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Morphological Studies on the Vagina and Vestibule of Dromedary Camels (*Camelus dromedarius*)

Estudios Morfológicos de la Vagina y Vestíbulo del Camello (*Camelus dromedarius*)

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SUMMARY: The camel (*Camelus dromedarius*) is an important multipurpose livestock species and its meat represents about 10% of the red meat consumption in Egypt. The reproductive efficiency of camel under natural condition is generally considered to be low. Sound knowledge about the tubular genital organs of this species might facilitate the application of new reproductive methodology. Our study was therefore conducted to investigate the morphology of mucosal surface of vagina and vestibule on camels using light, scanning and transmission electron microscopy. The mucosal surface of vagina consisted of stratified columnar epithelium with mucous secreting cells (goblet-like cells). SEM of vagina revealed the presence of longitudinal primary and secondary folds and small transverse folds. The columnar epithelium showed marked cell boundary and its apical surface was studded by a lot of microvilli. TEM confirmed the presence of microvilli at apical surfaces and showed some secretory granules in the supranuclear region of the columnar epithelium. The vestibule of dromedary camel was lined by stratified squamous keratinized epithelium. Basal lamina and stratum granulosum of this epithelium showed strong PAS positive reaction. SEM of vestibule revealed the presence of small longitudinal and fine transverse folds with a lot of mucous debris. However TEM of vestibule showed the stratified squamous keratinized epithelium with basal layer of cuboidal cells and superficial layers of squamous cells.

KEY WORDS: Dromedary camel; Vagina; Vestibule; Morphology.

INTRODUCTION

The camel (*Camelus dromedarius*) is an important multipurpose livestock species uniquely adapted to harsh arid and semi-arid areas that can be used for meat, milk, wool, hide production, transportation and as a source of entertainment, celebration and competition. There are about 24.5 million one-humped camels all over the world with 80% of them in Africa. In Egypt, camel meat presents about 10% of the red meat consumption and around 130,000 heads are raised in the arid and semi-arid governorates. The majority of the camels are imported from Sudan, and a lesser share from Somalia & Djibouti (The Statistics Division of the FAO, 2010). Although the dromedary camel plays an important economic role as a domesticated mammal in the arid regions of Africa, Asia and Australia, many aspects of its reproduction are still unknown (Zayed *et al*., 1995). The dromedary camel is a seasonal polyestrus animal with induced type of ovulation. Decreasing length of daylight appears to be the stimulus for seasonality in camels (Novoa, 1970; Sghiri & Driancourt, 1999). Generally, she-camels reach full maturity and can be bred at 5–7 years of age (Khatami, 1970).

The tubular genital organs of the mammalian female reproductive tract provide different luminal microenvironments that can act as an aid or a barrier to gamete transport and the first steps of embryonic development. The reproductive efficiency of camel under natural condition is generally considered to be low and a sound knowledge on ovarian dynamics and structure of tubular genital organs of camel is the key element to adopt modern reproductive technologies for improving fertility in camels (Abdulwahhab, 2003).

In camel (El-hariri *et al*., 1982; Farouk *et al*., 2012), rabbit (Carr, 1953; Forsberg, 1961), hamster (Hisano, 1977), murine (Arey, 1974; Kurita *et al*., 2001), human

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(Ulfelder & Robboy, 1976), the cranial (proper vagina) and caudal part of the vagina (vestibule) are of a different embryonic origin. The former develops from the Müllerian ducts whereas the latter is formed from the urogenital sinus. Mammalian sperm transport through the female genital tract appears to result from a sequence of sperm-microenvironmental interactions, which are not yet fully understood. Studies on the morphology of female reproductive tract and the studies of the structure and physical properties of genital mucus secretions may improve our understanding of these interactions. Thus, our study was carried out to shed more light on the morphology of mucosal surface of vagina and vestibule on camels using light, scanning and transmission electron microscopy.

