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Scanning Electron Microscopic Studies on the Uterus of Buffalo during Follicular and Luteal Phases of Estrous Cycle

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ABSTRACT

The present investigation was aimed to elucidate the surface ultrastructure of uterine horn, body and cervix of buffalo during follicular and luteal phase of estrous cycle. The present study was conducted on uterus of buffaloes during follicular and luteal phase of estrous cycle. The tissue samples fixed in Karnovsky's fixative and processed for viewing under scanning electron microscope. The luminal surface of uterus was folded into broad ridges and folds. The luminal surface was lined with ciliated and non-ciliated cells. Ciliated cells had many kinocilia during the follicular phase. The non-ciliated cells dominated both during follicular and luteal phases of estrous cycle. The non-ciliated cells were of two types *viz*: secretory and non-secretory type. The surface of the cervix was highly folded with ridges and deep crypts. A scanning electron microscopic examination of the cervix revealed exposed ridges composed of cell apices that alternate with deep folds or crypts. The exposed ridges consisted of the external apical membranes of the columnar epithelial cells that form the cervical lining. Ciliated cell surrounded by several non-ciliated cells from the posterior region of the cervix. The cells surrounding the ciliated cell were covered with much shorter projections or microvilli that characterize secretory cells. Ciliated cells terminated with a group of cilia that emerged from the apical cell membrane of the epithelial cells and projected towards the lumen of the cervix.

Keywords: Buffalo, follicular phase, luteal phase, scanning electron microscopy and uterus

In India milk is considered as a staple food for large vegetarian population and to make up the gap in production and demand, buffalo dairy systems are best available option (Balhara *et al*., 2017). Reproductive performance of buffalo is a major determinant in the efficient production of both milk and meat. The uterus is responsible for the sperm transport, luteolysis and control of cyclicity, provide environment and nourishment to zygote preceding implantation. It modifies itself to accept the conceptus and to provide protection throughout the pregnancy and also helps in expulsion of foetus and placenta at the time of parturition. It has been suggested that uterine environment regulates the development of embryos very early in gestation, when the physical capacity of uterus is not a developmental constraint, possibly via alteration in trophoblast binucleate cell number and structure (Sharma *et al.,* 2013).

The cervix has been postulated to function as a sperm reservoir and also to serve as a selective filter that prohibits transport of inactive or abnormal sperm but allows viable spermatozoa to enter the uterus during the periovulatory period, integral to maintenance of pregnancy and forming a barrier to the ascent of microorganism from vagina (Hafez and Kanagawa, 1973). The epithelial cells of the reproductive tract provide a barrier against pathogens and microbes and the secretions of tract contains anti-microbial peptides, cytokines, and chemokines responsible for the recruitment and activation of immune cells and together epithelial cells and immune cells provide protection through Toll-like receptors (Amjadi *et al*., 2014; Nott *et al.,* 2016).

During different phases of estrous cycle and pregnancy, the uterus undergoes characteristic changes under the influence of reproductive hormones. Scanning electron microscopic studies on the uterus have been done in cattle (Hafez. and Kanagawa, 1972) in African buffalo (Schmidt *et al.,* 2006), in sheep (Pathak *et al.,* 2008) and in Thai Swamp buffalo (Tienthai and Sajjarengpong, 2010) but the surface ultra structure of the luminal epithelium of the Indian buffalo uterus has not been described. The present study was designed to examine the surface ultrastructure of uteri of buffalo during follicular and luteal phases of the estrous cycle.

MATERIALS AND METHODS

Collection of samples

The present study was conducted on different parts (horn, body and cervix) of uterus of buffaloes (n=12). Samples were divided into being in follicular and luteal phases based on the surface features of the ovary. Samples were classified as being in follicular phase (n=6) of estrous cycle based on presence of well-developed tertiary follicle on the ovary and as luteal (n=6) with presence of a well-developed corpus luteum with few smaller surface follicles.

Processing of samples, viewing and imaging

The tissue samples were collected and washed in chilled 0.1M phosphate buffer (pH 7.2) and were fixed in Karnovsky's fixative (2.5% gluteraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer solution at pH 7.2) for 4-6 h. Fixed samples were washed in 0.1M phosphate buffer with 3 changes of 15 minutes each at 4ºC. Thereafter, the samples were dehydrated in ascending grade of acetone solutions i.e. 30%, 50%, 70%, 80%, 90%, 95% and 100% acetone (dry acetone) at 4ºC.

