muscle. Next is a layer of predominantly circular muscle, the stratum supravasculare. Finally a thin longitudinal layer, the stratum subserosum, lies adjacent to the perimetrium or adjacent connective tissue.

Perimetrium (serosa) is composed of peritoneum covering the uterine body and supravaginal cervix posteriorly, but anteriorly only the body. Over the most inferior quarter of the uterine length the peritoneum is separated posteriorly from the underlying uterus by loose cellular tissue and large veins.

2. Materials and methods

Seventy (70) fresh human fetuses without any gross abnormality, ranging from 14th to 40th gestational weeks (GW) were collected from the Department of Obstetrics and Gynecology, RIMS Hospital, Imphal, Manipur, India. The age of the fetuses were calculated from the obstetrical history, gross features and crown-rump lengths (CRL). The specimens were categorized into six age groups for easier study as Group I (14–18 weeks), Group II (18–22 weeks), Group III (22–26 weeks), Group IV (26–30 weeks), Group V (30–34 weeks) and Group VI (34–40 weeks). Fetuses were fixed in 10% formalin for 10–15 days. Sections of 7 μm thickness were cut with the Leika RM 2125 RT rotary microtome. Haematoxylin and

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Eosin (H&E), Masson's Trichrome, Van Giesons and Verhoeff's staining were done to differentiate the collagen fibers reticulin and elastic fibers and studied under trinocular compound light research microscope and microphotography were stored and analysed.

3. Results

3.1. 14th–18th GW (Group I)

At 14th GW, uniform homogenous stroma lined by uterine mucosa is identifiable (Fig. 1a). The mucosa is lined by distinct tall epithelium which is about 1/4th of the entire thickness of the wall of uterus. Lining epithelium is columnar with disposition of nuclei at different levels giving rise to pseudostratified columnar epithelium. The nuclei were round or ovoid vesicular and darkly stained with definite basement membrane (Fig. 1b). However, the lower part of the cervix is lined by stratified squamous epithelium. The epithelium to stroma ratio is 1:4. The lumen is seen as linear cleft between the anterior and posterior walls of the uterus. The connective tissue stroma is characterized by a homogeneous mass of undifferentiated mesenchymal tissue studded with blood vessels. The connective tissue stroma shows two zones, the inner zone being darker as cells are more compact and densely populated.

From 17th weeks, the uterine epithelium shows columnar cells with ovoid shaped nuclei arranged at basal, intermediate and superficial levels making it pseudostratified in appearance seen under higher magnification (x100). The cells have distinct cell boundaries. The basal surface of the epithelium rests on a distinct and continuous basement membrane.

The uterine glands start developing, seen as infolding of epithelium, more so in the cervical region giving early palmate appearance (Fig. 1c). The uterine stroma is composed of dense and compact undifferentiated mesenchymal cells. Each cell has oval nuclei with high nucleo-cytoplasmic ratio. Cells near the serosa (the outer zone), mostly elongated and fusiform are fibroblast cells, whereas cells towards the lumen (the inner zone), oval in shape are mesenchymal cells. The thickness of the inner zone is more than that of outer zone. The loosely arranged subepithelium connective tissue stroma is mainly composed of mesenchymal cells and collagen fibrils. Stromal cells are rounded to spindle shaped, with large nuclei and poorly represented cytoplasm, i.e. typical features of still undifferentiated cells.

The outer zone cells shows elongated nuclei and eosinophilic cytoplasm, showing features of differentiation of myoblast from mesenchyme. Myoblast shows spindle shaped tapering cytoplasmic ends and contact with neighboring myoblast shows linear arrangements of myofibrils which are distinguishable from collagen fibers and developed into early myofibrils and arranged randomly towards the deeper part whereas near the peripheral zone, they are arranged predominantly parallel to the collagen fibers. They are disposed in parallel to serosal surface. The collagen fibers and fibroblast cells were more distinctly differentiated with wavy in nature seen in Masson's Trichrome stained tissues. Blood vessel formation is seen.

3.2. 18–22 weeks (Group II)

The lining epithelium of the lumen is pseudostratified columnar in most areas. In higher magnification (x100), the uterine epithelium is made up of columnar cells with ovoid shaped nuclei arranged at different levels making it pseudostratified in appearance. At this stage, there is more tubular invagination of lining epithelium into the stroma. Under high power magnification the glandular elements differentiation are seen lined by pseudostratified columnar epithelial cells having elongated vesicular nuclei. The glandular differentiation is more active towards the cervical region as compare to the body (Fig. 2a).

