

Dept. of Anatomy & Histology,
Faculty of Vet. Med., Suez Canal Univ.,
Head of Dept. Dr. A.H.K. Osman.

HISTOGENESIS OF THE FETAL VAGINA OF THE EGYPTIAN WATER BUFFALO (*BOS BUBALUS L.*) (With 6 Figs.)

By
A.H.K. OSMAN
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التطور النسيجي لمهبل الجاموس الممـــــرى

عبد الحميد عثمان

تمت دراسة التطور النسيجي لمهبل الجاموس في عدد ١٨ من الأجنة التي تراوحت أطوالها بين ٢٦، ١٠٥ سم . ولقد أظهرت الدراسة أن الغشاء المخاطي للمهبل يكون املسا وخاليا من البروزات فسي الأجنة الصغيرة ولكنه سرعان ما يترامى الى عدد من الثنايا الطولية التي يزداد ارتفاعها بتقدم العمر . وتتكون الصفيحة الطلائية من طبقة واحدة من الخلايا العمودية التي تطابق مثيلتها في الرحم، مما يبعث على الاعتقاد أن كلا من المهبل والرحم لهما منشأ واحد . وهذه الخلايا تتميز بافرازاتها التي تحوى كلا من الجليكوجين والمخاط المتعادل والحمضي . وكما تتميز الصفيحة الأساسية وتحت الطلائية بوجود عدد من خلايا الدم البيض . الأمحولة والتي قد تعيق انتاج الألياف الغرويه مما يساعد على انتشار ونمو الشبكة الوريديه للغشاء المخاطي والتي تصل لتمام تميزها في الإجنة اليافعة ، ويصل الرداء العضلي لدروة نموه في الأجنة اليافعة كي يصبح الأكثر سماكا اذا ما قورن ببقية الطبقات المكونة للجدار المبهي .

SUMMARY

The histogenesis of the fetal vagina of the water buffalo in Egypt was studied from a series of female fetuses ranging from 26 to 105 cm CVRL. The luminal surface of the mucosa was regular in the 26 cm CVRL stage. It became thrown into longitudinal folds in the succeeding stages.

The entire epithelial lining was made up of a single layer of cylindrical cells identical to those lining the endometrium. Hence, it is suggested that it is Müllerian-derived. These cells were actively secreting glyco-gen, neutral and acid mucins, especially in the early stages.

Monocytes and other mononuclear leukocytes were a characteristic feature of the lamina propria-submucosa, and it was suggested that they may have an inhibiting effect on collagen synthesis, to induce the growth and proliferation of the mucosal venous plexus. The latter was in the form of a vascular layer at the border between the lamina propria and submucosa in full-term fetuses.

The muscular coat reached its greatest thickness in the full-term fetus; at that stage it was the thickest of the three coats composing the vaginal wall.

INTRODUCTION

The literature on the development of the vagina of ruminants is very sparse. Available data on its fetal differentiation were insufficient to be discussed with the data obtained in the present study. Several studies have been done on human development, and information gained from this study is best compared to what has been done in humans.

Although the development of the human vagina has been extensively studied, several conflicting views still exist concerning its embryonic derivation. FELIX (1912) suggested Müllerian origin. KOFF (1933) concluded a joint Müllerian and sinusal origin; but a Müllerian and Wolffian origin was reported by BULMER (1957) and WITSCHI (1970).

The vagina is considered to be mainly of Müllerian origin in the rabbit (CARR, 1953), sheep (BULMER, 1956), rat (CUNHA, 1975) and golden hamster (HISANO, 1977).

The work presented here aims to study the micromorphogenesis of the vagina in buffalo during the prenatal period, with emphasis on the histogenesis of the various vaginal coats.

MATERIAL and METHODS

Eighteen female water buffalo fetuses were collected from Cairo slaughterhouse. Their crown vertebral rump lengths (CVRL) ranged between 26 cm - 105 cm. The genital tract was dissected out and the vaginal canal between the vestibule and the caudal cervical fornix was removed, then fixed in 10% formol saline for 1 week. The canal was divided into 3 equal segments, each of which was embedded in paraffin. Each segment was serially sectioned at 5µm thickness and stained with H & E, PAS, Alcian blue, Best's carmine, Weigert's elastic stain, Alcian blue-van Gieson stain and Gordon and Sweet's reticulin stain (VACCA, 1985).

