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Scanning Electron Microscopic Studies of Endometrium in Buffalo (*Bos bubalis* L.) at Follicular and Luteal Phases

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ABSTRACT

This study was carried out to observe morphological changes of buffalo endometrium at follicular and luteal phases. Uterine tissues were collected from female buffaloes at abattoir and the selected estrous stages were categorized into the follicular (n = 5) and luteal (n = 5) phases. A scanning electron microscope (SEM) was used to study surface epithelial changes. The results by SEM examination, the ciliated and secretory cells with different patterns, i.e. abundant microvilli on the apical part or secretory protrusion in various degrees, distinctly increased at the follicular phase. Meanwhile, there were numerous secretory cells with stubby microvilli covered throughout the endometrial surface with secretory vesicles, which were observed numerously at endometrial glandular orifices during the luteal phase, at while the ciliated cells were scarcely seen. The buffalo endometrium obviously revealed modifications during the estrous cycle for physiological events, i.e. sperm transport, early embryonic development and implantation.

INTRODUCTION

Buffaloes were the main source of good quality meat and milk in Egypt and some other developing countries, despite this species were mostly reared under harsh socioeconomic condition and showed low productive and reproductive potentials *(1)*.

Reproduction was an important factor in economics of the animal production. Delivery of healthy offspring was the ultimate goal of a good breeding program *(2)*. Reproductive performance was economically important in dairy animals. It affected the average amount of milk produced per animal, per day, and the replacement frequency *(3)*.

Fundamental studies of endometrium in buffalo uterine horns had rarely been observed. Generally, the most important function of the uterine horns in ruminant was involved in sperm transport, implantation, pregnancy and parturition associated with adjustment of cellular structures and microenvironments *(4)*.

In buffalo cows, uterine fluid was produced by transudation of blood serum, uterine epithelial cells and endometrial glands. This fluid was very important for sperm maturation and capacitation including early embryonic development, in which blastocysts took a prolonged period before their attachment in endometrium *(5)*. In bovines, most cells and associated structures in the endometrial compartments undergo proliferation, differentiation and activity in correlation to fluctuation of steroid hormonal levels, throughout the estrous cycle *(6, 7)*. Therefore, supplementary information concerning the morphology of the buffalo endometrium was definitely required to distinguish normal physiological status, or events during cycling phases from abnormalities.

The purpose of this study was to investigate the morphological modifications by SEM approaches in buffalo endometrium at the follicular phase in comparison to the luteal phases of the estrous cycle.

MATERIALS AND METHODS

The present work carried out on 10 female genitalia of buffaloes at various ages (3-7years) which determined according to *(8)*. These genitalia were brought to the laboratory of the Scanning Electron Microscope institute of the faculty of agriculture, Mansura University in a cool container from an abattoir immediately after slaughter. The Female genitalia were classified into the follicular phase (days 18-20, n=5) and the luteal phase (days 5-10, n=5) based on the characteristics of the corpus luteum and the dominant follicle as described by *(9)*.

The uterine tissues (intercaruncular areas of uterine horns and body, cervix including Portio vaginalis cervicis) preserved in 2.5% glutaraldehyde were stored at 4°C for 24 hours and cut into small pieces. The samples were rinsed in distilled water, post-fixed for 1 hour in 1% osmium tetroxide in 0.1 M PBS (phosphate buffer saline), dehydrated in graded ethanol (30-100%) and substituted with acetone. The tissues were then subsequently subjected to critical point drying using liquid CO_2 substitution. Dehydrated samples were adhered to the stubs with clear nail polish, coated with gold-palladium, and analyzed under JEOL-JSM-6510 LV SEM (JEOL, Tokyo, Japan) at an accelerating voltage of 30 kV.

RESULTS

The endometrium had ciliated cells of various patterns, during the follicular phase, there were abundant microvilli on the apical part or secretory protrusions in various degree and the ciliated cells were more numerously detected.

During the luteal phase, the ciliated cells were scarcely distributed and in some areas of endometrium were absent. Moreover, the flat secretary cells of short microvilli could be discerned. The latter cells appeared as a mosaic-like in character. Meanwhile, the luminal surface of the uterus showed many secretory vesicular protrusions.