The loosely arranged subepithelial lamina propria is mainly composed of mesenchymal cells, few fibroblasts and collagen fibrils. Stromal cells which are rounded to spindle shaped, with large nuclei and poorly represented cytoplasm, i.e. typical features of undifferentiated elements are still present. In the outermost zone, fibrous strands with wavy pattern are seen in Masson's Trichrome stained tissues along with few fibroblast cells (Fig. 2b).

3.3. 22–26 weeks (Group III)

At 23rd week, in panoramic view the uterine lumen is cleft-like and distinctly identifiable. The epithelium is tall columnar with ovoid shaped nuclei arranged at different levels seen under higher magnification. Under high power magnification the glandular walls are seen lined by pseudostratified columnar epithelial cells having elongated vesicular nuclei with clear basement membrane. Early stage of palmate formation is apparent distinctly in the cervix. The outer zone primordial cells of myocytes with elongated

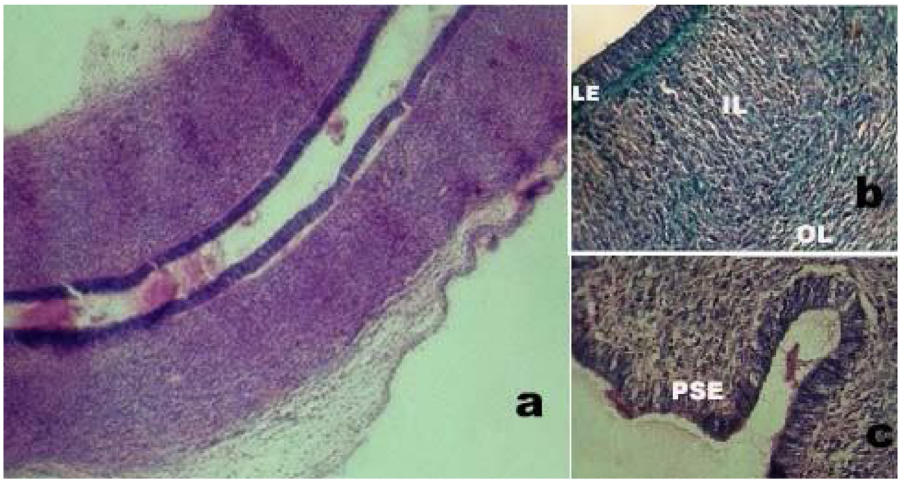

Fig. 1. Group I. a) Longitudinal section of uterine cavity at 14 weeks (Haematoxylin and Eosin x10) staining showing distinct tall epithelium lined mucosa. b) Longitudinal section (Masson's Trichrome x40) showing pseudostratified lining epithelium (LE) along with compact inner layer (IL) and sparse outer layer (OL). c) Longitudinal section of uterine gland at 17 weeks showing pseudostratified columnar epithelial (PSE) lining (H&E x40).

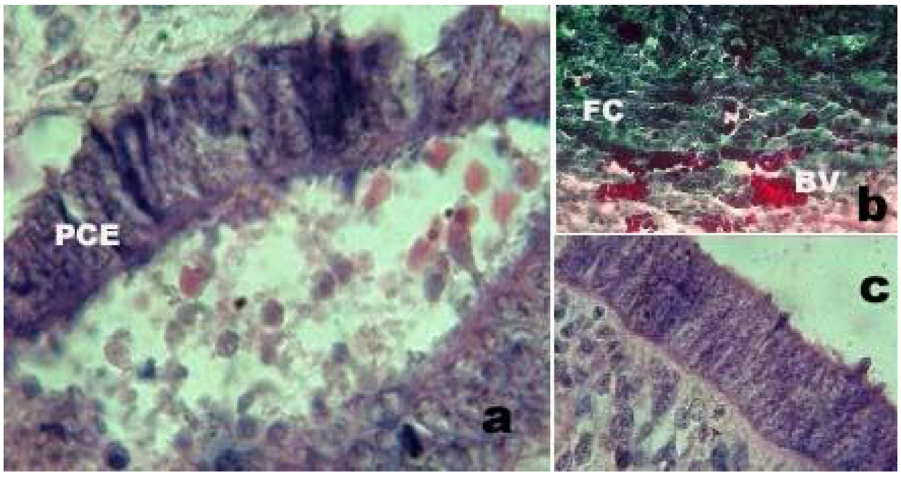

Fig. 2. Group II. a) Longitudinal section of developing uterine gland in cervical region with pseudostratified columnar epithelium (PCE) in 20 weeks uterus (H&E x100). b) Longitudinal section of differentiated fibroblast cells (FC) and blood vessels (BV) in 20 weeks uterus. Fibrous components seen predominantly (Masson's Trichrome x40). c) Longitudinal section of pseudostratified columnar epithelium with ovoid nuclei in 20 weeks uterus (H&E x100).

nuclei and more eosinophilic cytoplasm are recognized in groups, which are mostly myoblast cells (Fig. 3c).