RESULTS

26 to 29 cm stage:

The vaginal wall at the 26 to 29 cm CVRL stage was differentiable into the following layers: epithelial membrane; subepithelial mesenchymal layer; muscular coat; and outer adventitial covering (Fig. 1).

Along its entire length, the epithelial membrane was regular, with the exception of occasional shallow, V-shaped invaginations. The basement membrane was missing in such areas.

FETAL VAGINA, BUFFALO

The epithelium was made up of a single layer of cylindrical cells resting on a prominent PAS-positive and argyrophilic basement membrane. The large, oval nuclei of these cells were moderately basophilic, and occupied the basal two thirds of the cells. The supranuclear cytoplasm contained a faintly eosinophilic large globule; a more deeply eosinophilic infranuclear globule was also present (Fig. 2). The cytoplasm was strongly positive to PAS reaction and Best's carmine stain. The luminal epithelial surface was covered with a prominent alcianophilic coat. The vaginal lumen showed the presence of Best's carmine, alcian blue and PAS-positive homogeneous material.

The subepithelial mesenchymal layer (Lamina propria-submucosa) was very cellular. What appeared to be undifferentiated mesenchymal cells (and to a lesser extent, fibroblasts with retiform processes) were the principal types present in this region. Many histiocytes and other mononuclear leukocytes were also present (Fig. 2). The fibrous components consisted of collagenic and argyrophilic fibers. Thin-walled blood vessels were randomly distributed throughout the subepithelial region.

The muscular coat was made up of 6-15 layer of circularly-oriented smooth myocytes. This continuous coat was interrupted by the blood vessels crossing between the outer adventitial covering and the lamina propria-submucosa. The outer adventitial covering was structurally similar to the lamina propria-submucosa but the former was highly vascular and showed numerous hemopoietic cells of the leukoblastic series. This layer made up half to two-thirds of the total thickness of the vaginal wall.

31 to 37 cm stage:

From CVRL 31 to 37 cm, the mucosa was thrown into longitudinal folds (Fig. 3) with broad bases and narrow rounded summits. The epithelial cells showed a pronounced mitotic activity, and their luminal surfaces showed bleb-like protrusions indicating secretory activity. The lamina propria-submucosa was differentiable at this stage into a lamina propria with denser fibrous elements, and a submucosa with more dispersed cells and lower fibrous density (Fig. 4). The muscular coat became differentiated into inner circular and outer longitudinal layers, and it was interrupted by increasing numbers of blood vessels. The fibrous content of the outer adventitial coat was markedly increased.

40 to 45 cm stage:

At the 40 cm CVRL stage, the epithelial lining was highly vacuolated, and showed a slight drop in reactivity to PAS and Best's carmine stains. The mesenchymal cells in both the lamina propria-submucosa and adventitia had disappeared, and there was a substantial increase in the number of fibroblasts, argyrophilic and collagenous fibers. Many macrophages were observed crossing the epithelium to the vaginal lumen. Clusters of ganglionic cells were observed in close approximation to the outer longitudinal muscular layer.

46 to 68 cm stage:

From CVRL 46 to 68 cm, it was observed that the mucosal longitudinal folds became superficially divided into smaller ones by shallow furrows (Fig. 5). The epithelial lining showed a gradual loss of its vacuolation, with a substantially increased eosinophilia and a marked diminution in its reactivity to PAS and Best's carmine stain. A gradual decrease in the height of the epithelial cells accompanied by a substantial increase in the nuclear size comparable to the size of the cell.

With advancing age, the thin-walled blood vessels in the lamina propria-submucosa were markedly increased in number to form a well-developed venous plexus at the borderline between the lamina propria and submucosa in the full term fetus (Fig. 6).

Gradual increase in the thickness of the muscular coat at the expense of the overlying lamina propria-submucosa and the underlying adventitia was prominent, so that it became the thickest vaginal coat in the full-term fetus.

70 to 105 cm stage:

From CVRL 70 to 105 cm, additional longitudinal muscular layer was observed in some areas.