Cornuae uteri

During the follicular phase, the whole surface of the intercaruncular areas of the uterine horn could be observed as sponge like appearance with interference of several grooves. There were scattered ciliated cells immersed in plentiful number of swelling secretory cells (fig. 1). Meanwhile, in some areas of the endometrium, were covered with rounded apical prominences with clear microvilli. The latter were coalesced with each other forming rosette like appearance (fig. 2).

During the luteal phase, the surface of the endometrium was fissured with several grooves forming a mosaic-like shape (fig. 3). The ciliated cells were meager or absent, while the apical bulbous processes of secretory cells were abundant (fig. 4).

Corpus uteri

During the follicular phase, the whole surface of the intercaruncular areas of the uterine body had several halls at which coalescing cilia of high length could be observed. The latter were surrounded by rounded apical swelling of secretory cells (figs. 5 and 6).

During the luteal phase, the endometrium was distinguished by several rounded bulbous uterine vesicles, were protruded from the opening of endometrial glands (figs. 7and 8).

Cervix uteri

The mucosa had many longitudinal undulated primary folds of variable characters; large, intermediate and small folds could be detected and various orders (primary from which originated secondary). Secondary folds ran obliquely in the lateral walls of primary folds and branched toward basal areas (figs. 9, 10, 11 and 12). Several interconnected threads between the adjacent secondary folds (fig. 10).

The bottom between the primary folds showed oval or rounded shaped crypt. The latter, bordered by extensions of secondary folds extending from the walls of adjacent primary folds. The crypt had several deep rounded or oval opened pockets (figs. 11 and 12).

During the follicular phase, the folds of the cervix had scattered coalescing cilia of moderate length, which were surrounded by rosette like structure of swelling non ciliated secretory cells (fig. 13). The bottom between secondary folds had several halls with clear long wavy hair-like cilia could be discerned (fig. 14).

During the luteal phase, the surface of the cervical mucosa had several bulbous apical processes of non ciliated secretory cells forming rosette like appearance with a short microvilli could be detected **(fig. 15).**

Portio vaginalis cervicis

The mucosa had tall longitudinal thick primary folds of various sizes (large, intermediate and small folds), and various orders (primary from which originated secondary). Secondary folds ran obliquely in the lateral walls of some primary folds which were frequently converged, diverged, interconnected and present caudal triverged secondary folds (figs. 16 and 17).

In some areas of the same sample, present large thick smooth primary folds without secondary folds organization, with clear several deep longitudinal grooves (figs. 18 and 19).

During the follicular and luteal phases, the surface of Portio vaginalis cervicis had the same appearance as mentioned in the cervix (figs. 20 and 21).

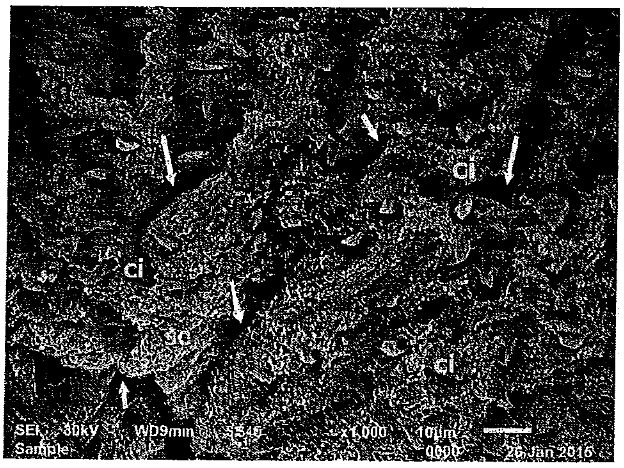

Fig. 1. A photomicrograph of epithelial surface of non gravid uterine horn (intercaruncular area) at the follicular phase showing, the increase of ciliated cells (ci) with long cilia and the distention of secretory cells (sc) with or without microvilli and presence of several grooves (white arrows). SEM, X 1, 000.

