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Histogenesis of the Vagina of the One-Humped Camel (*Camelus Dromedarius*): Morphological Evidence of the Histochemical Aspects

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Abstract

The current investigation was carried out to explore the sequence of prenatal histological and histochemical events associating with the morphogenesis of the vagina in dromedary camel. From 135 mm CVRL stage on, the vaginal canal was representing the caudal continuation of the cervical thickening. The vaginal mucosa was thrown into incompletely separated longitudinal folds as a result of incomplete separation of the interplical epithelium. The lamina epithelialis was made up of 2 to 4 layers of polygonal cells. At 185 mm CVRL stage, the vaginal epithelial lining on the summits and sides of the longitudinal folds had been invaginated into the underlying lamina propria resulting in the formation of secondary folds. The musculosa was in the form of interrupted, circular bundles of smooth myocytes that increased in thickness with increasing age. The adventitial vaginal covering was made up of mesenchymal cells and fibroblasts associated with argyrophilic, and fine collagenic fibers with many thin-walled blood vessels. At 210 mm CVRL stage, the muscular coat became differentiated into an inner circular and outer longitudinal layer of isolated bundles of smooth muscle fibers separated by stromal elements. At 465 – 630 mm CVRL stage, the developing vagina showed highly folded mucosa with stratified squamous epithelial lining containing glycogen material which was denser among the superficial epithelial layers. At the end of developmental stages, the lamina epithelialis became stratified squamous non-keratinized epithelium with slightly wavy basement membrane. The vaginal masculosa was differentiated into three layers; inner and outer longitudinal layers and middle circular one of smooth muscle bundles. With increasing age, a gradual increase of acid phosphatase, ATPase and Succenic dehydrogenase (SDH) reaction was observed especially in the epithelial lining and muscular coat of the vagina. A progressive increase of sudanophilic reaction and alkaline phosphatase activity was noticed among the different layers of the vaginal wall through the studied stages.

Keywords: Paramesonephric duct; Utero-Vaginal tract; Vagina; Histogenesis; Morphogenesis; Dromedar camel

Introduction

Patten [1], reported that the common primordium of the urethra and lower part of the vagina are outlined as the urogenital sinus, whereas the primordium of the upper portion of the vagina is associated with the uterine canal, which originates from the fused paramesonephric ducts. Sebe et al. [2], stated that, after the tip of the uterine canal reaches the urogenital sinusa, solid vaginal plate grows out from the dorsal aspect of the urogenital sinus.

It has been shown that uterine and vaginal epithelial morphological phenotypes (simple columnar and stratified squamous) are determined by uterine and vaginal mesenchyme [3]. In contrast to the simple columnar uterine epithelium, the differentiation response of vaginal epithelium is more complex and involves the generation and differentiation of multiple suprabasal cell layers [4].

Kurita et al. [5], revealed that at 16-18 days of gestation in the mouse, epithelial tissues of the female reproductive tract (uterine, Müllerian, and sinus vaginal epithelia) were recognizable and were beginning to show distinctive differences in gene expression.

Material and Methods

Specimen collection

The current work was carried out on the vagina of 41 female camel fetuses whose Crown Vertebral Rump Lengths (CVRL) ranged from 135 mm to 1230 mm (Table 1). These samples were freshly collected from El-Basateen (Cairo) and Belbes (El-Sharqeya) slaughter-houses, directly after the slaughtering of apparently healthy, pregnant she-camels. Their uteri were opened and then evacuated. The collected specimens were immersed directly as a whole into 10% neutral buffered formalin for 4 weeks. The formalin-fixed material was continuously transferred to freshly prepared fixative every week.

