Section: Anatomy

Original Research Article

A HISTOLOGICAL STUDY OF THE FOETAL CERVIX UTERI IN MANIPUR, INDIA

Rajkumari Kshemitra[1], Okram Sarda Devi[2], Kanmi Ningshen[3], N. Saratchandra Singh[4], Pinky Karam[5], T.D.Varneikip Chiru[6]

[1]*Assistant Professor, Department of Anatomy, Churachandpur Medical College, Churachandpur, Manipur, India*
[2]*Associate Professor, Department of Obstetrics and Gynaecology, Churachandpur Medical College, Churachandpur, Manipur, India*
[3]*Assistant Professor, Department of Physiology, Churachandpur Medical College, Churachandpur, Manipur, India*
[4]*Professor, Department of Anatomy, Regional Institute of Medical Sciences, Imphal, Manipur, India*
[5]*Assistant Professor, Department of Biochemistry, Churachandpur Medical College, Churachandpur, Manipur, India*
[6]*Assistant Professor, Department of Surgery, Churachandpur Medical College, Churachandpur, Manipur, India*

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Corresponding Author:
Dr. Rajkumari Kshemitra,
Assistant Professor, Department of Anatomy, Churachandpur Medical College, Churachandpur, Manipur, India.
Email: kshenista@gmail.com

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ABSTRACT

Background: Considering the clinical importance of the cervix uteri, updated knowledge of histogenesis in human foetuses of different age groups up to term would be of paramount value. Hence the present work was undertaken to document the sequence of events in the histological development and maturation of the cervix uteri during foetal life.

Materials and Methods: 50 human foetuses of 13 weeks to 38 weeks of gestational ages without any external anomalies were studied in the Department of Anatomy, RIMS, Imphal, Manipur.

Results: Histologically, the lining epithelium at 13 week was bilayer cuboidal or short columnar cells, from 14 weeks onwards, the lining epithelium were pseudostratified columnar ciliated epithelium except for the lower part of the cervical canal, which were lined by stratified squamous non-keratinized epithelium. From 31 weeks onwards, the pseudostratified columnar epithelium was gradually changed to simple columnar ciliated epithelium and from 34 weeks onwards, the lining epithelium were lined by simple columnar ciliated epithelium. The squamocolumnar junction was evident from 17 weeks onwards at the lower part of the cervical canal. Developing cervical glands or infoldings (crypts) were first evident from 15 weeks. The palmate appearance of the mucosal infoldings was first observed from 19 weeks onwards. The developing myometrial area, in the early age groups few myocytes are visible supported by collagenous fibres which becomes progressively thicker with advanced age groups.

Conclusion: The study documents the sequential histological maturation of the human foetal cervix uteri from 13 weeks to term, highlighting the epithelial differentiation, stromal organisation, glandular development and myometrial growth with in progressive age groups. Hence, it will help in understanding and add to the existing knowledge regarding the normal histological development of foetal uterine cervix.

Keywords: cervix uteri, cervical canal, squamocolumnar junction.

INTRODUCTION

In an adult uterus, the cervix uteri (Latin for neck) form the lower third of the uterus. It is about 2.5 cm in length, approximately 2 cm in diameter and comprises about 50% of the total uterine volume.[1,2] A fibromuscular junction, the isthmus separates the cervix from the corpus. The cervix extends downward and backward from the isthmus to the opening within the vagina. It is the least mobile part of the uterus and is divided into supravaginal and vaginal parts by the anterior wall of the vagina. The cervical canal in shape is fusiform longitudinally and it communicates with the main uterine cavity via the internal os and with the vagina by the external os. Two longitudinal ridges, one each on the anterior and

posterior walls of the canal gives off small oblique palmate folds which ascend laterally like the branches of a tree (arbor vitae uteri), interdigitates to close the canal.[3] The endometrium of cervix is continuous above with the uterine mucosa at the internal os and below with the vaginal mucosa through the external os.[4,5] The cervical mucosa measures 2-3mm in thickness and differs dramatically from the rest of the uterine endometrium in that it contains large, branched glands, also, it lacks the spiral arteries.[6] The endocervical canal is lined with tall, cylindrical mucus secreting columnar epithelium.[7] The mucous membrane of the cervical canal shows branching folds on its surface and comprises of an epithelium and a lamina propria. The endocervical cells invaginate into the cervical stroma to a depth of approximately 5 to 8 mm to represent crypt formation called endocervical glands.[2] The squamocolumnar junction is where the columnar secretory epithelium of the endocervical canal meets the stratified squamous covering of the ectocervix and is located at the external os before puberty.[1]

