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Microanatomical Characteristics of the Buffalo (Bos Bubalis L.) Uterine Tube: Stereomicroscopic and Scanning Electron Microscopic Studies

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Abstract

Introduction: the uterine tube (Tubae Uterinae) of buffalo was narrow and extensive flexuous tube lay very closely to the ovary. It was extended from the tubal extremity of the ovary to the tip of the uterine horn and was suspended in the mesosalpinx which was very thin, translucent and vascular**.** The uterine tube wasn't a simple passage for ova and sperm only, but also it possessed several important functions such as, it captured the ova released from the ovary and conveyed them toward the uterus. Moreover, it also conveyed the sperms in their ascent for fertilization which occurred normally in the ampulla of the tube

Methodology: the present investigation was carried out on twenty specimens of female genitalia of adult freshly slaughtered and apparently healthy buffaloes. The ages of the animals, ranged from 5 to 15 years. Ten specimens were dissected to perform the gross anatomy of the uterine tube, and stereomicroscopic examination, other ten specimens were used for scanning electron microscopic examination.

Result: the stereomicroscopic and scanning electron microscopic studies revealed that, the mucosa of the infundibulum was distinguished by small cords, which originated from the undulating anterior free border which converged distally forming primary longitudinal folds and then secondary folds ran obliquely from the lateral walls of these folds and branching toward the basal areas between folds to form cul-de-sacs and pockets. Frequent divergences or convergences or interconnections of folds were observed in the ampulla, more observed at ampullary-isthmic junction, and less frequent in the isthmus. The isthmic mucosa was characterized by folds, ridges, grooves and deep cul-de- sacs or pockets. The uterine tube of buffalo was subjected to cyclic change during the follicular and luteal phases of estrous cycle. In particular, the ciliated non secretory cells were extensive during the follicular phase, while non-ciliated secretory cells were extensive with several bulbous apical processes and large numbers of cilia were hidden during the luteal phase

Key words: **Microanatomical , Stereomicroscopic, Uterine tube, Buffalo, (Bos Bubalis L.), Scanning Electron Microscopi**

INTRODUCTION

The water buffalo or domestic Asian water buffalo (Bubalis bubalis) was a large bovine animal frequently used as live stock in the Indian subcontinent and also widely in South America, Southern Europe, Middle East, Northern Africa and elsewhere. All the domestic varieties and breeds descended from one common ancestor, the wild water buffalo.

The incidence of the total reproductive tract disorders reached 46% of the examined slaughtered buffaloes. The pathological abnormalities of the uterine tube and ovarian bursa represented 17.30% of these disorders. That reflected the importance of the uterine tube in the reproductive cycle of the animal under investigation **(17).**

The uterine tube wasn't a simple passage for ova and sperm only, but it possessed several important functions such as, it captured the ova released from the ovary and conveyed them toward the uterus. It also conveyed the sperms in their ascent for fertilization which occurred normally in the ampulla of the tube **(10).**

Also, the isthmus and uterotubal junction of the bovine uterine tube were involved in events of sperm transport; storage and capacitating that were needed for preservation of motility, viability and fertilizing ability of spermatozoa **(21).** Moreover, the bovine tubal fluid contained certain proteins and glycoproteins which were potential in sperm capacitation as well as the nourishment of the ovulated ova and the developing embryo **(20).**

The mammalian oviductal epithelium consisted of two morphologically distinct types of cells, ciliated and nonciliated. The nonciliated cells released the secretory proteins and glycoproteins materials into the lumen and their secretions formed the oviductal fluid. The ciliated cells played an important role in the transport of oocyte into the oviduct. **(8)** in bovine**, (19)** in rabbit and **(15)** and **(1)** in mammals**.**

By reviewing the available literature, there was a great shortage in the researches concerning the morphological studies of the uterine tube of buffalo in Egypt. This work was carried out to throw more light on the various aspects of the morphology of the uterine tube in buffalo using the modern techniques as a trial in solving some problems of its infertility to play a part in increasing the animal production and national income.

MATERIAL AND METHODS

The present work was carried out on twenty specimens of non pregnant female genitalia of adult, apparently healthy and recently slaughtered buffaloes. Their ages ranged between five to fifteen years.

These specimens were collected from different slaughterhouses from (Cairo, Zagazig and Belbeis) and farms (in case of emergency slaughter).

The reproductive organs were examined and the ovarian status of the estrous cycle, (i.e. follicular and luteal phases was determined by the morphological appearance of the corpus luteam **(7).**

The specimens were dissected to describe the gross anatomical studies of the different parts of the uterine tube and its ligaments as well as the ovarian bursa.