In the full term fetus, the adventitia covering the cranial third of the vagina, following the cervix, was externally lined with a single layer of attenuated mesothelial cells.

DISCUSSION

The histogenesis of the fetal vagina and the derivation of its epithelial lining are highly controversial. An essential preliminary is an understanding of the less-disputed development of the uterus (O'RAHILLY, 1973).

The early development of the uterovaginal canal of the buffalo in Egypt clarified that it originates from the fused Müllerian ducts. Therefore, this canal is entirely lined with simple columnar epithelium (OSMAN, 1989).

In the current investigation, the vaginal epithelial lining of the different developmental stages, up to full-term, was made up of simple columnar epithelium. Hence, it could be suggested that the vaginal epithelium of the buffalo is also of Müllerian origin.

Although there is general agreement of embryologists that the uterus of all species is derived from the fused Müllerian ducts, it is thought that the vaginal epithelium is derived from one or more of the following sources; the mesonephric (Wolffian)

FETAL VAGINA, BUFFALO

ducts, the paramesonephric (Müllerian) ducts, or the urogenital sinus (O'RAHILY, 1977).

There is general disagreement on the exact origin of the human vagina. FELIX (1912) has mentioned that its epithelium is of Müllerian origin, meanwhile, KOFF (1933) suggested a Müllerian origin to the upper portion and a sinusal origin to the lower segment. Derivation of the entire vagina from the urogenital sinus was reported by BULMER (1957) and FLUHMAN (1960). WITSCHI (1970) stated that the vaginal epithelium is of Müllerian and Wolffian origin; meanwhile FORSBERG (1973) claimed that it originates from sinusal or Wolffian epithelium.

CARR (1953) in the rabbits, BULMER (1956) in the sheep, CUNHA (1975) in the rat and HISANO (1977) in the golden hamsters have all pointed out that the major cranial part of the vagina is of Müllerian origin, but the minor caudal segment has a sinusal origin. On the other hand, KIMURA, *et al.* (1980) pointed out that the sinus epithelium grows out cranially underneath the vaginal Müllerian epithelium, if mice have been exposed to estrogen during fetoneonatal life. Recently, BOKE and DREWS (1983) declared that the interaction of the Müllerian and Wolffian ducts of mice embryos in organ culture is necessary for vaginal canal formation.

In the work presented here, the cylindrical cells lining the vaginal mucosa were characterized by luminal bleb-like protrusions, supranuclear cytoplasmic vacuolation, and positive reactivity to both PAS and Best's carmine stains. The luminal surface was covered by a prominent alcianophilic material. These features are suggestive of secretory activity. The contents of the vaginal lumen were PAS, Best's carmine, and alcian blue-positive. Hence, it can be concluded that the fetal vaginal epithelium of the buffalo secretes a mixture of glycogen and neutral and acid mucins, which may play a role in the growth and differentiation of the vaginal mucosa and prevent its luminal occlusion. Mucus and glycogen were reported in the guinea pigs (BURGOS and WISLOCKI, 1956) and in the albino mouse (NOMAGUCHI, 1965), neutral mucins in voles and guinea pigs (PERYT and SZUMOWSKA, 1968); neutral mucins, acid sulphate and sialic acid in the cows (IGUMNOV, 1967); glycogen, acid and neutral mucin in the rats (EJSMONT, 1968). Estrogen and progesterone are found to stimulate the formation and fluctuation of glycogen and mucosubstances in the vaginal epithelium (NOMAGUCHI, 1965; KENNEDY and ARMSTRONG, 1972).

The present findings revealed that the leukocytes and histiocytes were numerous among both the lamina propria-submucosa and the adventitial coat, with a decrease in the content of collagen fibers. The decrease of histiocyte concentration in older fetuses was accompanied by a substantial increase in collagenous fibers. It is suggested that these mononuclear cells may produce a collagen-inhibiting factor, reducing the formation of dense collagenic bundles. This reduction could induce the growth and differentiation of thin-walled blood vessels in the lamina propria-submucosa to form the well-developed venous plexus observed towards term. This suggestion coincides

with the findings of KITAMURA, et al. (1979) and ITO, et al. (1981), who mentioned that the cervical macrophage-monocytes produce collagenase, which inhibits collagen production, in order to induce cervical ripening and dilatation at the end of pregnancy. KITAMURA, et al. (1980) have reported that mononuclear leukocytes during pregnancy produce hyaluronic acid to degrade cervical collagen. In tissue culture, the cervical macrophages are able to produce collagenase to induce cervical ripening (ITO, et al. 1987).