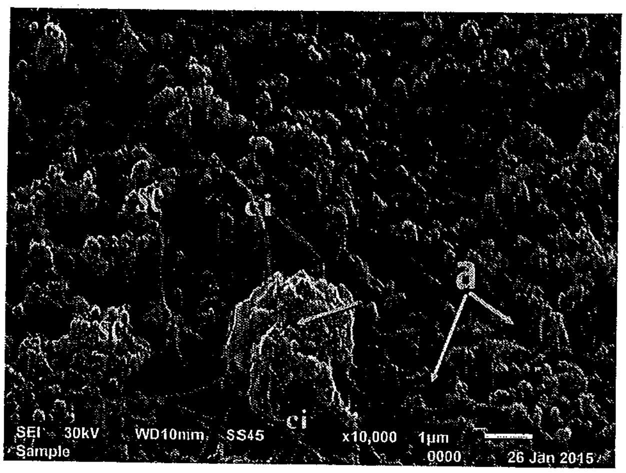

Fig. 2. A photomicrograph of the epithelial surface of intercaruncular area of uterine horn at the follicular phase showing, the whole epithelial surface were covered with microvilli of the flat secretory cells with a rosette shaped structure (yellow arrow); ciliated cells (ci); secretory cells (sc) and orifices of the excretory duct of the endometrial glands (a). SEM, X 10, 000.

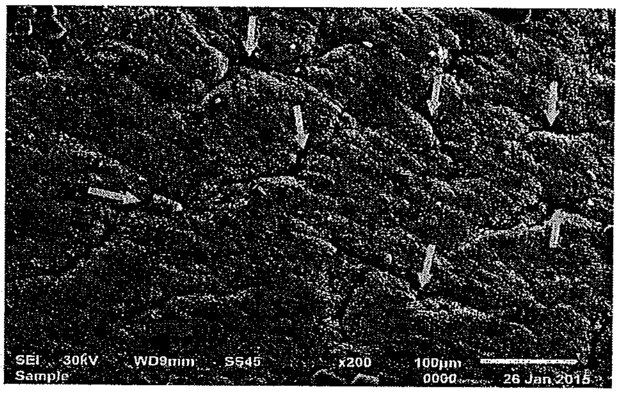

Fig.3. A photomicrograph of epithelial surface at intercaruncular area of uterine horn at the luteal phase showing, flat secretory cells covered with short microvilli without ciliated cells with apparent several detected grooves as a mosaic-like shape and orifices of the excretory duct of the endometrial glands (yellow arrows). SEM, X 200.

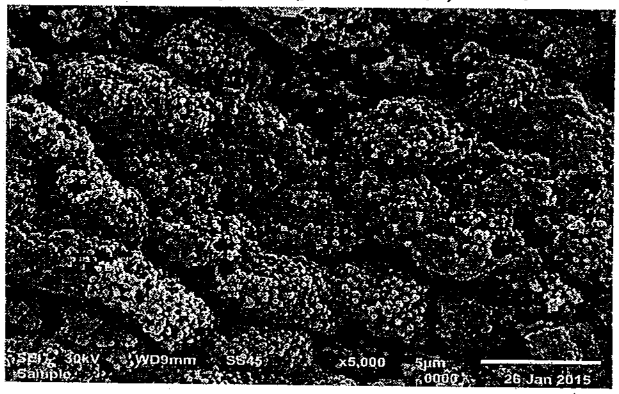

Fig. 4. A photomicrograph of epithelial surface of non gravid uterine horn (intercaruncular area) at the luteal phase showing, flat secretory cells covered with short microvilli had bulbous apical processes without ciliated cells. Several grooves as a mosaic-like shape. SEM, X 5,000.

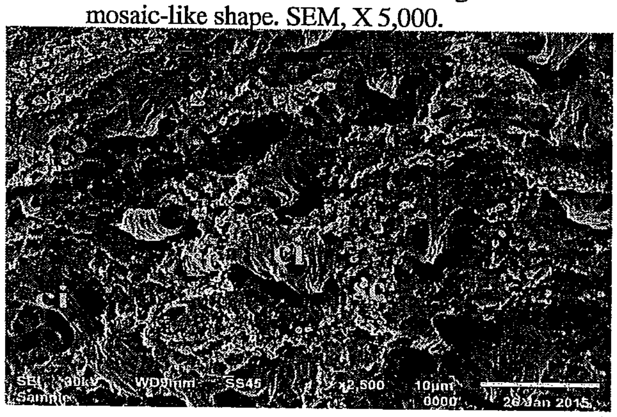

Fig. 5. A photomicrograph of epithelial surface of non gravid uterine body (intercaruncular area) at the follicular phase showing, the increase of ciliated cells (ci) with long cilia and the distention of secretory cells (sc) with or without microvilli with clear several grooves. SEM, X 2,500.

