

Histogenesis and histochemical observations on cervix of buffalo during prenatal life

VARINDER UPPAL¹ and K S ROY²

Guru Anged Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 114001 India

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ABSTRACT

The study was conducted on cervixes of 27 buffalo foetii collected from abattoir at different stages of gestation. The tissues were processed and sections were stained with hematoxylin and eosin, Masson's trichrome, Gridley's, Verhoeff's and Holmes methods. For histochemistry the sections were stained with periodic acid schiff, alcian blue and bromphenol blue for demonstration of neutral mucopolysaccharides, acid mucopolysaccharides and basic proteins. For lipid demonstration fresh cryostat sections were cut and stained with Sudan Black B and acid hematin. Primary folds of mucosa were observed at 21.0 cm CVR (121 days) and secondary at 35.0 cm CVR (152 days). Epithelium varied from simple columnar to pseudostratified columnar ciliated. At 13.5 cm CVR the cervico vaginal junction was lined by stratified squamous epithelium and extensive cellular mass in the lumen probably represented the part of vaginal plate. The propria submucosa comprised mesenchymal tissue in early fetal life which later on differentiated into different components of connective tissue. At 14.0 cm CVR the bundles of smooth muscles could be observed in tunica muscularis and a serosal layer comprised of differentiating mesenchymal cells and mesothelium could be seen. A moderate to strong reaction of PAS and weak to moderate reaction of Alcian blue/PAS was seen in tunica mucosa, muscularis and serosa in groups 1, 2 and 3. The reaction for basic protein, phospholipids and total lipids was weak to moderate in group I and increased with the progression of fetal age.

Key words: Buffalo, Cervix, Histogenesis, Histochemistry, Prenatal

Cervix or the neck of uterus comprised 3 layers (Priedkalns 1981). In literature, information is available on the histology and histochemical studies on cervix during postnatal life in buffalo (Singh 1983, Uppal and Roy 1997, Uppal and Roy 2000) and in cattle (Wordinger *et al.* 1972, Sato *et al.* 1982) but very scanty information is there on cervix during prenatal life (Baishya and Vyas 1993) so keeping in view the paucity of literature present project was proposed.

MATERIALS AND METHODS

The present study was conducted on cervixes of 27 female buffalo foetii of different gestational age collected from abattoir. After measuring the crown rump length of foetii, their approximate age was calculated by using the formula given by Soliman (1975). The samples were divided into 3 groups – group 1, foetii of CVR up to 20 cm; group 2, foetii of CVR between 20–40 cm; and group 3, foetii of CVR above 40 cm.

The tissues from cervix were fixed in Neutral buffered formalin and Bouin's fixatives and were processed for

paraffin blocks preparation by acetone benzene schedule (Luna 1968). The sections of 5–6 µm were cut with rotary microtome and stained with haematoxylin and eosin for histological studies, Masson's trichrome for collagen fibres, Verhoeff's for elastic fibres, Gridley's for reticular fibres, Holme's for neuronal elements, periodic acid Schiff for neutral mucopolysaccharides, alcian blue for acid mucopolysaccharides and bromphenol blue for basic proteins. The cryostat sections of 10–20 µm at –20°C were stained by Sudan black B to demonstrate lipids and acid haematin for phospholipids (Chayen *et al.* 1969).