MATERIAL AND METHOD

Collection of samples. Twenty genital tracts of normal non-pregnant adult female camels (7–13 years) were collected from Cairo and Kom Hamada slaughterhouses. Different samples were collected from the vagina and vestibule. Samples of the vagina were taken from the roof and floor cranial to the urethral opening, while the samples of vestibule were collected nearly 2 cm caudal to the urethral opening. The dentition was followed depending on the description of Williamson & Payne (1978).

Processing and Staining. The samples were immediately fixed in 10% neural buffer formalin, dehydrated in ascending grade of ethyl alcohol, cleared in xylene, embedded and blocked in paraffin. Thin sections (3-7 μm thick) were prepared and mounted on egg albumin-glycerin coated glass slides, dried and stained with Hematoxylin and eosin (H and E), Periodic Acid Schiff (PAS) and Crossmon's trichrome. All of the staining techniques employed were performed according to Bancroft & Stevens (1990).

Scanning electron microscopical examination. For scanning electron microscope (SEM), small tissue samples were collected from three different animals and immediately immersed in 4F1G fixative (2% formaldehyde, 1.25% glutaraldehyde in 0.1 M sodium cacodylate) at pH 7.2 and stored at 4ºC. The fixed samples were washed in 0.1 M cacodylate containing 5% sucrose, processed through tannic acid, and dehydrated in graded ethanol series. The dried samples (with carbon dioxide) were then attached to stubs with colloidal carbon and coated with gold palladium in a sputtering device. The samples were examined and photographed with Jeol SEM operating 15Kv in the Faculty of Medicine, Tanta University, Egypt.

Transmission electron microscopical examination (TEM). Samples (n=4) from both of vagina and vestibule were collected immediately after slaughtering. Pieces of 1 mm3 were cut then fixed immediately for 2 h at 4ºC in 6% solution of phosphate buffered glutaraldehyde at pH 7.4. After initial fixation, the tissues were washed in several changes of cold (4ºC) 0.1 M. phosphate buffer every 15 minutes for 2 h. The tissue was post fixed in 1% solution of osmium tetroxide in cold (4ºC) 0.1 M phosphate buffer (pH 7.2) for 2 h. Then they were rapidly dehydrated through ascending grades of ethyl alcohol then transferred to propylene oxide and placed overnight in a 1:1 mixture of propylene oxide and epoxy araldite. Then they were embedded in epoxy araldite. Semi-thin sections (1 μm) were cut firstly and stained with toludine blue and viewed with light microscope to select a suitable area for electron microscope examination. Then ultrathin sections (60-100 nm) were cut by a glass knife with LKB ultramicrotome then they were stained with uranyl acetate followed by lead citrate. The sections were examined with Jeol 100 CX electron microscope in the Faculty of Medicine, Tanta University.

RESULTS

Light microscopic examination. With light microscope (LM), the vaginal wall of dromedary camel was devoid of glands and consisted mainly of three layers: a mucosa, a muscularis, and an adventitia. A muscularis mucosa was not present and the tunica mucosa was therefore composed of both lamina epithelialis and lamina propria-submucosa. The vaginal mucosa was thrown into numerous folds (rugae), separated by furrows of variable depth. Connective tissue papillae from the underlying lamina propria were also projected into the epithelial layer (Fig. 1A).

Lamina epithelialis of vagina was lined by stratified columnar epithelium with mucous secreting (goblet-like) cells. Small number of migrating lymphocytes and other leukocytes were also seen. The superficial cell layer showed faint striated apical border while the basal cell layer was formed of cuboidal cells (Fig. 1B). The superficial layer of epithelium especially their apical borders and the majority of mucous secreting cells showed PAS positive reaction (Fig. 1C). On other hand, some of mucous secreting cells especially at the caudal part of vagina showed PAS negative reaction (Fig. 1D). Lamina propria-submucosa of vagina exhibited two distinct regions. The outer region immediately below the epithelium was a highly cellular loose connective tissue. The deeper region, adjacent to the muscular layer, was denser and contained many blood vessels (Figs. 1B and 1E).