The dehydrated specimens were then placed in the small stainless steel mesh baskets, which were placed in boat of critical point drying apparatus containing acetone and critical point drying was done at critical temperature (31.5ºC) at 1100 psi. There after the tissues were placed in desiccators, mounted on aluminum stubs, sputters coated with 35 nm thick layer of gold and were viewed under LEO 435 VP scanning electron microscope (Pathak *et al.,* 2008).

RESULTS AND DISCUSSION

Uterine horn and body

The luminal surface of uterus was folded into ridges and folds showing broad ridges with shallow and deep crypts (Fig. 1A). Unevenly distributed over the endometrial surface in the intercaruncular areas, a number of round to oval openings of endometrial gland were present (Fig. 1B). Cobblestone image of uterine surface epithelium at follicular phase in Thai buffalo was recorded (Tienthai and Sajjarengpong, 2010) whereas in the rabbits two different types of fold formations, mosaic like pattern in the body of uterus while wave like in the horn of uterus have been reported earlier (Hafez and Kanagawa, 1973). Similar observations on the endometrial glands have been reported using histomorphological and scanning electron microscopic studies in the Indian buffalo (Pathak and Bansal, 2012).

As seen by the scanning electron microscope, the endometrial surface was lined with ciliated and non-ciliated cells (Figs. 1C and 1D). The non-ciliated cells were either secretory cells or non-secretory cells. During both follicular and luteal phases of estrous cycle the non-ciliated cells dominated the cell types. The ciliated cells were rounded in surface outline. Most ciliated cells were convex and separated from each other by cell borders locating on a deep level. The cilia which covered the whole surface of the cell were clearly separated from each other. Ciliated cells overlapped the surface of secretory cells. These observations are in consonance with the findings in the uterus of Thai buffalo (Tienthai and Sajjarengpong, 2010). Presence of two distinct type of cell namely ciliated and non-ciliated cells were confirmed in buffalo uterine mucosa using transmission electron microscope (Pathak and Bansal, 2011), in swamp buffalo by scanning electron microscopy (Tienthai and Sajjarengpong, 2013) and in sheep using scanning electron microscopic study (Pathak *et al*., 2008).

The number and distribution of ciliated and secretory cells vary in body and horn of uterus as well as in different reproductive phases. At luteal phase, the epithelial surfaces were flat with microvilli and the cell boundaries are prominent and protruding into the uterine lumen. The cells were distinctly polygonal with microvillous surface