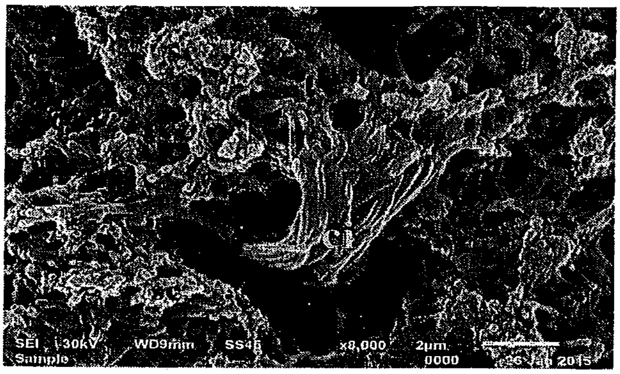

Fig. 6. A photomicrograph of epithelial surface of non gravid uterine body (intercaruncular area) at the follicular phase showing, the ciliated cells (ci) with long cilia and the distention of secretory cells (sc) with microvilli which had bulbous apical processes. Ciliated cells with cilia arising from a hall. SEM, X 8,000.

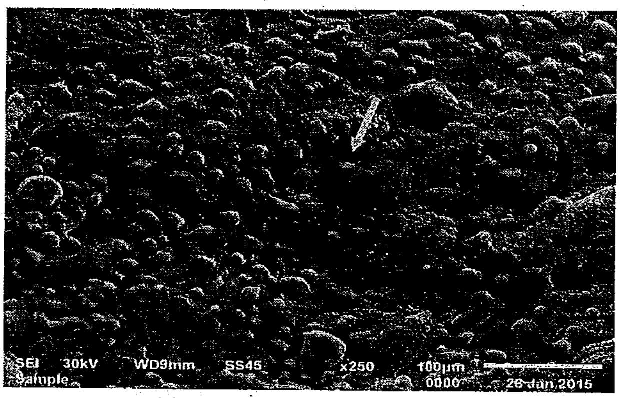

Fig. 7. A photomicrograph of epithelial surface of non gravid uterine body (intercaruncular area) at the luteal phase showing, variable spherical secretory vesicles projected at the surface epithelium and orifices of the excretory duct of the endometrial glands (yellow arrow). SEM, X 250.

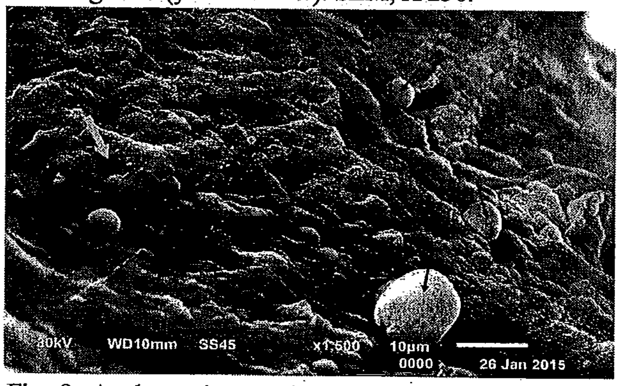

Fig. 8. A photomicrograph of intercaruncular epithelial surface of non gravid uterine body at the luteal phase showing, the spherical secretory vesicles (black arrows) of variable size, which were projected from endometrial glands and notice, orifice of the excretory duct of the endometrial glands (yellow arrow). SEM, X 1,500.

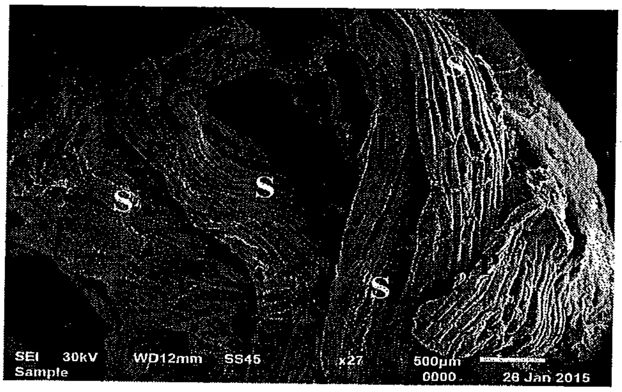

Fig. 9. A photomicrograph of epithelial surface of the cervix at the follicular phase showing, orientation of the longitudinal primary folds (p) with secondary mucosal folds (s). SEM, X27.