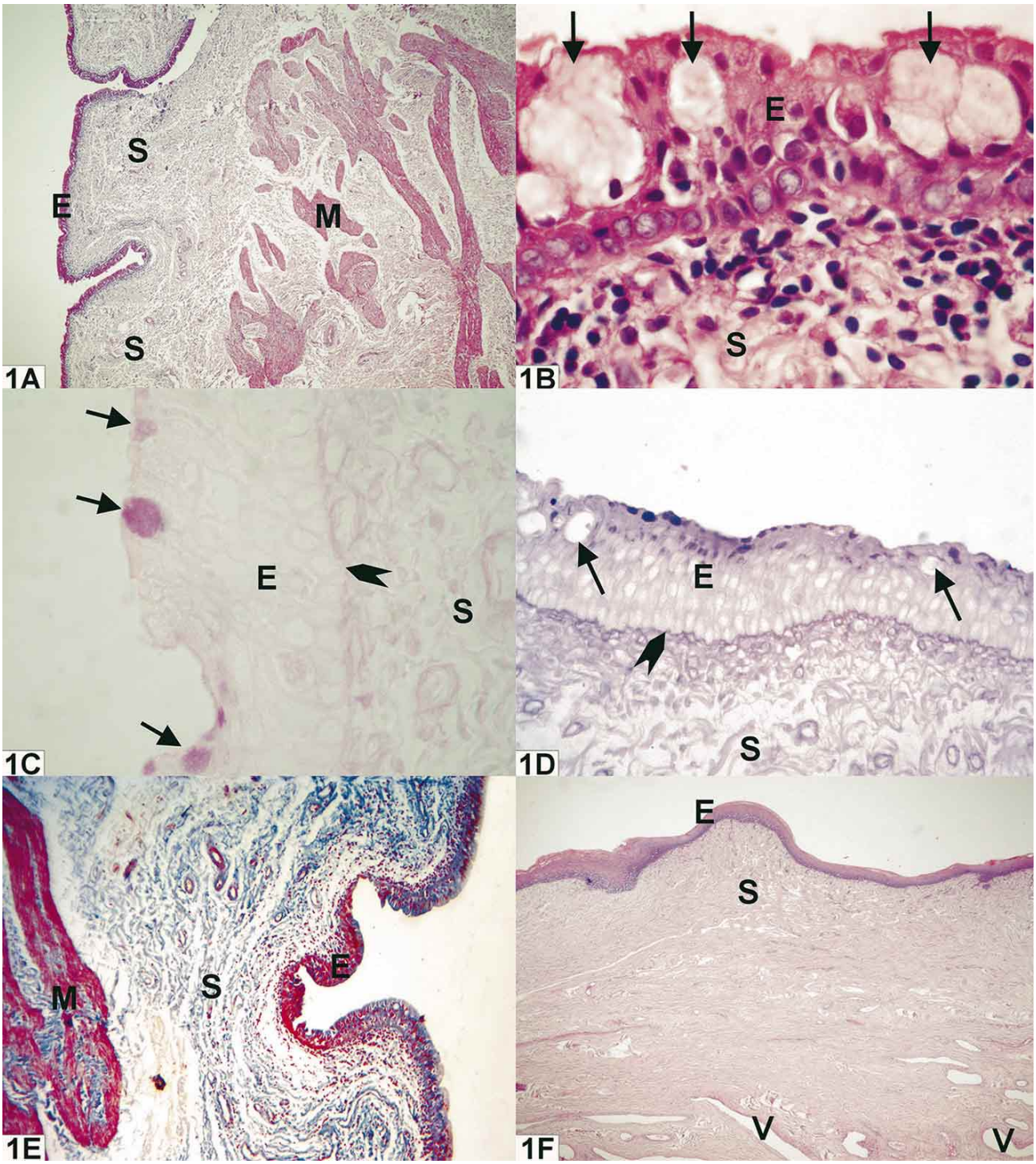

Fig. 1. A). LM of vagina of dromedary camel, showed lamina epithelialis (E) and lamina propria-submucosa (S) and tunica muscularis (M), H&E, X4. B). A higher magnification of panel A showed lamina epithelialis (E) and lamina propria-submucosa (S), and goblet cells (arrows) H&E, X40. C). PAS of vagina showed the positive reaction of goblet cells (arrows), basal lamina (arrowhead), and luminal surface of the lamina epithelialis (E) X40. D). PAS of vagina in some regions showed positive reaction of the luminal surface of the lamina epithelialis (E) and negative result of some goblet cells (arrows) X40. E). trichrome stain of vagina showed lamina epithelialis (E) and lamina propria-submucosa (S) and tunica muscularis (M), X4. F). LM of vestibule of dromedary camel, showed lamina epithelialis (E) and lamina propria-submucosa (S) and blood vessels (V) H&E, X4.

The vestibule was lined by stratified squamous keratinized epithelium and contained low folds (Figs. 1F and 2A). The basal lamina and the stratum granulosum of dromedary vestibule showed strong PAS positive reaction (Fig. 2B). The propria-submucosa of vestibule was highly vascularized and was consisted mainly of dense collagenous connective and some smooth muscle cells (Fig. 1F).

SEM and TEM examination. SEM of the vagina confirmed the data of light microscope where the mucosa was thrown into numerous incompletely separated longitudinal primary and secondary folds (rugae). Additionally it showed small transverse folds along the primary longitudinal folds (Fig. 2C). The columnar epithelium of lamina epithelialis showed marked cell boundary and its apical surface showed striated