At 24 week, stroma can be differentiated into two zones. The thickness of the inner zone to the outer zone is similar to the previous age group (Fig. 3a). In the inner zone undifferentiated stromal cells with rounded to spindle shaped, with large nuclei and poorly represented cytoplasm are disposed radially beneath the epithelium which is distinctly separated by well formed basement membrane. Under x40, as differentiated myocytes are distinctly stained purple by Masson's Trichrome stain these are easily identifiable from the rest of the mesenchymal cells. These myofibrils are sparsely distributed in the body. At this stage early myocytes and streaks of collagen fibers are seen only beneath the serosa.

3.4. 26–30 week (Group IV)

Endometrial epithelium shows infolding in the form of finger-like projection. The lumen is filled with these finger-like projections. Each projection has a core and stroma lined by the pseudostratified columnar epithelium. The cells have distinct cell boundaries with continuous basement membrane.

Differentiated glands are also visible in the submucosa. Under high power magnification (x100) the glandular walls are seen lined by pseudostratified columnar epithelial cells having elongated vesicular nuclei with clear basement membrane. It is surrounded by mesenchymal cells and fibroblasts.

3.5. 30–34 week (Group V)

The lining epithelium has become simple ciliated columnar in most areas with lesser distribution of columnar cells seen under x100 (Fig. 4b). The cells have distinct cell boundary. The differentiating glandular elements are seen lined by simple columnar ciliated epithelial cells having elongated vesicular nuclei with clear basement membrane (Fig. 4a). The connective tissue stroma of subepithelial lamina propria is loosely packed and mainly composed of mesenchymal cells fibroblasts and collagen fibrils. The stroma near the epithelium in the inner 1/3rd zone differentiation is minimal. Stromal cells which are round to spindle shaped, having large nuclei and poorly represented cytoplasm, i.e. typical features of undifferentiated cells are still present.

In the middle 1/3rd, myocytes adjoin each other and bundles of smooth muscle cells are oriented in different planes.

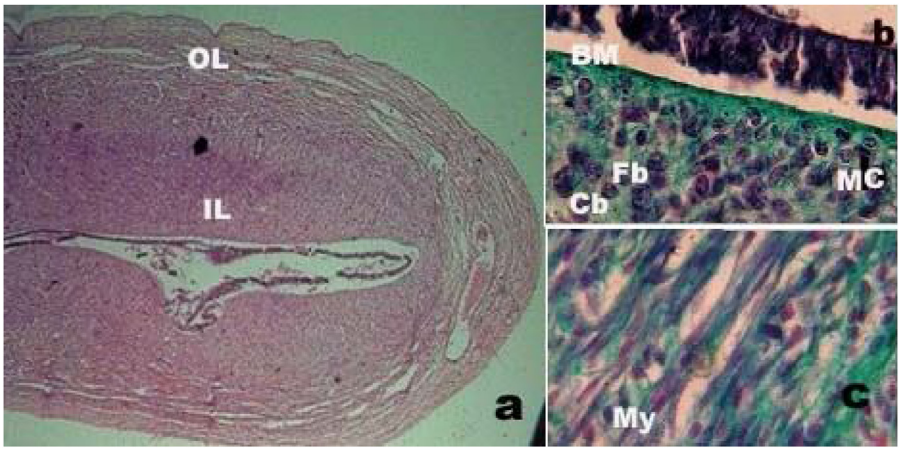

Fig. 3. Group III. a) Longitudinal section of 24 weeks uterus showing inner layer (IL) and outer layer (OL) cell thickness (H&E x5). b) Longitudinal section of compactly packed connective tissue with distinct basal membrane (BM), mesenchymal cells (MC), fibroblasts (Fb) and collagen fibrils (Cb) at 24 weeks (H&E x100). c) Longitudinal section of outer myocytes (My) in groups in 24 weeks uterus (Masson's Trichrome x100).