Ten specimens were used for stereomicroscopic examination. The uterine tube were elaborated from the mesosalpinx and infundibulocornual ligament then opened longitudinally with fine curved scissor then fixed in Petri dish then examined and photographed under forced light by light stereomicroscope **(ODRRECT, Tokyo, SEIWA Optical No. 6075000)**. The objective lenses were X 5, X10. X20 and X40.

The electron microscopic study was performed on ten specimens of the uterine tubes of adult buffaloes (6 in the follicular phase and 4 in the luteal phase).Small pieces as approximately 1mmX1mm represented the different parts of the uterine tube were perfused immediately with 0.9% physiological saline then put in 2.5% glutaraldehyde in 0.1 m sodium phosphate buffer, pH 7.3. Then all specimens were washed three times in 0.1 m sodium phosphate buffer, pH 7.3, for 10 min each, and post-fixed in 1% buffered osmium tetroxide for 2 h at 4°C. The specimens were again washed in 0.1 m sodium phosphate buffer, pH 7.3 (three times for 5 min each) and then in distilled water (two times for 5 min each), dehydrated in an increasing alcohol series, and dried to the critical point. The specimens were mounted in metal base, sputtered with gold in an Emitech K 550 Sputter apparatus, analysed and photographed under LEO 435 VP and JEOL JSM 5200 scanning electron microscopes at the Faculty of Medicine Tanta University.

The Nomenclatures used along the course of the present work were those adopted by **(18).**

RESULTS

Macromorphological examination

The uterine tube (Tubae Uterinae) of buffalo was narrow and extensive flexuous tube lay very closely to the ovary. It extended from the tubal extremity of the ovary to the tip of the uterine horn. The uterine tube suspended in the mesosalpinx which was very thin, translucent, vascular and large enough to envelope the ovary from the lateral side**.** It was a double peritoneal fold, enclosing the uterine tube in between; it was derived from the lateral surface of the mesovarium and continued with the mesometrium **(Fig. 1).**

There was another ligament shared in the fixation of the uterine tube called infundibulocornual ligament which extended from the wide side of the infundibulum to the tip of uterine horn. The ovarian bursa (Bursa ovarica) was a serous pouch bounded by the mesosalpinx laterally and proper ligament of the ovary, mesoovarium and ovary medially. It invested the ovary partially and its opening was directed ventromedially **(Fig. 1).**

Stereomicroscopic findings of the uterine tube

The mucosa of the fimbriated end of the wide side of the infundibulum carried on low, small and interconnected cords, which originated from the anterior undulating free border. These cords converged distally forming primary longitudinal folds of the wide side that fused and increased in height before reaching the abdominal opening of the uterine tube**.** The mucosa in the medial and lateral edges of the wide side exhibited three to seven tall longitudinal undulations **(Fig. 2)**.

The mucosa of the narrow side of the infundibulum exhibited numerous and tortuous folds that increased sharply in height from the free margin and converged toward the ampulla. Transitional areas between the wide and narrow sides exhibited high longitudinal folds **(Fig. 2)**. These primary longitudinal folds of the wide side communicated with longitudinal folds of the narrow side and those of the transitional area between both sides forming primary longitudinal folds of the infundibulum **(Fig. 2)**.

The primary longitudinal folds of the infundibulum could be differentiated into high, moderate or low folds**.** Secondary folds ran obliquely in the lateral walls of primary folds and branched toward basal areas between the folds with a slope that created cul-de-sacs (pockets) with different shapes and depth **(Fig. 3)**.

The primary undulated longitudinal folds from the infundibulum converged and entered the ampulla forming prominent primary folds (six to eight) with variable sizes and height as occurred in infundibulum. The complexity of the number and height of primary fold decreased toward the ampullary-isthmic junction. Secondary folds in the ampulla ran obliquely along the lateral walls of primary folds in the same orientation as occurred in the infundibulum. Convergence or divergence of primary folds was frequent in the ampulla **(Fig. 4)**.

The secondary folds in the infundibulum and ampulla either diverged or converged and then reconnected with each other forming a tree-like structures. The bottom of pockets was divided by tertiary folds that derived bilaterally from the wall of the secondary folds **(Fig. 4)**.

The mucosa of the isthmus possessed five to seven low, thick and parallel running longitudinal primary folds. Convergence or divergence of primary folds was less frequent in the isthmus. (**Fig. 5)**.

The primary longitudinal folds of the mucosa of the intramural part were markedly decreased in number as became fewer but broader and lower in height (**Fig. 6)**. The basement area between primary folds appeared nearly smooth with presence of irregular furrows with different depth and length (**Fig. 7)**.

Scanning electron microscopic findings of the uterine tube

The mucosa of the infundibulum had tall longitudinal wavy primary folds. Secondary folds ran obliquely in the bilateral walls of primary folds and branched towards the basal areas between these primary folds.