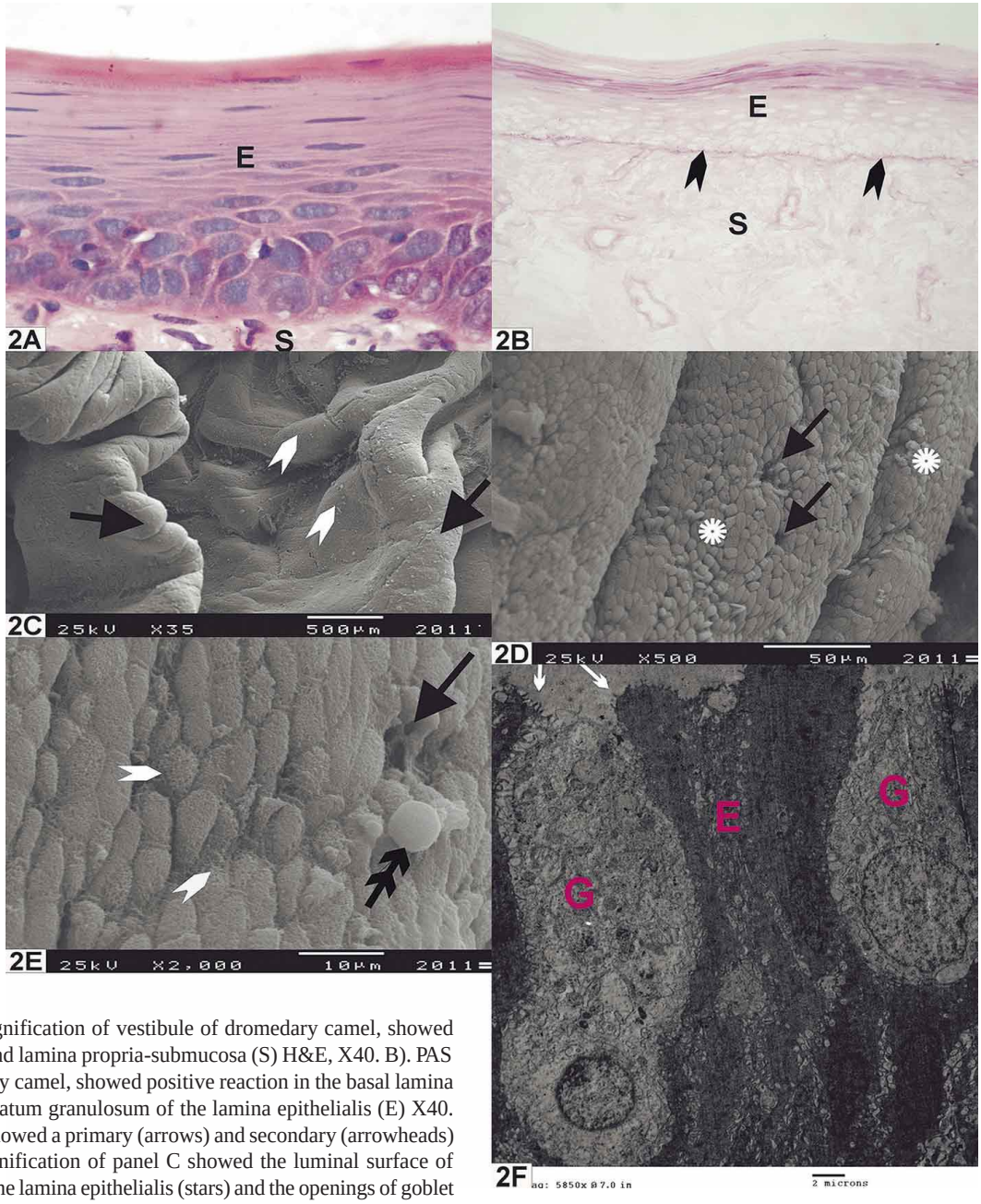

Fig. 2. A). A higher magnification of vestibule of dromedary camel, showed lamina epithelialis (E) and lamina propria-submucosa (S) H&E, X40. B). PAS of vestibule of dromedary camel, showed positive reaction in the basal lamina (arrowheads) and the stratum granulosum of the lamina epithelialis (E) X40. C). SEM of the vagina showed a primary (arrows) and secondary (arrowheads) folds. D). A higher magnification of panel C showed the luminal surface of columnar epithelium of the lamina epithelialis (stars) and the openings of goblet cells (arrows). E). A higher magnification of panel D showed striated luminal surface of columnar epithelium of the lamina epithelialis due to the microvilli (arrowheads), opening of goblet cells (arrow) and its secretion (double head arrow). F). TEM of proper, showed columnar epithelium of the lamina epithelialis (E), microvilli (arrows) and goblet cells (G).

appearance due to presence of short microvilli. Between the columnar epithelium, the openings of the mucous secreting cells were obvious and some of them were surrounded by mucous secretions (Figs. 2D and 2E). With TEM, the columnar epithelium of vagina showed short microvilli and some secretory granules in the supranuclear region (Figs. 2F and 3A). The mucous secreting cells (goblet-like cells) were large in size with large spherical basal nuclei. The cytoplasmic supranuclear region of these cells was filled with mucous secretion while their openings were large and surrounded by the columnar epithelium (Fig. 3A).

SEM of vestibule revealed the presence of small longitudinal and fine transverse folds. The surface of vestibule was covered with mucous debris and the cell border of squamous epithelium was well demarcated (Figs. 3B, 3C and 3D). TEM of dromedary vestibule showed the stratified squamous keratinized epithelium in which the basal cell layer was formed of cuboidal cells with spherical nuclei while the cells of the superficial layers were squamous with elongated nuclei. The stratum granulosum of the lamina epithelialis showed several granules and a lot of digitations along the cell borders (Figs. 3E and 3F).