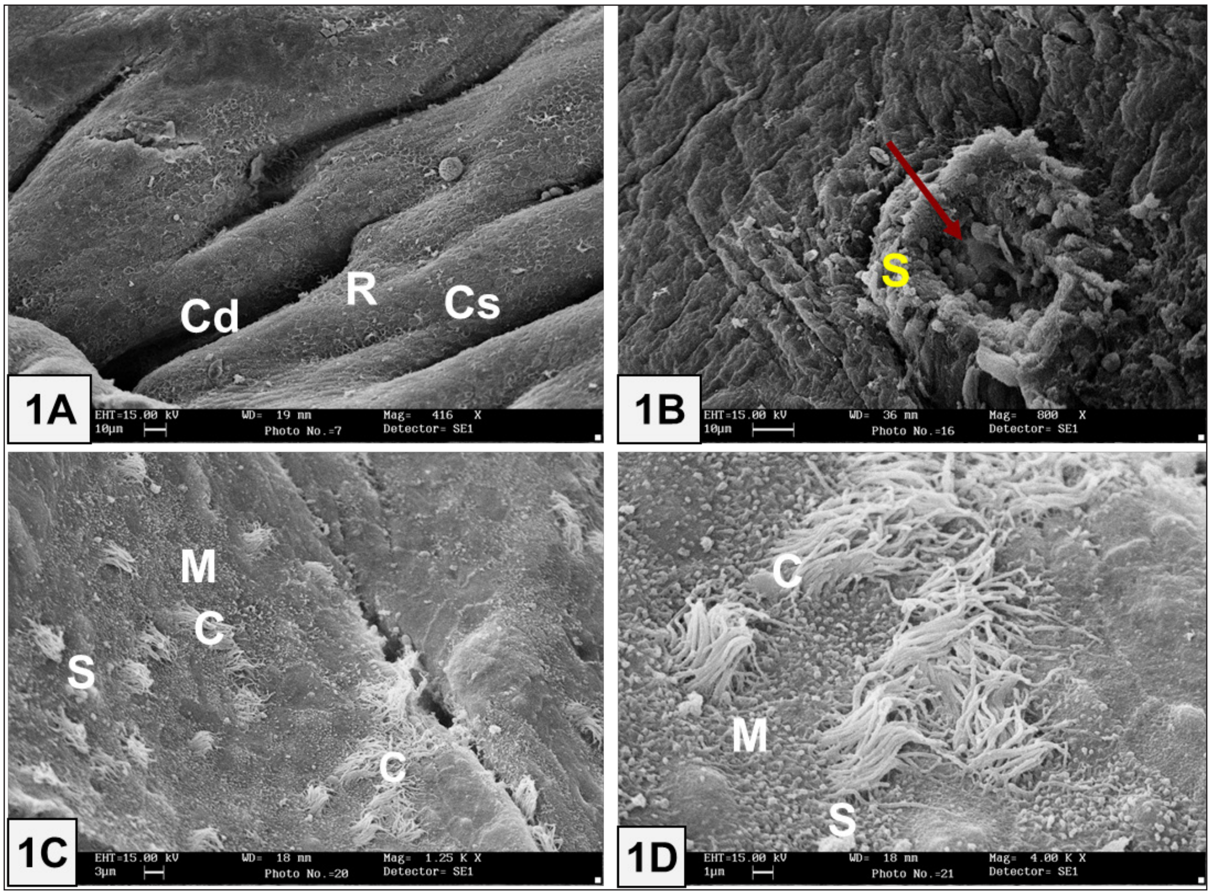

Fig. 1: Electron micrograph of buffalo uterine horn showing, **(A)** luminal surface folded into broad ridges (R) and crypts [Shallow (Cs) and deep (Cd)]. X 416; **(B)** endometrial gland openings (arrow) with secretory materials over the surface (S). X 1060; **(C)** showing ciliated cells with bunch of cilia (C), secretory cell with secretion (S) and microvilli over the non ciliated cells (M) during follicular phase. X 1250; **(D)** Ciliated cells with bunch of cilia (C), microvilli over the non ciliated cells (M) and secretory materials (S) during follicular phase. X 4000

and ciliated cells with few cilia at places (Fig. 2). Three types were cells viz. microvillous cells, ciliated cells and polygonal secretory cells were described in the mare endometrium (Samuel *et al*., 1979). Hexagonal shaped cells have been described in uterus of African buffalo which were masked by the presence of numerous microvilli (Schmidt *et al*., 2006). The occurrence of secretory activity during the follicular phase might be closely related with the estrogen level in the circulating blood.

It has been reported that that the morphology of endometrial cells of cow varied with change in the phase of estrous cycle (Benbia *et al*., 2017)

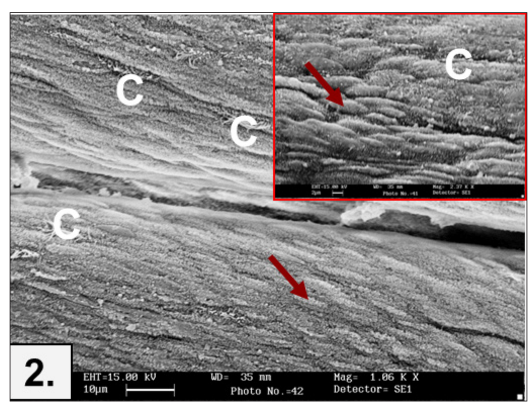

Fig. 2: Uterine horn during luteal phase showing polygonal non-ciliated cells (arrow) and ciliated cells with few cilia(C) X1060, Inset showing magnified view of polygonal non-ciliated cells (arrow) and ciliated cells with few cilia(C) X 2370