With increasing age, the muscular coat was characterized by progressive thickening to reach up to a peak in the full-term fetus where it became the thickest vaginal coat. Postmortem contraction of this coat may be the reason for the longitudinal mucosal folds of the vagina that are observed in histological sections.

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FETAL VAGINA, BUFFALO

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LEGENDS

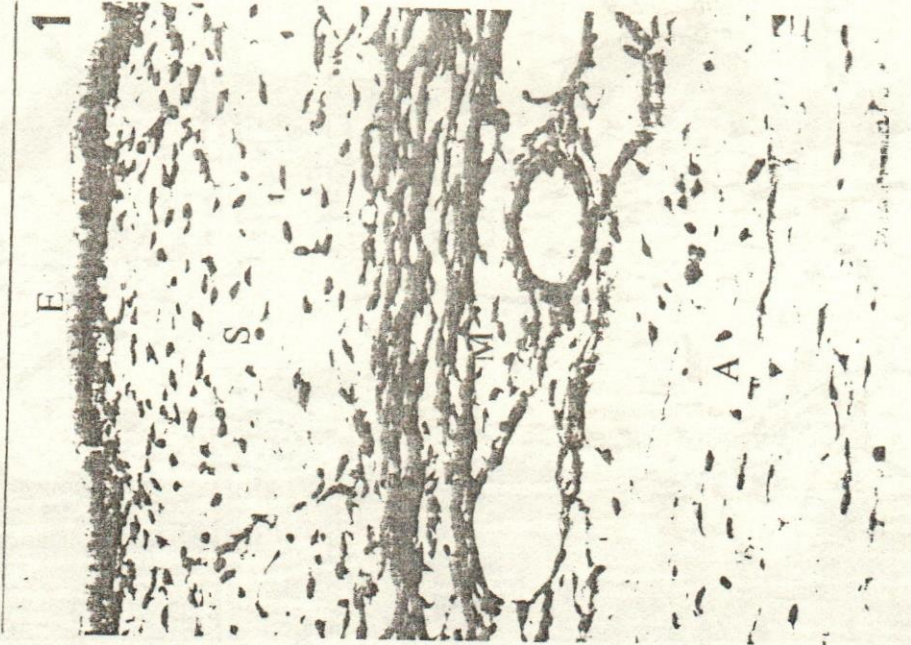
- Fig. (1):** Vaginal wall of the 26 cm CVRL fetus, showing lamina epithelialis (E); lamina propria-submucosa (S); muscular coat (M) and other adventitial coat (A). H & E X 100.
- Fig. (2):** Vaginal mucosa of the 28 cm CVRL fetus, showing vacuolated epithelial lining (E); lamina propria-submucosa with many histiocytes (arrow); and the vaginal lumen showing many macrophages (M). H & E X 200.

A.H.K. OSMAN

- Fig. (3):** Vagina of the 31 cm CVRL stage, showing mucosal longitudinal folds (arrow); strongly PAS positive epithelial lining (E); the lamina propria-submucosa (S) is invaded with many thin-walled blood vessels (V). PAS x 200.
- Fig. (4):** Vagina of the 37 cm CVRL stage, showing higher mucosal folds and the lamina propria-submucosa became differentiated into denser lamina propria (P) and looser submucosa (S); muscular coat (M); adventitia (A). Van Gieson X 100.
- Fig. (5):** Vagina of the 46 cm CVRL stage. The mucosal folds became superficially subdivided into smaller ones (arrow), thin-walled blood vessels (V) forming a plexus at the border between the lamina propria and submucosa; muscular coat (M). H & E X 100.
- Fig. (6):** Vagina of the full term fetus showing superficially divided mucosal folds (arrow); lamina propria-submucosa (S) with many thin-walled venous plexus (V), and thick muscular coat (M). H & E X 40.