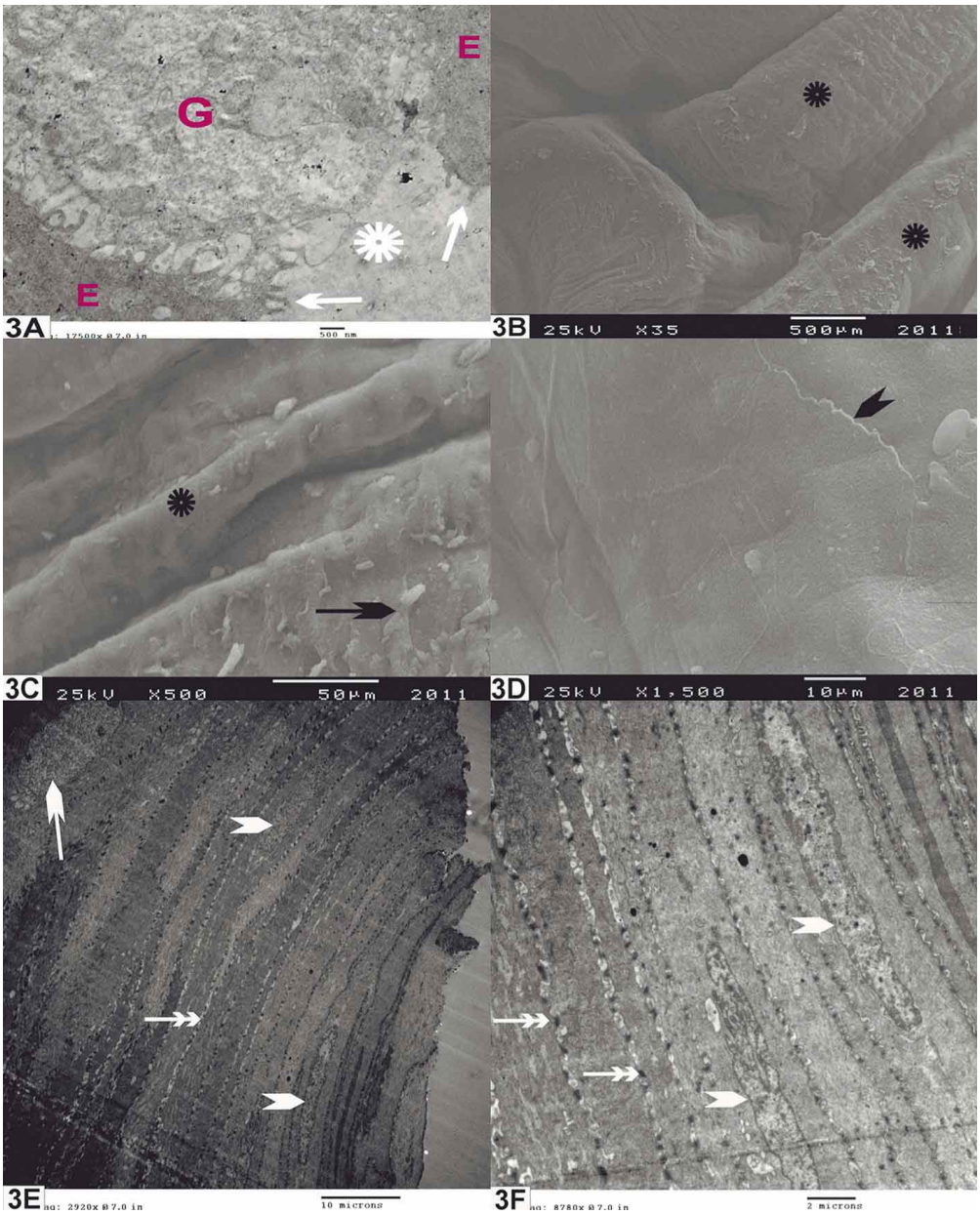

Fig. 3. A). TEM of vagina showed columnar epithelium of the lamina epithelialis (E) microvilli (arrows) and goblet cells (G) and its opening (star).

B). SEM of the vestibule showed primary folds of the mucosa (star).

C). A higher magnification of panel B showed the smaller folds (star) and mucous debris (arrow).

D). A higher magnification of panel C showed the mucous debris and the boundary of epithelium (arrow head).

E). TEM of vestibule showed the basal cell layer with round nuclei (arrow), superficial layers with elongated nuclei (arrow-heads) and digitations of cell boundary (double head arrow).