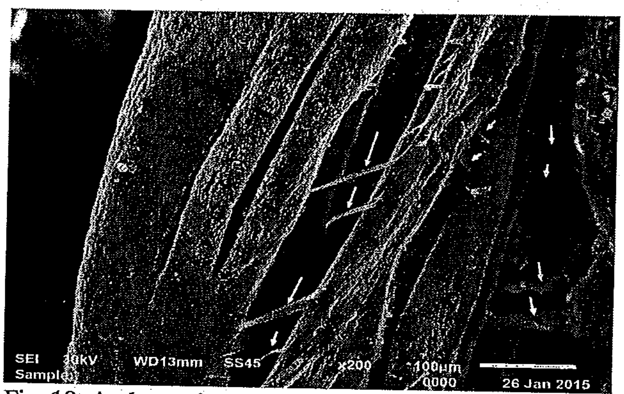

Fig. 10. A photomicrograph of epithelial surface of cervix at the follicular phase showing, longitudinal primary folds (p); secondary folds (s) were oriented along the lateral walls of the primary folds. There were thin interconnecting threads joining between the secondary folds (white arrows). SEM, X200.

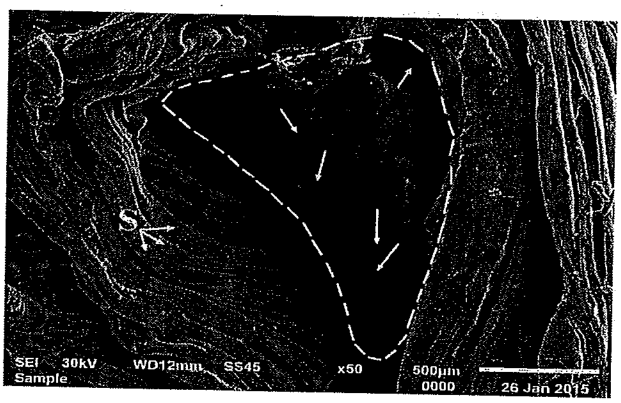

Fig. 11. A photomicrograph of epithelial surface of cervix at the follicular phase showing, longitudinal primary folds (p), orientation of secondary folds (s) along the lateral walls of the primary folds. Also there was a clear crypt (dotted area) with several pockets (white arrows). SEM, X50.

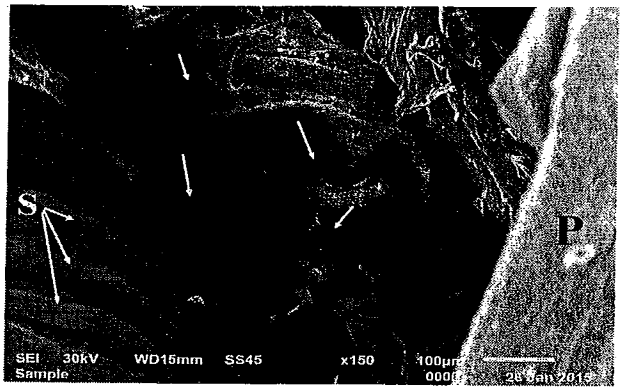

Fig. 12. A photomicrograph of higher magnification of (48) of cervix at the follicular phase showing, longitudinal primary folds (p), with lateral orientation of secondary folds (s) in between them clear pockets (white arrows). SEM, X150.

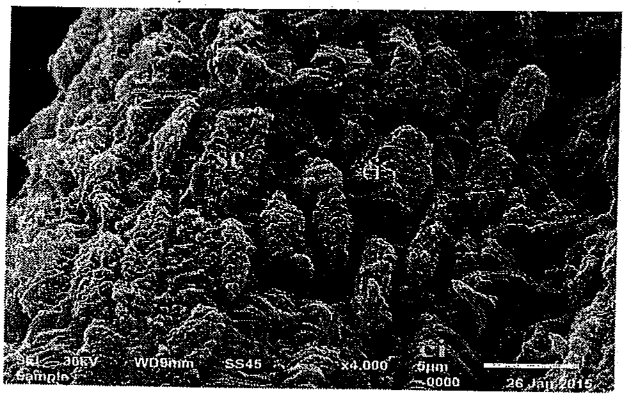

Fig. 13. A photomicrograph of cervical secondary folds at the follicular phase showing, clear ciliated cells of a medium length cilia (ci) and secretory cells (sc).SEM, X 4, 000.