Tissue processing and histochemistry

Preserved samples were briefly rinsed into 70% ethanol, then dehydrated through a graded series of ethanol (75% , 80% , 90%, 95%, absolute alcohol I, II and III) at 8 hour intervals, cleared in 3 changes of xylene (4 hours each), then embedded in paraffin wax (melting point 60°C). The vaginal specimens were serially sectioned at 5-7 µm thickness. The prepared sections were stained using the following stains: Harris heamatoxylin and Eosin, Masson's trichrome, Gomori's stain, Periodic-Acid Schiff (PAS) technique, Combined Alcian blue – PAS technique, Combined aldehyde fuchsin-alcian blue method, Toluidine blue and Best's carmine method.

Enzymatic and lipid histochemistry

Freshly collected specimens of the vaginal canal of 240, 630 and 990 mm CVRL camel fetuses were taken and immersed in liquid nitrogen (-196°C), put in cryostat at -20°C and cut into 10 um thick sections, then subjected to the following reactions for detection of different enzymes and lipids: Nitro-Blue Tetrazolium (NBT) method for demonstration of succinic dehydrogenase, Gomori's lead method

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for detection of acid phosphatase, Calcium cobalt method for demonstration of alkaline phosphatase, modified method for detection of adenosine-triphosphatase (ATPase) and Sudan black-B for detection of sudanophilic lipids.

The afore-mentioned histological and histochemical techniques were followed as outlined after, Bancroft et al. [6] and Carson [7].

Photomicroscopy

Representative photomicrographs were taken using Olympus BX41 research optical photomicroscope fitted with an

Olympus DP25 digital camera, Japan

Quantitative measurements

The magnification scale bare of the photomicrographs and the quantitative morphometric measurements included the height of epithelium, the thickness of mucosa-submucosa, musculosa and whole wall of the vaginal canal were taken using a digitizer calibration software; image analysis program (Olympus CellSens, ver. 1.5; image J Basics, Ver. 1.38 and Tsview Software, ver. 6.2.4.5).

Statistical analysis

All collected numerical measurements were statistically analyzed by Pearson's correlation coefficient according to Cohen [8], using statistical Package for Social Science (SPSS) software program version 16.0 [9].

Results

135 - 200 mm CVRL stage

At the 135 mm stage, the vaginal canal was representing the caudal continuation of the cervical thickening. The vaginal wall comprised an inner mucosa-submucosa, middle musculosa, and an outer adventitia (Figure 1).

Its mucosa was thrown into incompletely separated longitudinal folds due to the incomplete separation of the interplical epithelium (Figures 1 and 2). The lamina epithelialis was thicker on the plical summits and tended to be thinner on their sides. It was made up of 2 to 4 layers of polygonal cells (Figure 2), with relatively small euchromatic nuclei and faintly eosinophilic, PAS positive cytoplasm (Figure 3). The subepithelial propria-submucosa was predominantly cellular without a sharp line of demarcation between both areas; however, the propria was comparatively denser than the narrower submucosa (Figure 1). The main cellular elements were mesenchymal cells, fibroblast, and few mast cells (Figure 4). The intercellular matrix was made up of fine argyrophilic fibers which were more condensed at the epithelio-stromal interface (Figure 5). The stromal amorphous ground substance was moderately alcianophilic (Figure 3) from 185 mm stage on, it was observed that the vaginal epithelial lining on the summits and sides of the longitudinal folds had been invaginated into the underlying lamina propria resulting in the formation of secondary folds (Figure 6). A further development from 185 mm CVRL stage, the musculosa was in the form of interrupted, circular bundles of smooth myocytes that increased in thickness with increasing age (Table 2) (Figure 6).

The adventitial vaginal covering was made up of mesenchymal cells and fibroblasts associated with argyrophilic and fine collagenic fibers with many thin-walled blood vessels.

210 - 300 mm CVRL stage

Progressive differentiation of the different layers of the vaginal wall was observed during these stages. This differentiation included an increase in the width of the vaginal lumen, gradual increase of the vaginal epithelial height (Table 2) and progressive differentiation of the muscular layers (Figures 7 and 8).