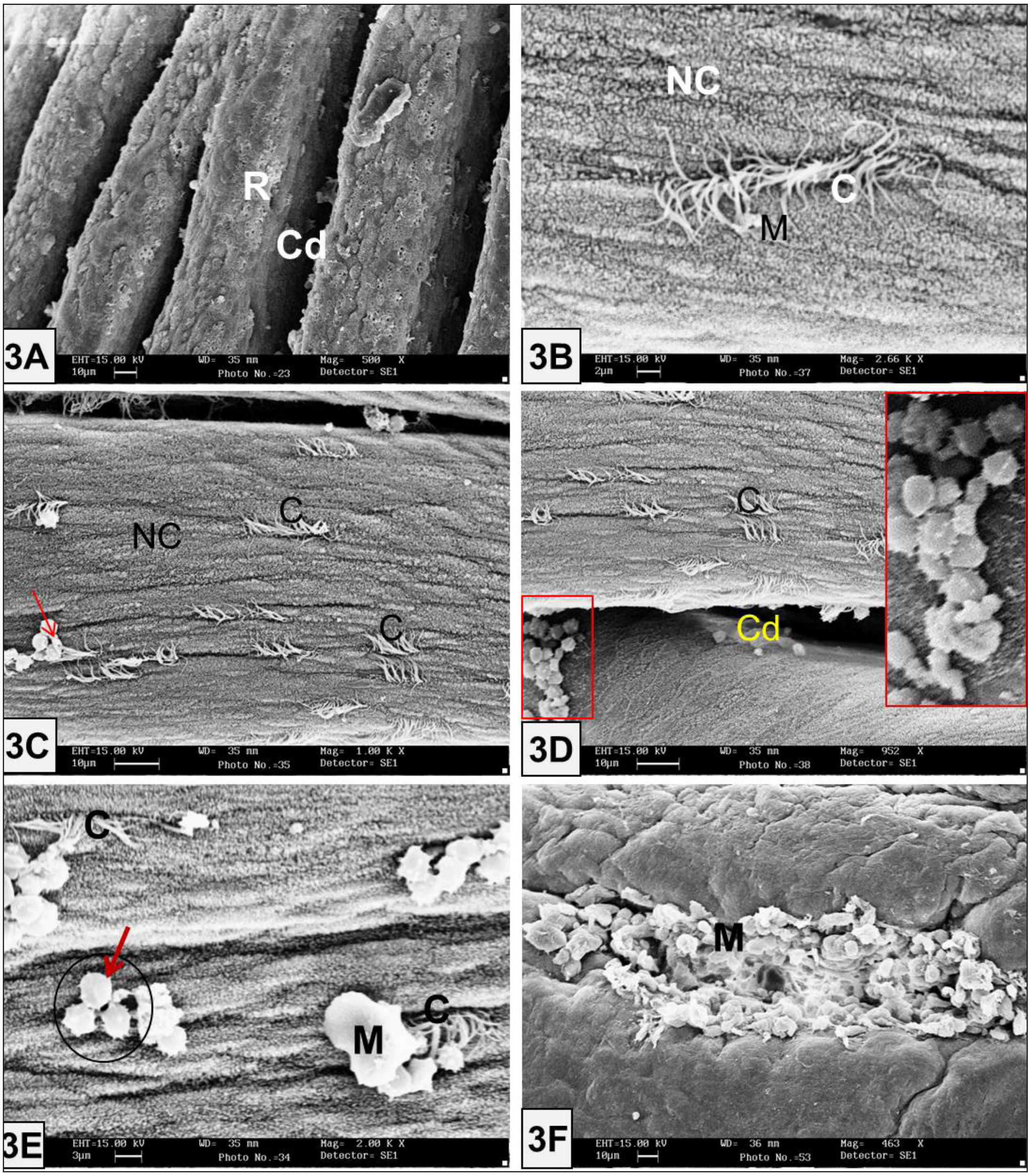

Fig. 3: Electron micrograph of buffalo cervix uteri during follicular phase showing, **(A)** ridges (R) alternating with deep crypts (Cd) X 500; **(B)** ciliated cells with bunch of cilia (C), non-ciliated polygonal cells (NC). X 2660; **(C)** ciliated cells with bunch of cilia (C), mucoid secretions near ciliated cells (M), group of lymphocytes with surface spikes (encircled area with arrow) (M). X 2000; **(D)** ciliated cells with bunch of cilia (C), deep crypts (Cd). X 2000. Inset showing magnified view of clumps of lymphocytes; **(E)** ciliated cells with bunch of cilia (C), mucoid secretions near ciliated cells (M), group of lymphocytes with surface spikes (encircled area with arrow) (M). X 2000. F. large mass of mucous in the cervical crypt (M). X 463

The secretory cells had polygonal outline and smooth apical blebs in which a number of cavities were visible. The non-ciliated non-secretory cells were the most common cell type in both the phase of the estrous cycle and were covered with microvilli. El Basset *et al*. (2016) recorded numerous microvilli on the apical part or secretory protrusion in various degrees, distinctly increased at the follicular phase and numerous secretory cells with stubby microvilli covered throughout the endometrial surface with secretory vesicles in larger numbers near orifices of endometrial glandular during the luteal phase and the ciliated cells were scarcely reported in buffalo endometrium.

The ovoid protrusions were present on the epithelial cells endometrium (Fig. 1D). The apical smooth surfaced blebs of the secretory cells were occasionally pedunculated and apparently budding off into the uterine lumen. It has been established that the estrogen caused an increase in length and number of microvilli including the amount of secretory droplets while the progesterone hormone affected the uterine epithelial cell flattening and a decrease the shortening of microvilli in uterine horn of Thai swamp buffalo (Tienthai and Sajjarengpong, 2010). The results of present investigation were in accordance with the findings described in Thai buffalo, bovine and other species in which the morphological changes of the endometrial epithelium are under the control of ovarian hormones.