MATERIALS AND METHODS

A cross-sectional study was conducted in the Department of Anatomy, RIMS, Imphal, Manipur, India from September 2015 to August 2017 after taking formal approval of Institutional Ethics Committee. 50 normal female human foetuses of 13-38 weeks of gestational ages which were products of medically terminated pregnancy and stillborn normal human foetuses were collected from the Department of Obstetrics and Gynaecology, RIMS, Imphal, Manipur. India. The age of the foetuses was calculated from the crown- rump lengths (CRL) and were categorized into 4 age groups: Group A (13–18 weeks), Group B (19–24 weeks), Group C (25–30 weeks), Group D (31–38 weeks). The tissues were subjected to routine histological processing and stained with Haematoxylin and Eosin (H&E), Masson's Trichrome and Verhoeff's and studied under trinocular compound light microscope and microphotographs were taken and stored. Data obtained were analysed statistically using SPSS version 21.

RESULTS

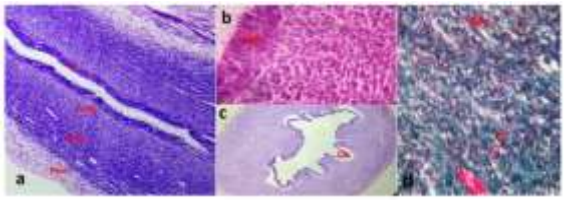

Figure 1: Group A (a) longitudinal section (H&E. x10) of 13 weeks cervical canal showing distinct lining epithelium with endometrium (Endo), myometrium (Myo), Perimetrium (Peri) (b) longitudinal section (H&Ex40) of 14 weeks cervical canal lined by pseudostratified columnar epithelium (PSE) (c) cross section of the cervix uteri at 18 weeks (H&E. x10) showing the cervical glands (CG) infolding in the endocervical region. (d) cross section of the cervix uteri at 18 weeks (Masson's trichome. x100) showing the myocyte (MY) supported by collagenous fibres (CF).

GROUP - A (13-18 Weeks): At 13 weeks, the developing cervix uteri was lined by short columnar or cuboidal cells arranged in 2 layers. The cellular component was more abundant than the fibrous component of the area. Few myocytes are visible supported by collagenous fibres. Along with it very few thin-walled blood vessels were observed [Figure 1a]. At 14 weeks (x40), the developing mucosa of the cervix uteri was observed to be lined by pseudostratified tall columnar epithelium [Figure 1b]. The lower part of cervical canal was lined by mosaic of flattened cells. At 15 weeks, the lining epithelium invaginated the underlying subepithelial area of endometrium, thus indicating the first appearance of the developing cervical glands.

At 17 weeks, the developing mucosa of the cervix uteri was lined by pseudostratified ciliated columnar cells and the lower part of the cervical canal was lined by stratified squamous epithelium of non- keratinized variety resting on a basement membrane. Different stages of developing gland formation are visualized at this stage. At 14 to 17 weeks, greater number of developing myocytes were observed supported by few thin collagenous fibres in the developing myometrial area. At 18 weeks, the developing glands were visualized as outpocketings of the lining epithelium. Deeper parts of the glands were lined by pseudostratified columnar epithelium and the superficial part or surface of the glands were lined by simple columnar epithelium [Figure 1c]. The developing myometrium were formed by more developed myocytes [Figure 1d].

GROUP - B (19-24 Weeks): At 19 weeks, the lower part of the cervical canal was lined by stratified squamous non-keratinized epithelium. Increasing number of developing glands appeared like branches of trees, thus, the first appearance of the early plica palmatae. The mouths of the developing glands were lined by simple columnar epithelium (Fig.2a). The developing endometrial area was seen much thicker compared with the earlier age group. The stromal cell

predominates and the collagenous fibres becomes progressively less in this area.

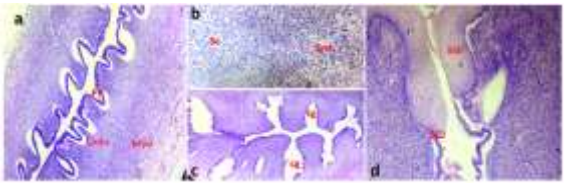

Figure 2: Group B (a) longitudinal section of the cervix uteri at 19 weeks (H&E. x10) showing the early appearance of plicae palmatae (PP) in the endocervical region with clear distinction of Endometrium (endo) & myometrium (myo) is seen. (b) longitudinal section of cervix uteri at 20 weeks (H&E. x40) showing the interface between the endometrium with stromal cells (Sc) and myometrium with smooth muscle cells (Sm). (c)longitudinal section of cervix uteri at 21 weeks (H&E. x10) showing developing cervical glands. (d)longitudinal section of lower part of cervical canal at 21 weeks showing the simple squamous non keratinised epithelium (SSE) and the squamocolumnar junction (SCJ). H&E. x40.