The vaginal epithelium was made up of several layers of polygonal cells differentiated into darkly stained basal zone overlapped by three to five layers of pale stained cells (Figure 7). There was a slight reaction to ATPase (Figure 9A), moderate sudanophilic reaction (Figure 9B) and strong reaction to SDH (Figure 9C) in the surface epithelium. The propria-submucosa became thicker and comprised predominance of cellular elements in addition to fine argyrophilic and collagenic fibers. The intercellular matrix was still alcianophilic and negatively reactive to aldehyde fuchsin (Figure 9D).

During these stages, the muscular coat was characterized by a considerable increase in its thickness, ranged about 68.57 to 87.754 μm (Table 2), it was made up of circularly arranged layers of smooth myocytes (Figure 8). The latter was in the form of isolated bundles separated by stromal elements and it showed strong reaction to SDH (Figure 9C).

The vaginal adventitia showed a pronounced increase in its fibrous content particularly the wavy, thin collagenic bundles (Figure. 8).

310 - 450 mm CVRL stage

During these stages, no further qualitative changes were noticed. There was an increase in the epithelial height (51.609 μm), mucosa-submucosa (419.455 μm) as well as the whole thickness (613.024 μm) (Table 2).

The vaginal epithelial lining showed strong PAS reaction (Figure 10A). The propria-submucosa showed relative increase in collagenic fibers on the expense of the argyrophilic ones (Figure 10B, 10C). There was a marked decrease in the stromal alcianophilia (Figure 10D) and an increase in the number of the subepithelial mast cells.

The muscular wall became thicker (Table 2) and the smooth myocytes showed weak PAS reactivity. The adventitia showed an increase in its fibrous, nervous and vascular content (Figure 11).

465 - 630 mm CVRL stage

During this stage, the developing vagina showed highly folded mucosa (Figure 12) with stratified squamous epithelial lining containing Best's carmine positive, granular material (glycogen). The latter was denser among the superficial epithelial layers (Figure 13).The surface epithelium showed strong acid phosphatase (Figure 14) and moderate ATPase reaction (Figure 15).

The propria-submucosa showed appreciable amount of alcianophilic amorphous ground substances in addition to collagenic fibrers included in its matrix (Figure 16). The submucosa showed marked increase in its vascular elements which formed a vascular layer in close proximity to the underlying muscular coat (Figure 16).

The masculosa was differentiated into an inner circular and outer longitudinal layer of interrupted bundles of smooth myocytes (Figure 16). The latter cells showed the presence of Best's carmine positive granular material (Figure 13), moderate ATPase reaction (Figure 15) and strong acid phosphatase reaction (Figure 14).

640 - 1230 mm CVRL stage

There are few differences in the vaginal architecture when compared to the previous stage. The lamina epithelialis became stratified

squamous nonkeratinized with slightly wavy basement membrane (Figures 17 and 18). It comprised a stratum basale, stratum spinosum and a superficial stratum squamosum. The lamina propria-submucosa became distinctly differentiated into highly cellular, alcianophilic subepithelial layer and a wider, predominantly fibrous, deeper zone containing numerous vascular elements (Figures 17 and 19). It was noticed that increasing the amount of collagenic fibrous content was associated with substantial decrease of the reticular fibers in the propria sub-mucosa.

At the end of this stage, the vaginal musculosa was differentiated into three layers; inner and outer longitudinal layers and middle circular one of smooth muscle bundles (Figure 20).

The adventitial layer was composed of different types of connective cells and fibers, in addition to many vascular and neural elements.

Discussion

The present study demonstrates that the camel's vaginal canal can be identified at the 135 mm CVRL stage representing the caudal continuation of the cervical thickening. El-Tayeb [10], recorded a differentiation between the cervix and anterior vagina at 28.5 cm CVRL of camel fetus which was earlier in human, Arey [11] has recorded such differentiation at 11.7 cm CVR length. In camel, El-Hariri et al. [12], mentioned that the portio vaginalis uteri and vagina proper were well differentiated at 40 – 45 cm CVRL fetus stage.

El-Hariri et al. [12] showed that the goblet cells couldn't be detected in the vaginal epithelial lining during the fetal developmental stages of the dromedary camel. This observation was similar to our result.