F). A higher magnification of lamina epithelialis of vestibule showed superficial layers with elongated nuclei (arrowheads) and digitations of cell boundary (double head arrow).

DISCUSSION

To our knowledge, this study represents the first report on SEM and TEM of vagina and vestibule in the dromedary camel. The mucosa of vagina was lined by stratified columnar epithelium with mucous secreting (goblet-like) cells as revealed by both LM and TEM. These data are concurrent with a previous study on camel vagina which showed the epithelium as stratified cuboidal or stratified columnar (Singh *et al*., 2005). On the contrary, other study stated that the lining epithelium of the anterior 4/5 of vagina of adult she-camel were lined by stratified cuboidal to columnar epithelium with an extensive lymphocytic infiltration, while the remainder posterior 1/5 was lined by thick stratified squamous epithelium with partial keratinization (Awad *et al*., 1982). Additionally, the lining epithelium of the developing proper vagina differed from the vagina of adult she-camel where the epithelium become stratified squamous non-keratinized in the late stage of development (El-hariri *et al*.; Farouk *et al*.). Moreover, in lama (a member of camelidae) the epithelium of vagina is stratified columnar epithelium with goblet cells (Feder *et al*., 1999).

Our results on the epithelium of the dromedary camel vagina are also controversial to that found on other species of animals. Collectively, the mucosa is lined, throughout, by a stratified squamous epithelium in all species except the cow. In the anterior portion of the vagina of the cow, the epithelium is stratified columnar with goblet cells. In carnivores, the epithelial cells become keratinized during estrus (Bacha & Bacha, 2000). On bovine (Trautman & Fiebiger, 1957; Marion & Gier, 1960) and buffaloes (Bareedy, 1977; Badawy *et al*., 1978; Osman, 1990: Ayen *et al*., 2003) the vaginal epithelium in the caudal region consists mainly of stratified squamous non-keratinized epithelium while, cranially, the epithelium is simple columnar and continuous with the cervical epithelium. Moreover, the epithelium in the caudal part of cow vagina is 3–6 layers thick with luminal squamous or cuboidal cells and is thicker than the cranial regions where the epithelium was 1–4 layers thick with luminal cuboidal or columnar cells (Blazquez *et al*., 1987a). In ewe (Zourgui & Elze, 1976; Vojtic, 1997) and in African dwarf goat (Ola *et al*., 2006), the vaginal epithelium is stratified and differentiated into basal, parabasal, intermediate and superficial cell layers. In sow, the epithelium of the tunica mucosa consists of three to five layers of stratified squamous cells. The mucosa-submucosa had areolar tissue which appeared spongy due to the presence of many blood vessels. Both the arteries and veins are lined only with endothelium, being devoid of a muscular coat (Bal & Getty, 1972). In rabbit, the vaginal wall is distinctly lined by simple columnar cells (Dehkordi & Parchami, 2012). The human vaginal mucosa consists of a thick, non-keratinizing, stratified, squamous epithelium containing cells laden with glycogen, as well as a smaller number of cells of other types, such as macrophages and Langerhans cells (Costin *et al*., 2011).

Our study revealed the presence of mucous secreting cells (goblet-like cells) within the epithelium of vagina. The majority of goblet cells and the luminal columnar epithelium showed PAS positive reaction. This observation was also reported in the vagina of cow (Dellmann & Brown, 1976). However goblet cells in the vagina of buffalo were not seen (Bareedy). The luminal cells in the cranial regions of cow vagina are filled to a greater or lesser extent with neutral, sialated and sulphated mucins (Blazquez *et al*., 1987a). Previously, other studies reported the absence of goblet cells in the vaginal lining epithelium during the fetal developmental stages of the dromedary camel (El-hariri *et al*.; Farouk *et al*.). Although the actual role of these PAS positive material is not completely clear, some reports might provide a reasonable interpretation. The vaginal epithelium synthesizes and accumulates glycogen under the effect of estrogen and the glycogen is deposited in the lumen of the vagina when the vaginal cells desquamate. Bacteria in the vagina metabolize glycogen and form lactic acid, which is responsible for the usually low pH of the vagina. The acidic vaginal environment provides a protective action against some pathogenic microorganisms (Junqueira & Carneiro, 2005).