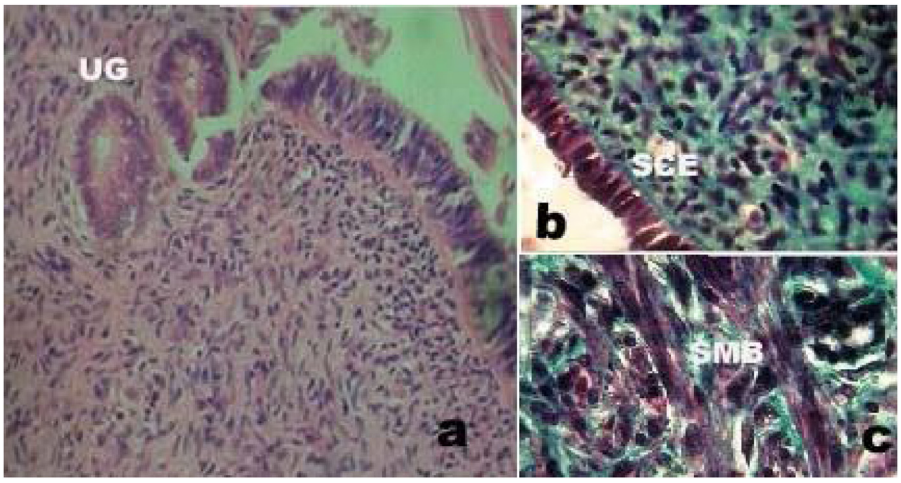

Fig. 4. Group V. a) Longitudinal section of developing uterine glands (UG) in cervical region at 32 weeks (H&E x40). b) Longitudinal section of simple columnar epithelium (SCE) of uterus at 32 week (Masson's Trichrome x100). c) Longitudinal section of developed smooth muscle bundles (SMB) in uterine body at 32 weeks (Masson's Trichrome x100).

The connective tissue stroma of cervix is composed mainly of collagen fibers. The smooth muscle bundles in the cervix are fewer in amount as compared to the body of uterus (Fig. 4c).

In the perimetrium collagen fibers along with the fibroblast cells and very few mesenchymal cells are seen in Masson's Trichrome stained tissues. Larger blood vessels, with well developed tunica media are seen in this zone.

3.6. 34–40 week (Group VI)

The lining epithelium has become simple columnar ciliated epithelium but still pseudostratified columnar epithelium is also seen. The cells have distinct cell boundary. A continuous basement membrane is clearly seen as in previous group.

The gland differentiation is still seen. The glandular wall are seen lined by simple columnar ciliated epithelial cells having elongated vesicular nuclei with clear basement membrane and just underneath it fibroblast cells are seen. Numerous glands are seen in the endometrium. Very few glands are seen reaching the myometrium (Fig. 5b). Fibromuscular stroma is mostly made of well-developed smooth muscle cells. The smooth muscle bundles in the cervix are fewer in amount as compared to the body of uterus.

Elastic fibers are not visible anywhere as seen under Verhoeff's stain except in the tunica media of the arteries and arterioles (Fig. 5c).

4. Discussion

The primodial uterus is formed by the fusion of the lower part of the paramesonephric duct by 10th week of gestation. The uterine development and function depend on epithelial mesenchymal interactions.[2] These interactions provide local control and coordination of morphogenetically important cell behaviors, including movement, adhesion, differentiation, and proliferation.[3]

The younger fetuses at 12th week of gestation, uterine epithelium appeared pseudostratified, consisting of basal, intermediate and superficial nuclei. At 18th week the epithelium of the lower part of cervix is made up of mosaic of flat or slightly raised polygonal cells.[4]

At 12 weeks of gestation, mesenchymal cells are distributed sparsely in the uterine mesenchyme. They are mostly round in shape with high nucleo-cytoplasmic ratio. Smooth muscle is not identified at this stage. By 14th weeks two layers of mesenchymal cells can be seen; the cells near the serosa are most abundant and elongated, whereas the cells towards the lumen are sparse and