Cervix

The surface of the cervix was extensively folded. A scanning electron microscopic examination of the cervix revealed brad ridges that were separated with deep crypts (Fig. 3A). These folding patterns were present both in follicular and luteal phase of estrous cycle. Similar observations have been recorded in the cervix of ewe (Wergin, 1979) and in cattle cervix uteri (Hafez and Kanagawa, 1973).

The exposed ridges consisted of the luminal membranes of the columnar epithelial cells that formed the cervical lining. These lining epithelial cells were of two types namely, ciliated and non-ciliated cells. Ciliated cell surrounded by several non-ciliated cells were observed. Ciliated cells terminated with a cluster of cilia that emerged from the cell membrane of the epithelial cell and projected into the lumen of the cervix (Fig. 3B). Ciliated cells have also been observed in the cervical mucosa of the sheep (Pathak *et al.,* 2008).

The cells surrounding the ciliated cell were covered with much shorter projections or microvilli that characterize secretory cells. The mucoid secretions from the cells were observed on the cervical surface and were seen near the ciliated cell. The presence of secretory material near the ciliated cells suggested the role of cilia in the flow of mucous (Fig. 3C). This secreted mucous on the endometrial surface might help in the smooth passage of the spermatozoa. Similar observations have been reported in ewe (Wergin, 1979).

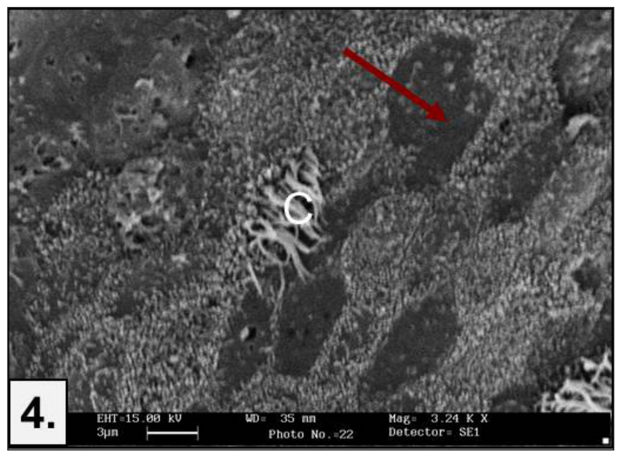

Fig. 4: Cervix uteri during luteal phase showing ciliated cells with bunch of cilia (C), cells with eroded apical surface (arrow). X3240

On the luminal surface of cervix clumps of lymphocytes were observed. These cells were polygonal in appearance with spikes on their surfaces. These cells were present in the grooves between mucosal ridges and these clumps were larger in the deep clefts between mucosal ridges (Figs. 3D, 3E). Presence of lymphocytes and other polymorphonuclear cells was the essential component of the immune defense system of the female reproductive tract of buffalo.

These cells might be responsible for preventing ascend of infectious agent. Presence of immune cells in the cervical mucous of buffalo has been reported earlier and it was hypothesized that because of this type of defense system of reproduction in buffalo the infection rate to reproductive tract is lower than the cattle. The percentage of lymphocytes, neutrophils and basophils in luteal phase were reported higher than during the follicular phase in buffaloes (Ayen *et al*., 2012).

Large mass of mucous secretions were observed in the crypts of cervix uteri (Fig. 3F). This suggested the opening of cervical glands in the cervical crypts. During the luteal phase the number of ciliated cells reduced. The apical parts of secretory cells were eroded suggesting the apocrine type of secretions. These cells have already undergone secretion during the follicular phase of estrous cycle (Fig. 4).

Reduced number of ciliated cells during the luteal phase of estrous cycle has been explained as deciliation under the hormonal influence. The cervix uteri of rabbits sustained only a patchy loss of cilia 18 months following ovariectomy (Riches *et al.,* 1975). Deciliation has been reported in the reproductive tract of the rhesus monkey but not in the human. The uterine cilia in rhesus monkey underwent true degeneration during the influence of progesterone but were renewed following an elevation of estrogen production (Hafez, 1972).

CONCLUSION

The study characterized the surface ultrastructure of horn, body and cervix of uterus of buffalo during follicular and luteal phases of estrous cycle. Mucosa was thrown into broader folds and these folds were lined with ciliated and non-ciliated cells. Non-ciliated cells were of secretory and non-secretory in nature. At places opening of the endometrial glands were observed. Lesser number of ciliated cells was observed in the follicular phase while larger number secretory cells were observed in the luteal phase of estrous cycle. These changes might be correlated with the level of estrogen and progesterone hormones in the circulation during these phases of cycle.

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