Our work demonstrated that the vaginal epithelial lining was made up of 2 to 4 layers of polygonal cells at 135 mm CVRL stage then became stratified squamus non keratinized epithelium with slightly wavy basement membrane. It comprised a stratum basale, stratum spinosum and a superficial stratum squamosum. El-Hariri et al. [12], stated that the lining epithelium of the vagina proper differed from the vagina of adult she-camel. They were lined with stratified cuboidal to columnar epithelium with an extensive lymphocytic infiltration at the anterior 4/5 of this organ, while the remainder posterior 1/5 was lined with thick stratified squamous epithelium with partial keratinization [13]. In 180 mm CVRL human fetus, Bulmer [14], claimed that the vagina is lined by a very thick stratified squamous epithelium in which it is possible to distinguish four cellular zones. Buchanan et al. [5] showed that in contrast to the simple columnar uterine epithelium, the differentiation response of vaginal epithelium is more complex and involves the generation and differentiation of multiple suprabasal cell layers.

The vagina showed epithelial lining, showed strong PAS reaction, contain Best's carmine positive, granular material (glycogen) which was denser among the superficial epithelial layers. Our finding was similar to Bulmer [14], who showed that the glycogen materials are extremely abundant in the vaginal epithelium in all but the basal zone of cells. It is generally located as large granules, sometimes almost filling the cytoplasm of the cell, restricted to part of the cell on the proximal side of the nucleus. In addition, the cell walls of the three superficial zones show a positive PAS reaction.

A further development from 185 mm CVRL stage, the vaginal masculosa was in the form of interrupted, circular bundles of smooth myocytes that increased in thickness with increasing age. At 640 mm CVRL stage, the vaginal masculosa was differentiated into three layers; inner and outer longitudinal layers and middle circular one of smooth muscle bundles.

The quantitative measurements throughout the randomly selected specimens have clarified that there was a progressive increase in all the studied parameters during the early vaginal developmental stages. The statistical analysis has given a highly significant positive correlation between the CVRL, epithelial thickness, mucosal-submucosal thickness, muscular coat thickness as well as the vaginal wall thickness (Table 3) (Chart 1).

With developing age, the mucosal epithelial lining and the muscular coat of the vaginal wall showed a gradual increase in acid phosphatase activity. The acid phosphatase is a lysosomal enzyme concerning with the digestion of the foreign substances and with antagonism of any bacteria. The Lysosomal Acid Phosphatase (LAP) is synthesized as a type I membrane glycoprotein and targeted to lysosomes via the plasma membrane [15].

There was a gradual increase of the alkaline phosphatase activity among the different layers of the vaginal canal. There is a probable role of alkaline phosphatase in glycogen synthesis and the transport of secretary substances across cell membranes [16]. Vacco [17], said that the alkaline phosphatase function is to break down phosphate esters by hydrolysis therefore it is involved in the process of energy release.

Our findings revealed that there was a progressive increase of the sudanophilic reaction of all layers of the vaginal wall. Pearse [18], stated that sudan black-B possessed a greatest advance for staining phospholipids and natural fats. Padykula and Gauthier [19], explained that the lipid droplets appeared to be associated with mitochondria.

With increasing age, a gradual increase of ATPase reaction was observed especially in the epithelial lining and muscular coat of the vaginal canal. The ATPase is a specific alkaline phosphatase enzyme plays a role in the oxidative phosphorylation [20] and one important property is its hydrolytic ability of ATP [21]. This indicates the proportional correlation between the amount of mitochondrial ATPase and the intensity of reaction of the enzymes.

With advancing age, a progressive increase of Succenic dehydrogenase (SDH) reaction was noticed in the muscular coat as well as the vaginal epithelial lining. Regarding SDH as a mitochondarial enzyme, its increase in activity denotes the high metabolic activity of the cell [22]. Davies and Gunn [23], provided that the density of the staining with sudan black-B always corresponds with succinic dehydrogenase activity.

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