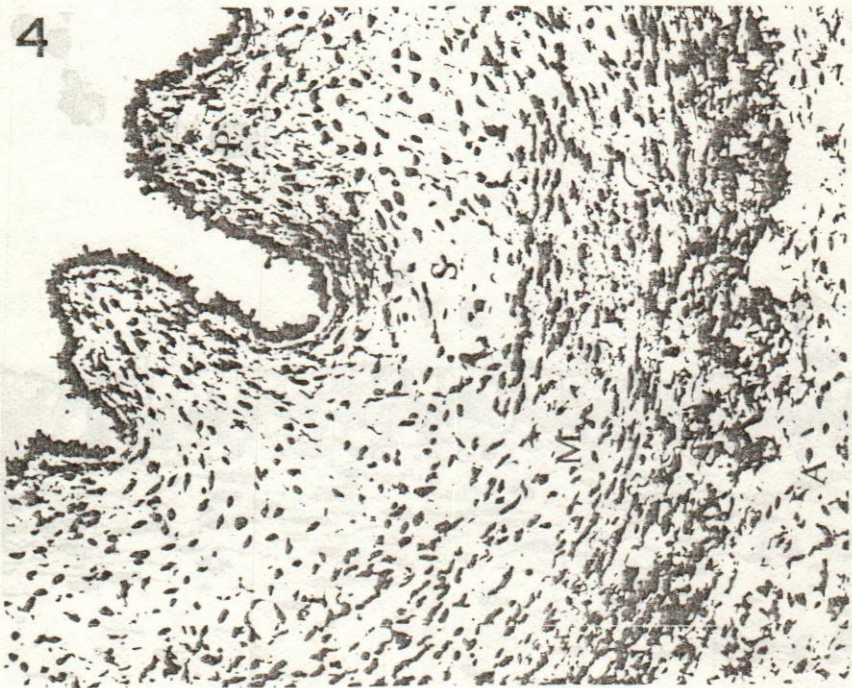
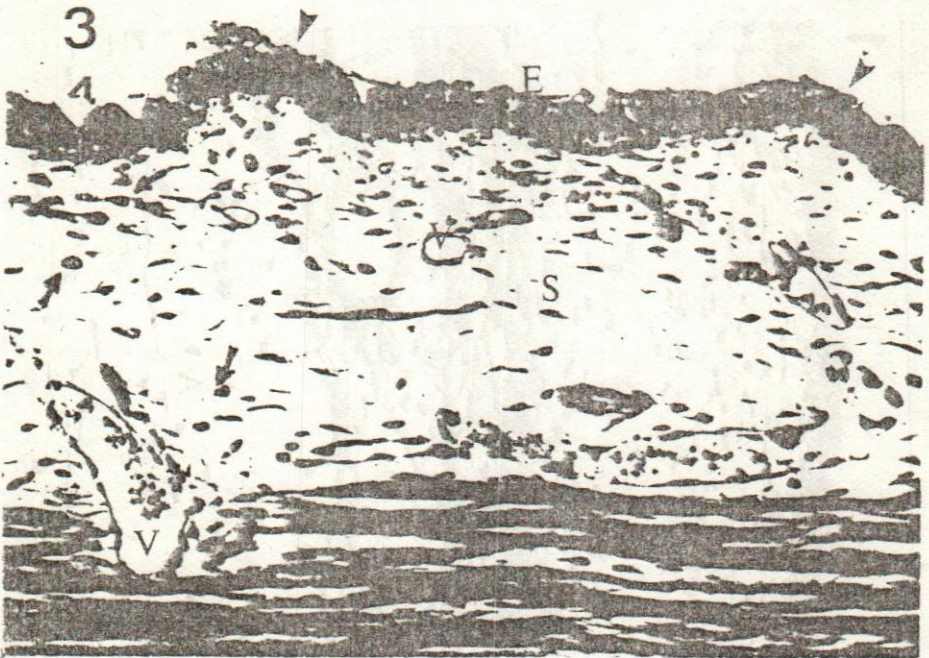
LEGENDS

FETAL VAGINA, BUFFALO



2





FETAL VAGINA, BUFFALO