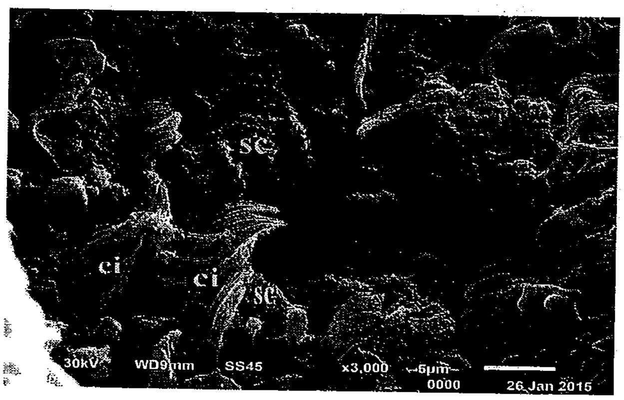

Fig. 14. A photomicrograph of epithelial surface between cervical secondary folds at the follicular phase showing, long wavy cilia (ci) which obscure the flat secretory cells (sc) with short microvilli. SEM, X 3,000.

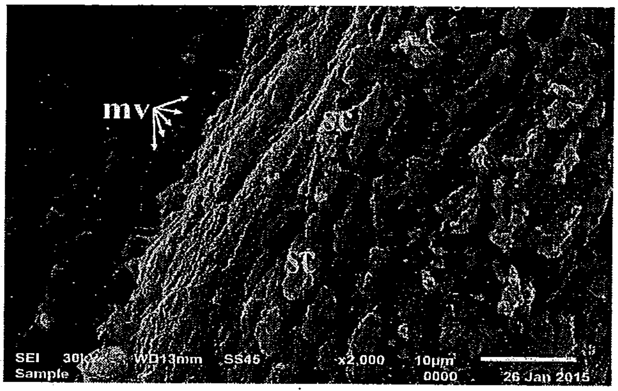

Fig. 15. A photomicrograph of cervical epithelial surface at the luteal phase showing, bulbous apical processes of the flat secretory cells (sc) and short microvilli (mv). SEM, X 2,000.

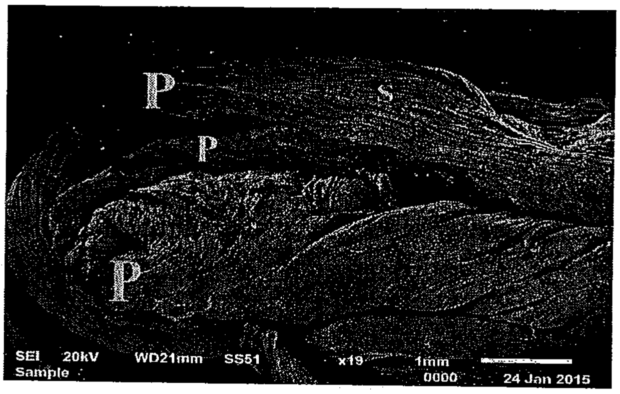

Fig. 16. A photomicrograph of buffalo Portio vaginalis cervicis at the follicular phase showing, longitudinal primary folds (p), orientation of secondary folds (s) along the lateral walls of the primary folds. SEM, X19.

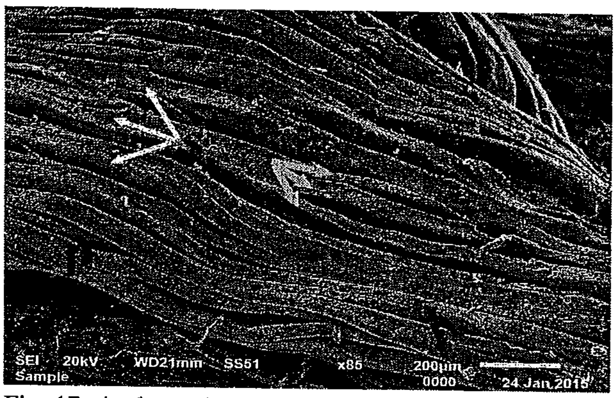

Fig. 17. A photomicrograph of Portio vaginalis cervicis at the follicular phase showing, longitudinal primary folds (p), orientation of secondary (s) folds along the lateral walls with clear divergence (red arrows) and trivergence (yellow arrows) of secondary folds. SEM, X85.