At 20-24 weeks, the lining epithelium was simple columnar ciliated epithelium. However, pseudostratified columnar epithelium was still seen lining certain areas of the cervical canal. In the lower part of the cervical canal, the columnar epithelium changed abruptly into stratified squamous non-keratinized epithelium at the squamocolumnar junction [Figure 2d]. The developing glands are seen to branch [Figure 2c]. From 19 to 23 weeks, the developing myometrium becomes progressively thicker compared with the earlier age group. A clear distinction between the endometrium and myometrium can be delineated at age group [Figure 2a]. The ratio of the thickness of the endometrium to the myometrium is about 2:1. At 24 weeks, the developing myometrium is composed of well organised smooth muscle fibres, which are arranged in bundles, oriented in an irregular manner throughout this area supported by fibroblast and thin collagenous fibres [Figure 2b]. The perimetral area in this age group is more fibrous and less cellular, consisting mainly of collagenous fibres with sparsely arranged fibroblast. Large thin-walled blood vessels are seen to be progressively increasing.

GROUP - C (25-30 WEEKS): From 25-27 weeks, in the lower part of the cervical canal, squamocolumnar junction (SCJ) was seen at the level of the external os. The stratified squamous non-keratinized epithelium is seen lining the external surface (Exo cx) of the cervix. [Figure 3a].

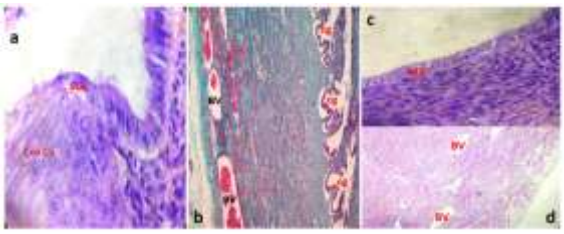

Figure 3: Group C (a) longitudinal section of cervix uteri at 25 weeks (H&E. x100) showing the squamocolumnar junction (SCJ) at the level of the external os. (b) longitudinal section of cervix uteri at 25 weeks (Masson's trichome. x10) showing the crypt like invagination of the cervical glands (cg). (c) longitudinal section (H&E. x40) of cervix uteri at 28 weeks showing the pseudostratified lining epithelium conforming towards simple columnar epithelium (SCE) (d) longitudinal section (H&E. x10) of cervix uteri at 28 weeks showing the developing smooth muscle bundles and wide lumen blood vessels (BV) in the developing perimetral area.

The cervical glands are also more developed, highly branched and the epithelial outpouching are more in depth (crypt like) but not reaching the myometrium [Figure 3b]. Gradual increase in the number of vascular elements is observed in this zone. The myometrium is much thicker and the ratio of the myometrium to the endometrium becomes 2:1. At 28 weeks, the mucosa in the upper part of cervical canal of the cervix uteri was seen conforming to simple tall columnar ciliated epithelium [Figure 3c]. The endometrial area was much thinner and the myometrial area was progressively thicker and much better organised. The vascular elements are increased and more organized in the form of thin-walled capillaries to thicker walled arterioles [Figure 3d].

GROUP - D (31 -38 WEEKS): At 31-32 weeks, the mucosa of the cervix uteri is lined mostly by simple columnar ciliated epithelium [Figure 4a]. The cervical glands are also more developed, highly branched and the epithelial outpouching are seen more in depth but not reaching the myometrium. The myometrium is much organized and appears thicker, more condensed and solid, smooth muscle cells are arranged in compact bundles, with collagenous fibres interspersed in different directions [Figure 4c].

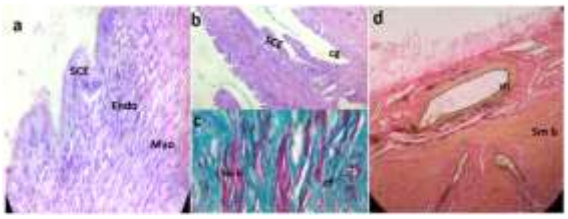

Figure 4: Group D (a)longitudinal section (H&E. x40) of cervix uteri at 32 weeks showing the lining epithelium by simple columnar ciliated epithelium (SCE) with thinner endometrium (endo) and thicker myometrium (myo). (b) longitudinal section of cervix uteri at 34 weeks (H&E. x40) showing the crypt like cervical glands (cg) lined by transformed simple columnar epithelium (CSE). (c) longitudinal section of cervix uteri at 32 weeks (Masson's trichome x100 oil) showing the myometrium with smooth muscle bundles (Sm b) interspersed between collagenous fibres (cf). (d)longitudinal section of cervix uteri at 36 weeks (Verhoeff's stain x40) showing the elastic fibres in internal elastic lamina (IEL) of a muscular artery present in the perimetrium.