These basement areas between primary folds showed a strong degree of organization as a net of secondary folds that diverged or converged or interconnected with each other in different directions from which originated tertiary folds with pockets (cul-de-sacs) present in-between them which had several shapes (**Figs. 8 and 9).**

The epithelial surfaces of the infundibulum at the follicular phase were richly ciliated. The cilia of the ciliated cells covered most of the apical surface and extended above most of the apices of the non ciliated cells. The cilia of ciliated cells were more numerous along the bilateral walls and in apical areas of the longitudinal primary folds than in basal areas. These cilia were not uniform in length, not evenly distributed and made clumping organization. The apical surface of the nonciliated cells were gently rounded and appeared as bulbous apical processes which concealed by the cilia of ciliated cells (**Fig. 10).**

In the luteal phase, the bulbous processes of nonciliated cells predominated in the epithelium. The majority of the processes of nonciliated cells were elliptical in shape and of a various sizes. The cilia appeared to be markedly decreased or partly hidden below the bulbous processes of nonciliated cells (**Fig. 11).**

The mucosa in the ampulla had high prominent primary folds that alternated with less prominent lower folds with small transverse rib-like secondary folds originated on lateral walls of adjacent primary folds which frequently converged or diverged and interconnected in basal areas. These secondary folds in the ampulla ran obliquely along the bilateral walls of primary folds in the same orientation as occurred in the infundibulum **(Fig. 12).** Tertiary folds extended from the walls of adjacent secondary folds bordering pockets (cul-de-sacs) like structures (**Fig. 13)**.

The mucosa of the isthmus was furrowed longitudinally into low and thick primary folds. Secondary folds arose from the bilateral walls of each primary fold in the isthmus form oblique angles and these secondary folds differed from that in the infundibulum and ampulla as they were less branching without converge or diverge. Numerous deep pockets (cul-de-sacs) of different shapes were apparent in basal areas between secondary folds. The bases of the pockets were flat and contained openings of tight deep crypts bounded by small tertiary ridges **(Figs. 14 and 15)**.

The epithelium in the ampulla and isthmus at the follicular phase showed extensive ciliation. The ciliated cells were evenly distributed on the epithelium than the nonciliated cells which appeared as in the infundibulum. The cilia were not fairly uniform in length and not equally distributed, usually extending above the apices of the nonciliated cells **(Fig. 16).**

At the luteal phase, the epithelium of the ampulla and isthmus was entirely covered by the bulbous processes of nonciliated cells. The cilia were concealed by the bulbous processes of the nonciliated cells extended beyond the tips of the cilia. Most of the processes of the nonciliated cells

were polyhedral in shape and with variable sizes **(Fig. 17).**

Fig. (1): A photomacrograph of formalinized genitalia of buffalo (left side) showing, infundibulum (1); wide side of infundibulum (1'), narrow side of infundibulum (1"), ampulla (2), isthmus (3), tip of the uterine horn (4), ovary (o), mesosalpinx (a), mesovarium (b), proper ligament of the ovary (c), ovarian bursa (double black arrows), abdominal opening of uterine tube (inserted needle) and infundibulocornual ligament (white arrows).

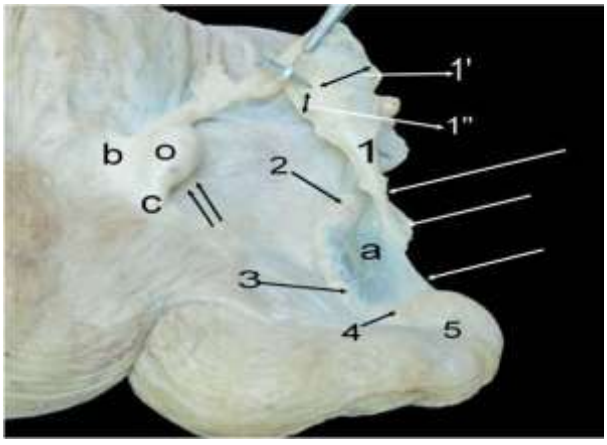
Fig. (2): A photomicrograph of the luminal surface of the infundibulum showing, numerous low and interconnected mucosal cords of the wide side (a), longitudinal folds of the wide side (b), longitudinal folds of the transitional area (c), longitudinal folds of the narrow side (d) and longitudinal primary folds of the infundibulum (e). Stereo. X5.