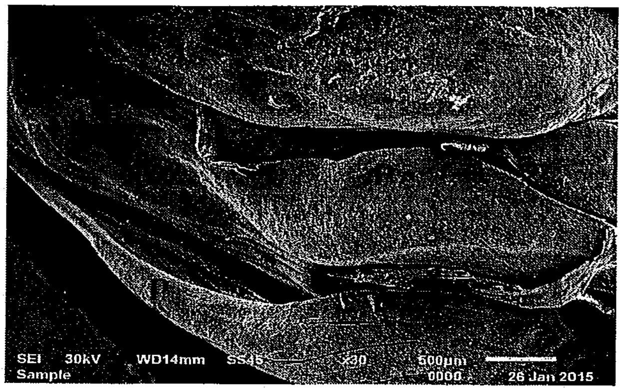

Fig. 18. A photomicrograph of buffalo Portio vaginalis cervicis at the follicular phase showing, smooth longitudinal primary folds (p), with clear secondary folds (s). SEM, X30.

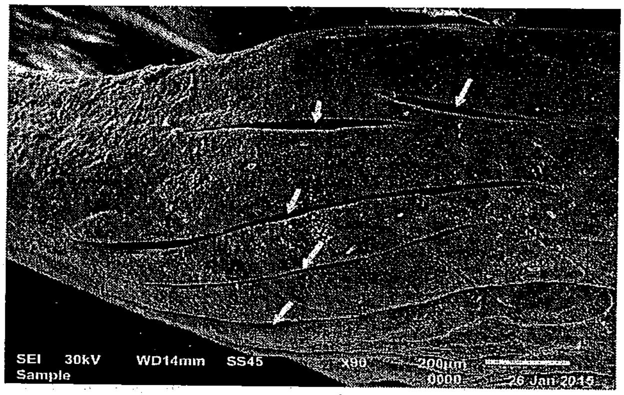

Fig. 19. A photomicrograph of higher magnification of (18) showing, smooth longitudinal primary folds (p) with apparently detected longitudinal grooves (white arrows). SEM, X90.

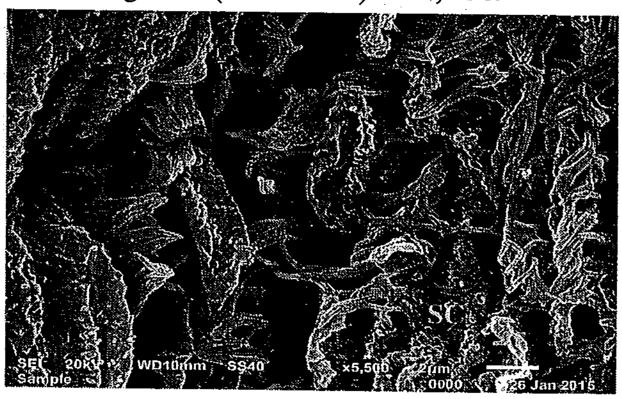

Fig. 20. A photomicrograph of epithelial surface of buffalo Portio vaginalis cervicis (secondary folds) at the follicular phase showing, Ciliated cells with long cilia (ci) and secretory cells (sc) with apical bulbs. The long cilia obscure the underlying secretory cells. SEM, X 5,ᵒ00.

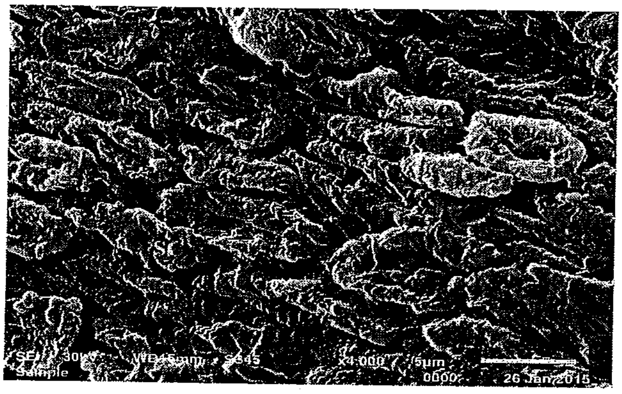

Fig. 21. A photomicrograph of epithelial surface of buffalo Portio vaginalis cervicis at the luteal phase showing, flat secretory cells (sc) with apical bulbs. SEM, X 4,000.