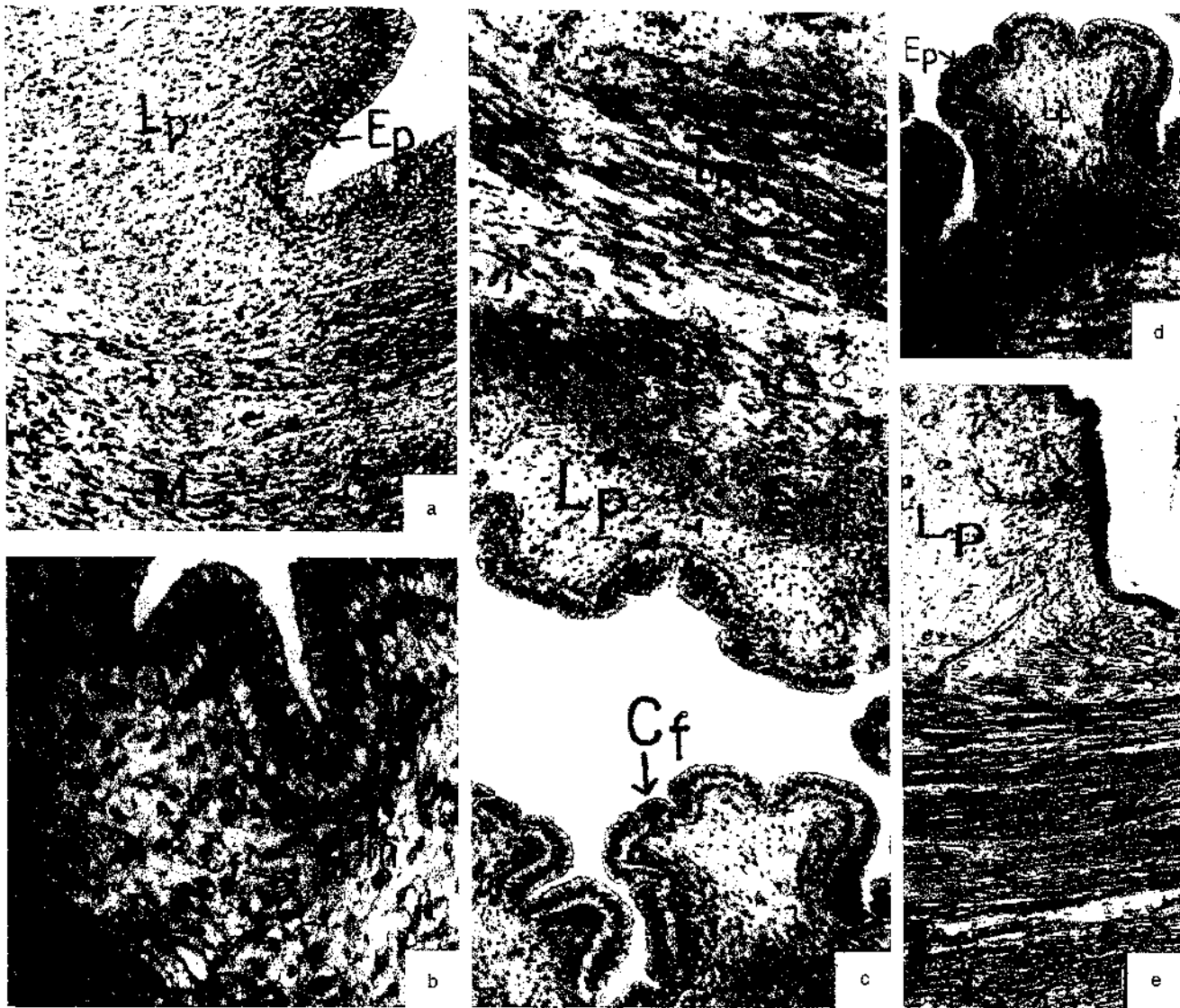
RESULTS AND DISCUSSION

Histogenesis

In group I the lumen of the cervix was giving a star shaped appearance (Fig.a). The primary folds were first observed at 21.0 cm CVR (121 days) and secondary at 35.0 cm CVR (152 days) whereas Baishya and Vyas (1993) reported formation of primary folds at 85 days, secondary folds at 94 days and tertiary folds at 130 days in Surti buffalo fetus.

The average total thickness of the wall of the cervix in groups 1, 2 and 3 was 638.44 ± 27.93 µm, 843.92 ± 77.05 µm and 2546.10 ± 416.50 µm, respectively. The thickness of cervical wall also includes the height of the mucosal folds.

Present address: ¹Associate Professor; ²Emeritus Scientist (ICAR).