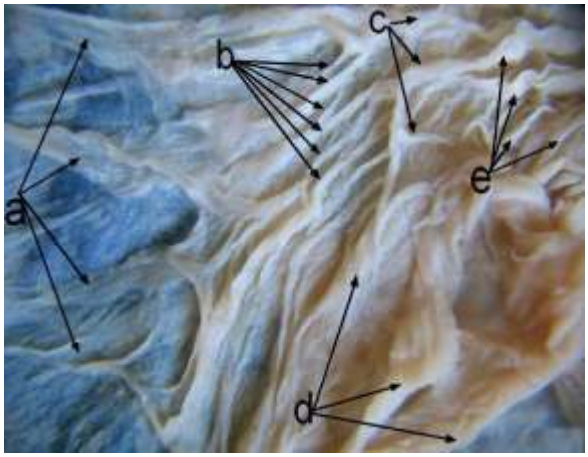
Fig. (3): A photomicrograph of the luminal surface of the infundibulum showing, the distal evolution of cords (a) forming longitudinal primary folds of various sizes; high fold (b1), moderate fold (b2), low fold (b3), secondary folds (c), pockets (arrows). Stereo.X 10.

Fig. (4): A photomicrograph of luminal surface of the ampullary portion of the uterine tube of buffalo showing, the size variation of primary longitudinal folds (a), secondary folds (b) and tertiary folds (black arrows) with pockets inbetween. Stereo. X20.

Fig. (5): A photomicrograph of the luminal surface of the isthmus of the uterine tube of buffalo showing, wall of the isthmus (a), parallel-running longitudinal primary folds (b) with round-shaped luminal projections (black arrows) and secondary folds (arrow heads) Stereo.X5.

Fig. (6): A photomicrograph of the luminal surface of the intra-mural part of the uterine tube of buffalo showing primary longitudinal folds (arrows) Stereo.X20.





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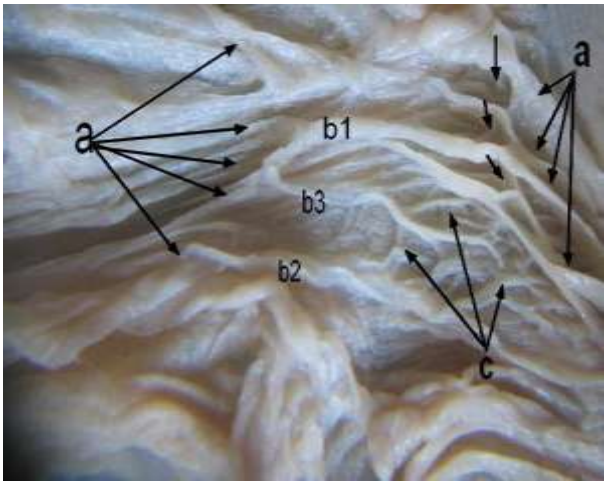

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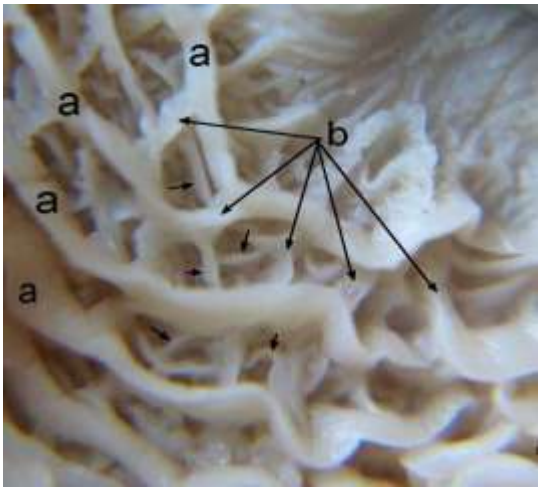

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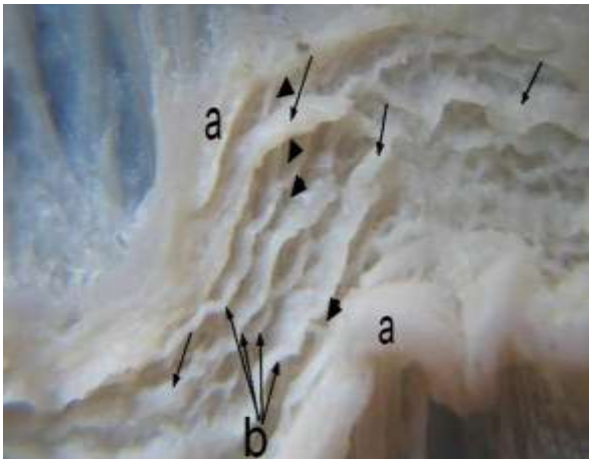

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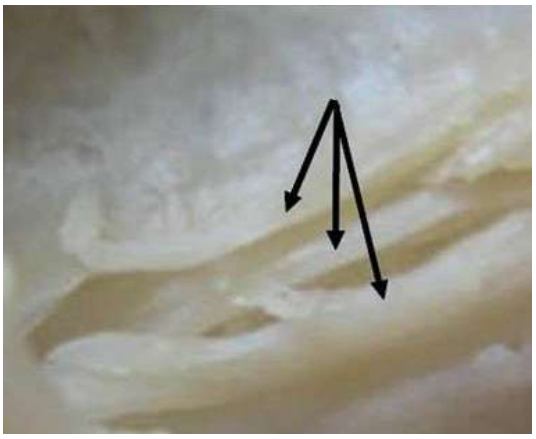


Fig. (5)

Fig. (7): A photomicrograph of the luminal surface of the intra-mural part of the uterine tube of buffalo showing primary longitudinal folds (a) and secondary furrows (arrows) Stereo.X40.