At 34-38 weeks, the lining epithelium is simple columnar ciliated epithelium. The developing cervical gland is highly branched, forming crypt like infolding and increasing in number and in depth [Figure 4b]. Few glands are seen reaching the myometrium. The endometrial area is seen decreased in area due to the increase in the myometrial thickness. The vascular element is seen to have enhanced in this age group. In this age group, the perimetrium is also seen to be well organized with more fibrous component then cellular component. The vascular elements are seen well developed and organised consisting of increased number of thin walled to thicker walled blood vessels. Elastic fibres are seen in muscular arteries as internal elastic lamina which are present in the outermost perimetral area [Figure 4d]

DISCUSSION

The present study documents the sequential histological maturation of the human foetal cervix uteri from 13 weeks to term, highlighting the epithelial differentiation, stromal organisation, glandular development and myometrial growth with in progressive age groups.

The epithelial lining of the developing endocervix in the early age groups shows considerable variability. Previous studies described a layer of flatten cells at 12 weeks,[8] and a monolayer blastema like epithelium of undifferentiated cuboidal cells and ciliated cells at 14 weeks.[9] In contrast, the present study identified a lining epithelium composed of short columnar and cuboidal cells arranged in 2 layers giving an appearance of stratification. This suggest that epithelial maturation may commence earlier than previously reported.

From 34 weeks onwards, the epithelial pattern in the present study closely resembled that of the adult cervix, with simple columnar epithelium in the upper cervical canal and non-keratinized stratified squamous epithelium in the lower portion. These findings support earlier observations of epithelial differentiation towards term.[10]

Earlier study reported pseudostratified of the endocervical epithelium with higher, apically convex and shorter basal cells at 20 weeks and glandular development with evident plicae palmatae at 22nd week,[8] the present study observed pseudostratified tall columnar cells lining the endocervical canal as early as 14 weeks onwards and first formation of cervical glands was seen at 15 weeks with evident plicae palmatae formation at 21 weeks.

In early human foetuses, the squamo-columnar junction resides in the upper part of the cervical canal, closer to the uterus than the vagina,[11] and gradually descends toward the vaginal surface with advancing gestation.[12] The present study, the squamocolumnar junction was evident at the lower part of the cervical canal at 19 weeks and descents down towards the external os of the cervix.

The wall of the cervix has three layers- mucosal layer, muscular layer and serosa layer.[10] Consistent findings were observed in the present study, where in all the specimens (13-38 weeks), three zones were delineated – an inner developing endometrial area, a middle developing myometrial area and an outer perimetral area.

Earlier studies have described the myometrial layer of the prenatal uterus increases in thickness with advancing gestational age.[13,14] In the present study, the endometrium-to-myometrium ratio was 2:1 between 19 and 24 weeks and 1:2 by 25 weeks, with the myometrium predominating at term. Histologically, the cervical myometrium was composed mainly of abundant smooth muscle fibres interspersed with sparse collagenous fibres.

CONCLUSION

Histologically, in the 13-week foetus, the lining epithelium was arranged in two layers of short columnar or cuboidal cells. From 14-18 weeks, showed pseudostratified columnar ciliated epithelium in the upper part of the cervical canal and stratified squamous non keratinized epithelium in the lower part of the cervical canal. From 19 weeks onwards, the pseudostratified columnar ciliated epithelium was gradually conformed towards simple columnar epithelium at different areas of the upper part of the cervical canal. From 34 weeks onwards, the lining epithelium resembled that of the adult. The squamocolumnar junction was evident from 17 weeks onwards at the lower part of the cervical canal where the pseudostratified columnar epithelium changed abruptly into stratified squamous non-keratinized epithelium. At 25 weeks, the squamocolumnar junction was seen to descend downwards towards the external os. Developing cervical glands or infoldings (crypts) were first

evident from 15 weeks. The palmate appearance of the mucosal infoldings was first observed from 19 weeks onwards. The developing endometrium was seen consisting of undifferentiated mesenchymal cells supported by few collagenous fibres at 13 weeks. From 19 weeks onwards, the endometrium became thicker than the myometrium and was composed of more cellular components and less fibrous components. From 27 weeks onwards, the developing endometrium appears thinner than the myometrium. From 19 weeks onwards, well organized smooth muscle bundles were observed. From 25 weeks onwards, the thickness of the myometrium progressively increases than the endometrium, with a ratio of 2:1. At term, the myometrium was abundant with bundles of smooth muscle fibres oriented obliquely or parallel to the outer serosa layer. The developing perimetrium were evident in all the specimens. The vascular elements were seen increasing with progressive increase in the gestational age. The present study will be helpful in understanding and add to the existing knowledge regarding the normal histological development of foetal uterine cervix.

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