Figs. a-e. (a) Cervix of 14.0 cm CVR (91 days) foetus showing epithelium (Ep), differentiating lamina propria submucosa (Lp) and smooth muscle cells (M). H & E. $\times 175$; (b) Cervix of 35.0 cm CVR (152 days) foetus showing collagen fibres (Cf) in basement membrane (BM) and lamina propria (arrow). Masson's trichrome. $\times 350$; (c) Cervix of 35.0 cm CVR (152 days) foetus showing cervical folds (Cf), lamina propria (Lp) and tunica muscularis (Tm). H & E. $\times 87.5$; (d) Cervix of 35.0 cm CVR (152 days) foetus showing epithelium (Ep), lamina propria (Lp) and tunica muscularis (Tm). H & E. $\times 175$; (e) Cervix of 79.0 cm CVR (252 days) foetus showing collagen fibres in lamina propria submucosa (Lp), tunica muscularis (Tm) and tunica serosa (Ts). Masson's trichrome. $\times 87.5$

The thickness of wall of cervix in all the groups differed significantly ($P < 0.05$) from each other.

Lamina epithelialis mucosae: In group 1 at 14.0 cm CRL (91 days) the epithelium was mostly simple columnar with cilia at some places (Fig. a). The basement membrane was well developed. In group 2 and 3 the epithelium varied from simple columnar to pseudostratified columnar ciliated (Fig. c). Intraepithelial infiltration of lymphocytes at certain places could be observed. The height of the epithelium decreased with the progression of fetal age. This supports the earlier findings of Baishya and Vyas (1993) in the Surti buffalo fetus. Eida (1961) has claimed that at 185 μm not only the vagina

but also the cervical canal is lined by stratified squamous epithelium so that the squamocolumnar junction is situated very high. The extensive cellular mass in the lumen in the present study at 13.5 cm CVR (89 days) may represent the part of the vaginal plate.

The average height of the epithelium of cervix in groups 1, 2 and 3 was $35.33 \pm 1.57 \mu\text{m}$, $29.54 \pm 2.13 \mu\text{m}$ and $21.71 \pm 1.01 \mu\text{m}$, respectively and they differ significantly ($P < 0.05$) from each other. Desjardins and Hafs (1969) reported the epithelium of bovine newborn to be $19.10 \pm 0.7 \text{ mm}$. So the values of the group 3 are in close agreement with the values of Desjardins and Hafs (1969).

Propria submucosa

The propria submucosa in group 1 at 14.0 cm CVR (91 days) comprised differentiating mesenchymal cells with large number of blood capillaries (Fig. a). The collagen fibres were fine and few in group 1 but fibers increased in group 2 (Fig. b). Fine reticular fibers could also be observed in the basement membrane and propria-submucosa and their quantity increased with the progression of fetal age.

Elastic fibers were few and mainly present in the wall of blood vessels. Red blood cells aggregates could also be seen in the propria (Fig. d). In group 3 at 42.0 cm CVR (168 days), the submucosa consisted of loosely arranged connective tissue and differentiating smooth muscle cells (Fig. e) and at 79.0 cm CVR (252 days), the propria submucosa comprised fibroblasts, connective tissue fibers, blood vessels and myelinated- and non-myelinated nerve fibers. Some of the smooth muscle cells from the inner circular layer could be seen extending into the propria submucosa in the later period of fetal life. Baishya and Vyas (1993) also reported reticular and collagenous connective tissue framework with a few elastic fibres in the propria submucosa of the cervix in Surti buffalo fetus.

Tunica muscularis

In group 1 at 14.0 cm CVR (91 days), the mesenchymal cells were found differentiated into smooth muscle cells and the bundles of muscle cells could be seen at locations (Fig. a). Baishya and Vyas (1993) have also reported the development of muscularis in Surti buffalo fetus of 94 days. The differentiation of mesenchymal cells into smooth muscle cells was better in the cervix than the uterus at a time. Szamborski and Laskowska (1968) based on the histochemical studies indicated that the development of the cervix precedes that of the corpus during fetal life. The differentiation was better in group 2 and a large number of muscle cells could be seen intermingled with the loosely arranged connective tissue (Fig. c). In group 3 at 79.0 cm CVR (252 days) the circular layer of smooth muscle cells could be well appreciated (Fig. e) whereas at 105.0 cm CVR (310 days), a circular and longitudinally arranged layer of smooth muscle cells separated by a layer of blood vessels could be seen. A small amount of elastic fibers among the smooth muscle cells could also be observed at this stage.