Fig. (8): A photomicrograph of the infundibulum showing, longitudinal primary folds (a), orientation of secondary folds along the bilateral walls (b) with clear divergence or convergence or interconnections of the secondary

Fig. (6)

folds (arrows). Scanning electron microscope SEM, X35.

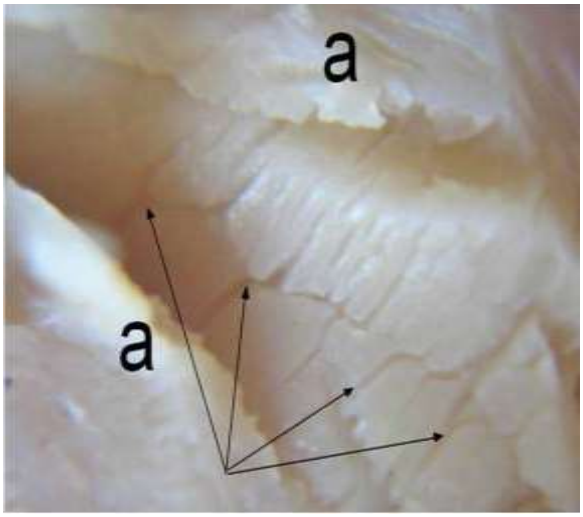
Fig. (9): Higher magnification of boxed area in fig 8 showing, longitudinal primary folds (a), orientation of secondary folds along their bilateral walls (b) , tertiary folds (c) and pockets (cul-de-sacs) (d) SEM, X100.

Fig. (10): A photomicrograph of epithelial surface in the apical area of a primary infundibular

fold in the follicular phase showing, the predominance of ciliated cells over secretory cells which are partially concealed by the surrounding cilia. Ciliated cells with clumping cilia (ci) and secretory cells (arrows) SEM, X2000.

Fig. (11): A photomicrograph of epithelial surface lining the pockets formed between infundibular folds in the luteal phase showing, predominance of bulbous apical processes of secretory cells (p) and cilia of ciliated cells (ci) SEM, X1500.

Fig. (12): A photomicrograph of ampulla showing, longitudinal primary folds (a) and secondary folds along their walls (b) SEM, X35.



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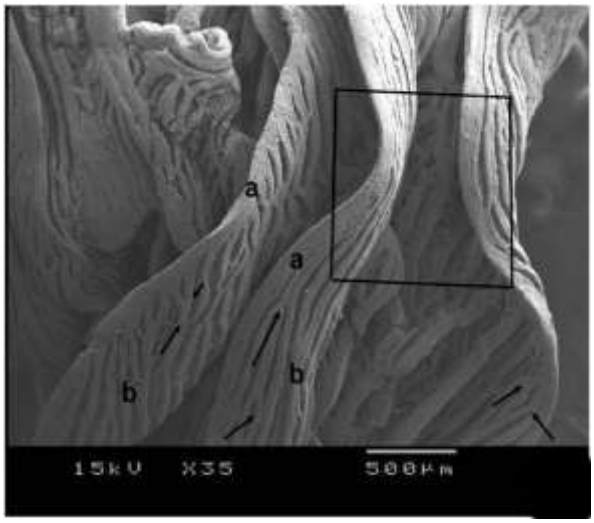

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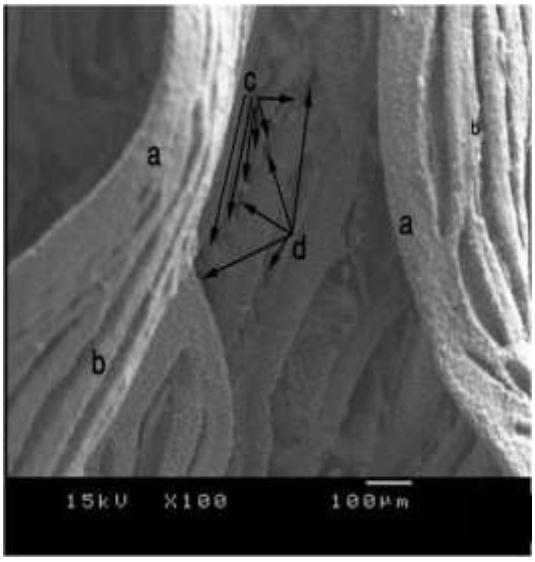