The luminal columnar cell layer of vagina of dromedary during this study showed a striation on its luminal surface that was confirmed by TEM to be microvilli. This observation is similar to the previous data on camel (Sing *et al*.), bovine (Steukers *et al*., 2011), gerbil (Kress *et al*., 1989), mouse (Lamb *et al*., 1978), and rat (Centola, 1978) vagina.

In the present study, the vestibule of dromedary camel was lined by stratified squamous keratinized epithelium. These results are completely parallel to previous studies in camel (Singh *et al*.) and mare (Hammond & Wodzicki, 1941). Other approaches are also partially concomitant with our results whereas the posterior 1/5 of camel vagina is lined with thick stratified squamous epithelium with partial keratinization (Awad *et al*.). In cow, the vestibular epithelium is stratified squamous containing variable numbers of lymphocytes and polymorphonuclear leucocytes. However, the lymphatic nodules associated with the epithelium are most commonly observed around the clitoris. In addition, branched mucus-secreting major vestibular glands contain neutral, sialated and sulphated mucins are reported (Blazquez

et al., 1987b). Experimentally, no significant changes in the epithelial thickness of bovine vestibule measured during cultivation are detected. However, a clear non-keratinized stratified squamous epithelium and a few zones containing a stratified columnar to cuboidal epithelium are observed (Steukers *et al*.).

In conclusion, the epithelium of the vagina of dromedary camel is only stratified columnar epithelium with goblet cells. However, the vestibule of dromedary camel was lined by stratified squamous keratinized epithelium. Further studies are also needed to investigate the suspected alteration in vagina during different stages of the reproductive cycle.

SAYED-AHMED, A.; ELSHAFEY A. & ABD-ELMAKSOUD, A. Estudios morfológicos de la vagina y vestíbulo del camello (*Camelus dromedarius*). ***Int. J. Morphol., 32(4)***:1156-1163, 2014.

RESUMEN: El camello (*Camelus dromedarius*) es una importante especie de ganado de usos múltiples y el consumo de su carne corresponde al 10% aproximadamente del consumo de carne roja en Egipto. La eficiencia reproductiva del camello, bajo condiciones naturales, se considera generalmente baja. El conocimiento adecuado sobre los órganos genitales tubulares de esta especie podría facilitar la aplicación de una nueva metodología de reproducción. Por lo tanto, se llevó a cabo este estudio para investigar la morfología de la superficie de la mucosa de la vagina y el vestíbulo en camellos, utilizando luz, escaneado y microscopía electrónica de transmisión. La superficie de la mucosa de la vagina está formado por epitelio columnar estratificado con células secretoras mucosas (células en copa). La microscopía electrónica de barrido (SEM) de la vagina reveló la presencia de pliegues primarios y secundarios longitudinales y pequeños pliegues transversales. El epitelio columnar mostró un límite celular marcado y su superficie apical se evidenció salpicado por una gran cantidad de microvellosidades. La microscopía electrónica por transmisión (TEM) confirmó la presencia de microvellosidades en las superficies apicales y mostró algunos gránulos secretores en la región supranuclear del epitelio columnar. El vestíbulo del dromedario está revestido por epitelio estratificado queratinizado, de tipo escamoso. La lámina basal y el estrato granuloso de este epitelio mostraron una fuerte reacción PAS positiva. La microscopía electrónica de barrido (SEM) del vestíbulo reveló la presencia de pequeños pliegues transversales longitudinales y finos, con gran cantidad de restos de mucosidad. Sin embargo, la microscopía electrónica por transmisión (TEM) del vestíbuloreveló un epitelio queratinizado escamoso estratificado, con una capa basal de células cúbicas y capas superficiales de células escamosas.

PALABRAS CLAVE: Camello dromedario; Vagina; Vestíbulo; Morfología.

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