DISCUSSION

With respect to the changes, the characters of ciliated and secretory cells in buffalo endometrium during the estrous cycle, there were many differences in their number, activity and shape. Our work agreed with that mentioned by ***(10)*** in cows, ***(11)*** in bitch, ***(12)*** in mares and ***(13)*** in buffalo.

In our study, the uterine epithelial surface of buffalo showed a smooth surface of secretory cells with or without microvilli and prominent mosaic-shape boundaries in the luteal phase, whereas the ciliated cells were more observed in the follicular phase. The results were in a line with those mentioned by ***(13)*** in buffalo. These investigations supported the theory that the epithelial cell modification was induced by ovarian steroid hormones, and could be concluded that, when the buffaloes reach to puberty, the uterine epithelial surface would be changed rapidly. This suggestion was supported by ***(14),*** they reported that, the number of ciliated cells increased during the ovulatory phase, to reach its maximum of about 20%, and then declined. The latter authors suggested that, the increase in ciliated cells during ovulatory phase was attributed to high secretion of estrogen hormone.

In the present investigation, the uterus of buffalo made 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 and large numbers of cilia were hidden during the luteal phase. Similar cyclic changes had been observed by ***(13)*** in buffalo. The latter authors observed that, the epithelial cells were convex and separated from each other by cell borders locating in deep levels. The epithelial cells were covered by numerous microvilli and the ciliated cells were scarcely found among these cells. These results were in agreement with that detected in the follicular phase in our work. Moreover, ***(13)*** at the luteal phase stated that, the epithelial surface was flat with very low number of microvilli and the cell boundaries were prominent and protruded into the lumen. This was in a line with that discerned in our research.

Wu et al., ***(15)*** found that, the luminal surfaces of the secretory cells were largely obscured by cilia. This agreed with detected in our findings. ***(15)*** showed that, the rare presence of cilia in porcine cervical epithelium in the estrous cycle. This in contrast with that had been recorded in other mammalian species. The ciliated cells had been observed in the cervical mucosa of cow. This was in agreement with our work as well as ***(15, 16).*** These results attributed to the species variations.

It was known that the microvilli in the ruminant uterine epithelium were important to the implantation process. This due to the interdigitation of cytoplasmic projections of trophectoderm cells and uterine epithelial microvilli, which occurred during apposition ***(17).*** Moreover it was possible that the microvilli associated with absorption and electrolyte transport during uterine metabolism ***(18).***

In this respect, these many secretory cells were observed in luteal phase in the present investigation in comparison to cilia, as many investigators suggested that, the ciliated cells were only normal cellular components of the uterine surface epithelium, and there was no clear evidence of their function in endometrium *(10, 11, 14, 19)*. Moreover *(20,21)* suggested that, the uterine horns played a major role in sperm transport after insemination, sperm maturation and perhaps sperm capacitation before they travel toward the utero-tubal junction (UTJ) to be stored till the fertilization.

There was a relationship between morphology of secretory cells and the activities of the endometrial glands could be observed. So, in this respect, the cytoplasmic protrusions of secretory cells in varying degrees were noticed at the follicular phase but the secretory vesicles from the endometrial glands were clearly observed on the glandular openings during the luteal phase. Similar cyclic changes had been observed by *(22)* whereas *(23)* suggested that these protrusions in cow uterine epithelium were principally associated with secretory activity and involved in merocrine.

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الملخص العربى

دراسات بالماسح الضوئي المجهرى الالكترونى لبطانة الرحم فى الجاموس فى مرحلتى جراف والأصفرية

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أوضحت الدراسة بالماسح الضوئي المجهرى الالكتروني أن الغشاء المخاطي للرحم في الجاموس يتغير دوريا خلال مرحلتى جراف والأصفرية من دورة الشبق. وقد كانت الخلايا المهدبة الغير إفرازية كثيرة خلال مرحلة جراف، بينما الخلايا الغير مهدبة الإفرازية كانت كثيرة خلال المرحلة الأصفرية كما لوحظ ان الخلايا المهدبة كانت قليلة فى هذة المرحلة. وقد تميز الغشاء المخاطي لعنق الرحم بعدد من الطيات الصغيرة، وهذة الطيات تتقارب لتشكل طيات طولية ابتدائية وأخرى ثانوية، تخرج خروجا مباشرا من الجدران الجانبية لهذه الطيات والمتفرعة باتجاه المناطق القاعدية بين الطيات الابتدائية لتشكيل طريق مسدود الجيوب.