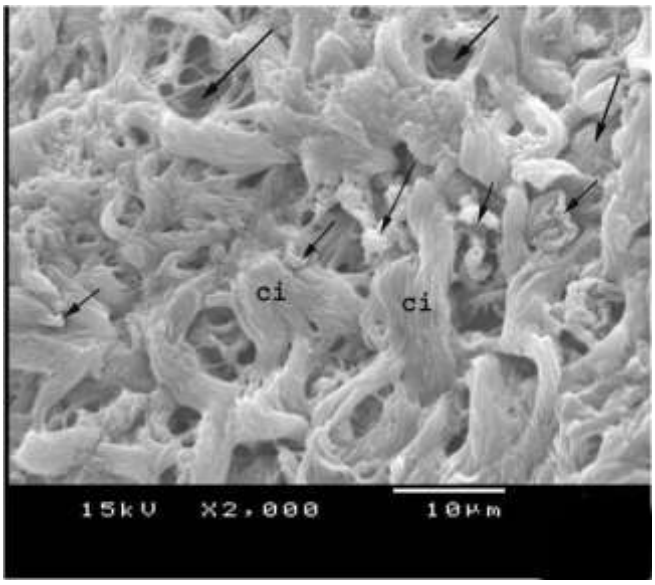

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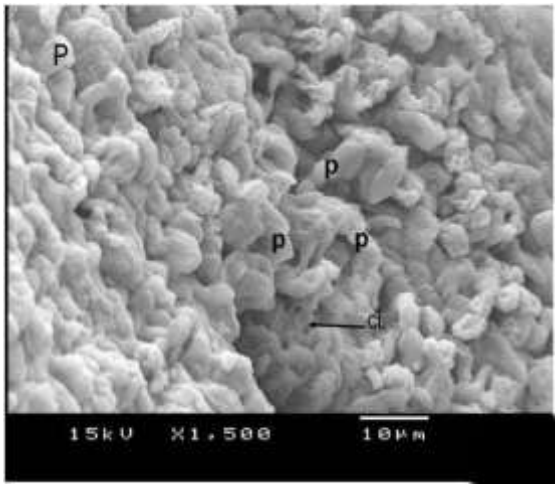

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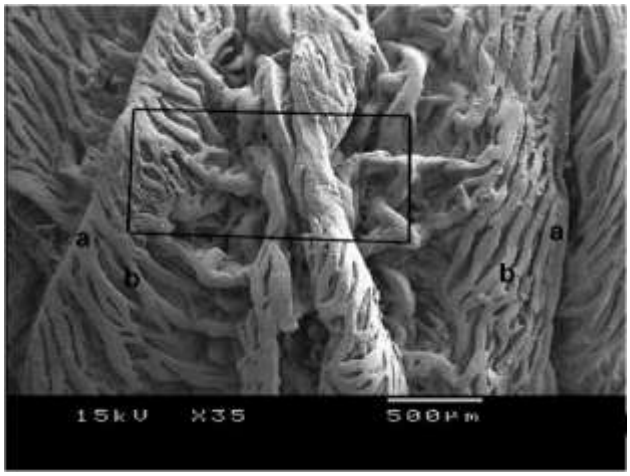


Fig. (11)

Fig. (12)

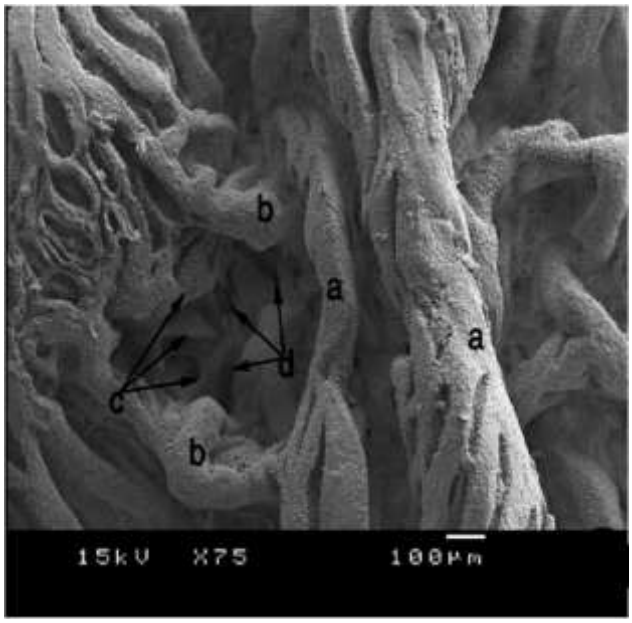
Fig. (13): Higher magnification of the boxed area in fig. 12 of ampulla showing, longitudinal primary folds (a), secondary folds along their walls (b), tertiary folds (c) and pockets (cul-de-sacs) (d). SEM, X75.