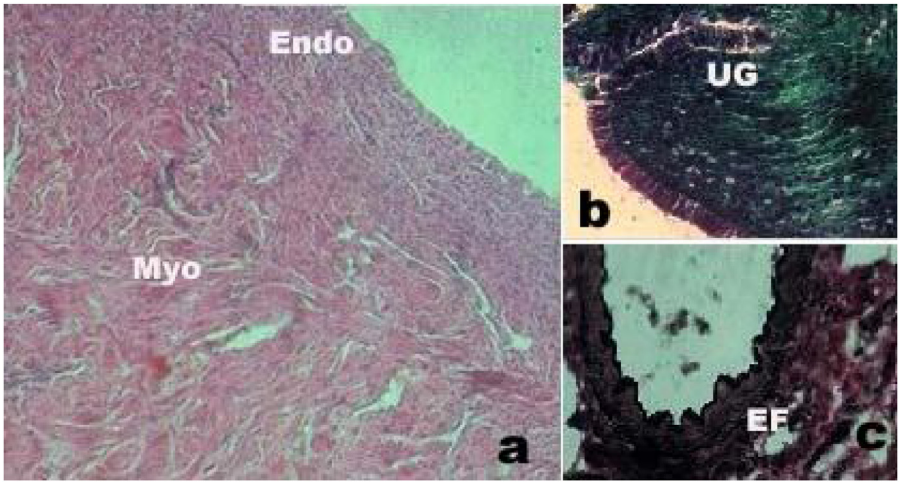

Fig. 5. Group VI. a) Cross section of endometrium (Endo) and myometrium (Myo) thickness at 39 weeks uterus (H&E x10). b) Cross section of uterine gland (UG) of 38 weeks uterus (Masson's Trichrome x100). c) Cross section of artery showing elastic fibers (EF) in tunica media in 38 week (Verhoeff's stain x40).

round in shape. Rich vascular networks are observed between the outer layer and the serosa. The mesenchymal cell with round shape having high nucleo-cytoplasmic ratio are abundantly present. This connective tissue stroma shows an inner zone mostly consisting of mesenchymal cells and an outer zone with more of fibroblasts which is spindle shaped, and few of mesenchymal cell. From 14th to 20th weeks spindle shaped cells of the outer layer gradually increased in number. At 24 week of gestation, the inner and outer zone thickness becomes similar. By 28 week of gestation the outer layer thickness increases due to development of more smooth muscle bundles.

At 26 weeks the thickness of the outer layer increased markedly and at 31–40 weeks bundles of cells like smooth muscles are obvious in this layer. This observation implies that the outer mesenchymal layer of the body of the fetal uterus gives rise to the myometrium and that the inner layer corresponds to the endometrial stroma of the adult uterus. These findings are similar to these observations by Konishi I.[5]

The uterine smooth muscles originate from the undifferentiated mesenchymal cells and at 12 weeks of gestation.[6] Moreover, immature smooth muscle cells are identified in the region between the myometrial stromal layers both at 26 and 32 weeks, when bundles of almost matured smooth muscles cells are already formed. These observations imply that undifferentiated mesenchymal cells which develop into smooth muscle cells may exist in the inner layer of the fetal uterus and that smooth muscle differentiation may occur in the junctional area between the myometrial and endometrial stromal layers. The smooth muscle bundles originate from the undifferentiated mesenchymal cells by 24 weeks of gestation in the subserosal area. The undifferentiated cells are seen arranged radially directing towards the inner zone. In the middle third, the smooth muscle bundles are well developed oriented in different directions - parallel, oblique and circular pattern. In the outer one-third, beneath the serosa, majority is parallel and intertwined to serosa. These observations imply that the mesenchymal cell further away from the epithelium differentiate earlier. This results in differentiation of myometrium commencing from periphery towards the inner aspect.

The endometrial gland development is very superficial and starts appearing by 20–22 weeks of gestation. In this study the endometrial gland starts appearing from 17 weeks but the development is seen superficial in agreement with the findings reported by Koff AK.[6]

The endometrial histoarchitecture at birth resemble that of adult, though less developed. Neonatal endometrial luminal epithelium is low columnar or cuboidal, and glandular epithelium is sparse and limited to the adluminal stroma.[7,8] However, in the present study, the glandular epithelium is lined by pseudostratified columnar epithelium in the early weeks of gestation. At term it is lined by simple columnar epithelium.

Although initiated during fetal life, endometrial gland proliferation in the human uterus is completed postnatally, in a manner similar to that observed for domestic ungulates.[9,10] Thus, genesis of endometrial gland in the human fetus and neonate involves differentiation of glandular epithelium from luminal epithelium and development of glandular epithelium through endometrial stroma to the myometrium. Similar finding were noted in this study.

The study result and observations of fetal uterine mesenchyme suggest that the undifferentiated mesenchymal cells around paramesonephric duct have the potential to develop into both smooth muscle cells and endometrial cells.

5. Conclusion

Histologically, the epithelium throughout the fetal uterine lumen except the lower part of the cervix is lined by pseudostratified columnar epithelium. The endometrial glands start appearing from 17 weeks onwards. In early weeks it is lined by pseudostratified columnar epithelium and by term it is replaced by simple columnar epithelium. The palmate folds of the epithelium appear by 17 weeks in cervical canal. Seen.The smooth muscle bundle appears by 24 weeks of gestation. The myometrium which is visible in three zones increases in thickness with advancing foetal age. From 32 weeks onwards the pseudostratification gradually changes to simple columnar epithelium. At term, very few pseudostratified columnar epithelium is seen.

Conflicts of interest

All authors have none to declare.

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