Tunica serosa: A serosal layer comprised differentiating mesenchymal cells, and a mesothelium could be seen at 14.0 cm CVR (91 days). Baishya and Vyas (1993) have also reported the development of serosa in Surti buffalo fetus of 94 days. A large number of bundles of myelinated- and non-myelinated nerve fibres and ganglionic cells could also be observed in the outer part of cervix. In group 2 and 3 well developed collagen fibers and few reticular fibers were evident (Fig. e). The elastic fibers were mainly present in the wall of blood vessels. Uppal and Roy (2000) observed that

Table 1. Histochemical observations on the cervix of buffalo during prenatal life

Histochemical Moety Structure	Group 1					Group 2					Group 3				
	PAS	AB PAS	Basic protein	Phospho lipid	Sudano phili Lipid	PAS	AB PAS	Basic Protein	Phospho lipid	Sudano phili Lipid	PAS	AB PAS	Basic protein	Phospho lipid	Sudano phili Lipid
Lamina epithelialis	++/++++	+/++	+/++	+/++	+	++/++++	+/++	++/++++	++/++++	+/++	++/++++	+/++	++/++++	++/++++	+/++
Propria submucosa	+/++	+	+	+	+	+/++	+/++	+/++	+/++	++/++	+/++	++/++	+/++	++/++	++/++
Tunica muscularis	++/++++	+/++	+/++	+	+	++/++++	+/++	++/++++	++/++++	+/++	++/++++	+/++	++/++++	++/++++	+/++
Tunica serosa	+/++	+/++	+	+	+	+/++	+/++	+/++	+/++	+	+/++	+/++	+/++	+/++	+

+, weak; ++, moderate; +++, strong.

the serosa of new born buffalo calf cervix comprised of loose connective tissue lined by mesothelium.

Histochemical studies

The histochemical localization of different moieties in cervix of buffalo during different stages of prenatal life has been summarized in Table 1.

Neutral mucopolysaccharides (NMPS): The lamina epithelialis showed a moderate to strong reaction for NMPS whereas the reaction was weak to moderate in propria and serosa in all the groups. The basement membrane was strongly positive. Tunica muscularis also showed a moderate to strong PAS reaction for NMPS. These NMPS are either glycogen or glycoproteins utilized as source of energy during cellular differentiation and growth. Davies and Kusama (1962) reported PAS-positive basal cells in the cervical epithelium and Uppal and Roy (1997) observed moderate to strongly PAS positive tunica mucosa and muscularis in new born buffalo calf.

Acid mucopolysaccharides: Lamina epithelialis, propria submucosa, muscularis and serosa showed a weak to moderate reaction for acid mucopolysaccharides in all the groups. The luminal contents were weakly PAS-positive. Uppal and Roy (1997) also observed moderately alcianophilic tunica mucosa and muscularis in cervix of new born buffalo calves.

Weak to moderate alcianophilia reflects the area of more glycosaminoglycans synthesis, degradation and morphogenetic activity as explained by Silbertstein and Daniel (1984).

Basic proteins: In group 1, epithelium and muscularis showed a weak to moderate reaction and propria and serosa showed a weak reaction for basic proteins. In groups 2 and 3, the reaction in epithelium and muscularis was moderate to strong and weak to moderate in propria and serosa. Uppal and Roy (1997) observed moderate to strong reaction in epithelium, cervical glands and luminal contents for basic proteins, whereas in the propria the reaction was weak and muscularis was moderately positive in neonatal buffalo calves.

Lipid: In group 1 weak sudanophilia was observed in epithelium, propria, muscularis and serosa of cervix during prenatal life. In group 2 and 3, moderate to strong positive sudanophilic fine lipid droplets were observed in epithelium and muscularis whereas propria and serosa showed a weak to moderate sudanophilia. The mucosa and muscularis of neonatal buffalo calf were also reported to be moderate to strong positive for Sudan black B and Oil red O (Uppal and Roy 1997).

Phospholipids: In group 1 all the layers were weakly positive for phospholipids and in group 2 and 3 fine granular moderate to strong activity of phospholipids could be seen in the epithelium and muscularis, whereas propria and serosa were weak to moderately positive.

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