Fig. (14): A photomicrograph of isthmus showing, longitudinal primary folds (a) and secondary folds along their walls (b). SEM, X50.

Fig.(15): Higher magnification of the boxed area in fig 14 of isthmus showing, longitudinal primary folds (a), secondary folds along their walls (b) and complex system of pockets in the spaces between secondary folds (c) and narrow crypts (white arrows). SEM, X200.

Fig. (16): A photomicrograph of epithelial surface in the apical area of a primary ampullary fold in the follicular phase showing, the predominance of ciliated cells over secretory cells which are partially concealed by the surrounding cilia. Ciliated cells (ci) and (p) bulbous processes of secretory cells.SEM, X 2000.

Fig. (17): A photomicrograph of epithelial surface lining the pockets between secondary ampullary folds in the luteal phase showing, predominance of bulbous apical processes of secretory cells (p) over the ciliated cells (ci). SEM, X1500.



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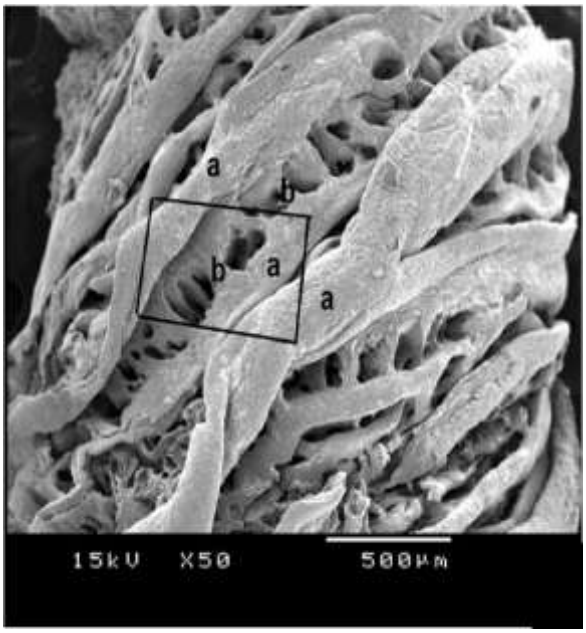

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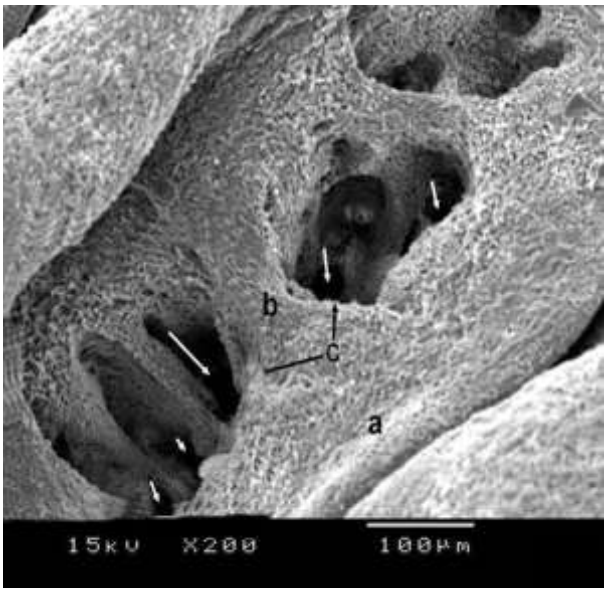

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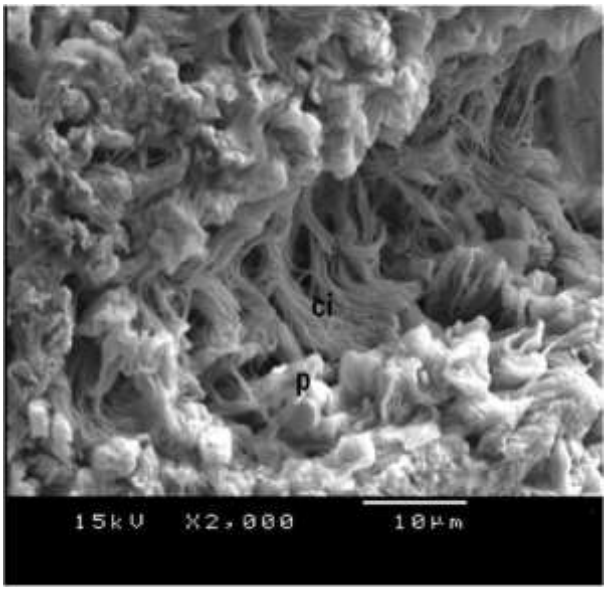

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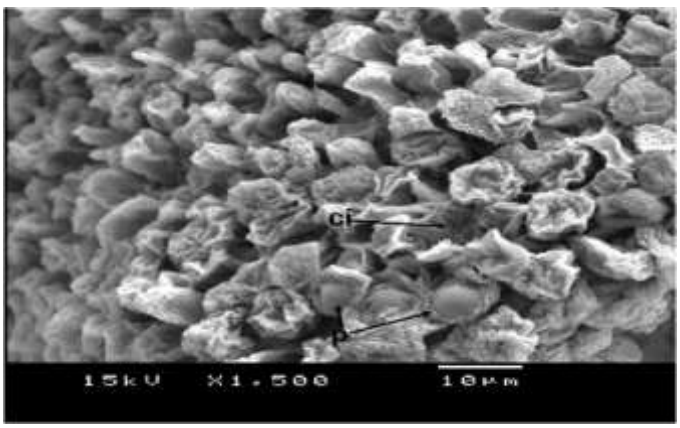

Fig. (17)

DISCUSSION

The present investigation showed that, the mucosa of the buffalo's uterine tube was formed of a complex arrangement of the mucosal folds which branching and spreading across the entire length of the tube forming a network. These folds decreased in number and height toward the uterus. This results were in the same line with those observed by **(11), (14) and (12)** in mammals**.**

Concerning the mucosa of the wide side of the infundibulum, the present findings revealed that, it had small cords originated from the undulating anterior free border. These cords converged distally forming seven to nine tall primary longitudinal folds. That wasn't in agreement with the statements of **(25)** in bovine who recorded that, the wide side of the infundibulum exhibited three to eight tall longitudinal folds.

The primary longitudinal folds of the wide side of the infundibulum communicated with longitudinal folds of the narrow side and transitional area between the both sides forming primary longitudinal folds of the infundibulum. The secondary folds run obliquely from the lateral walls of these primary folds. These secondary folds branched toward the basal areas forming cul-de-sacs (pockets).The arrangement of primary and secondary folds of the infundibulum simulating the results which were obtained by **(16)** in virgin heifers and **(25)** in bovine.

In the present investigation, the primary longitudinal folds of the ampulla were six to eight that simulated in a great extent with the findings of **(25)** in bovine as they mentioned that, high prominent primary mucosal folds of the ampulla were six to ten in number. The orientation of the primary and secondary folds was similar to that described in bovine by **(25).** Moreover, the pockets (cul-de-sacs) which were observed during the present work between the tertiary folds which originated on the bilateral wall of the secondary folds, could not met within the available literatures.

In the animal under investigation, the Cilia of ciliated cells were more numerous along the lateral walls and in apical areas of the longitudinal folds than in basal areas between folds of the infundibulum. Such result was in a line with those mentioned by **(25)** in bovine. This arrangement of cilia was explained by **(19)** in rabbit, as they played an important role in the pickup of ovulated eggs.

The secondary folds in the infundibulum and ampulla after arising from the primary folds, they either diverge or converge and then reconnected with each other forming a tree-like structure .This result was in agreement with findings by **(25)** in bovine**.**

In the present study, frequent divergences or convergence or interconnections of folds were observed in the ampulla, ampullary-Isthmic Junction, and less frequent in the isthmus. Such observation was in accordance with **(13)** and **(25)** in bovine.

The present work revealed that, the isthmic buffalo mucosa was characterized by folds, ridges, grooves and deep cul- de- sacs or pockets in addition to grossly constriction of the isthmus. Such observation was in accordance with **(13)** and **(25)** in bovine. This observation explained that this area acted as a physical barrier to the ascent of

spermatozoa so the isthmus considered being a functional sperm reservoir **(13).**

The primary longitudinal folds of the intramural part of the uterine tube were markedly decreased in number as became fewer, broader, more flatten and wider. Such observation was in the same line with **(13)** and **(25)** in bovine.

The basement area between primary folds of the intramural part of the uterine tube appeared nearly smooth with presence of irregular furrows of different depth and length. This organization of the basement area weren't mentioned in any other available literatures during the course of this work.

The present study revealed that, the uterine tube of buffalo was subjected to cyclic change during the follicular and luteal phases of estrous cycle. In particular, the ciliated non secretory cells were extensive during the follicular phase, while non ciliated secretory cells were extensive with several bulbous apical processes and large numbers of cilia were hidden during the luteal phase. Similar cyclic changes had been observed by **(22)** in bovine**,** (**3)** in cow, **(4)** in goat, **(9)** in mare and **(2)** in Chinese Meishan pig ,**(23)** and **(24)** in Thai swamp buffalo.

The increasing of the secretory cells during the luteal phase was explained by **(5)** in goat and **(6)** in bovine as the oviductal epithelial cells secreted an oviduct-specific glycoprotein. Some of these oviduct-specific glycoproteins were associated with the zona pellucida of ova and\or the surface of spermatozoa and might play an important role in fertilization, early embryonic development, and functions of spermatozoa

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