



A transmission electron microscopic study on the buffalo uterus during follicular and luteal phases of estrous cycle

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Transmission electron microscopic studies on the uterus have been made by Larsen (1962) in rabbit, Nilsson (1962), Fetaih *et al.* (1992) in camel and Wick and Kress (2002) in marsupials and Schimdst (2006) in African buffaloes but little work has been done on the Indian buffalo uterus. The present work was intended to throw light on transmission electron microscopic studies on uterus of buffalo in follicular and luteal phases of estrous cycle.

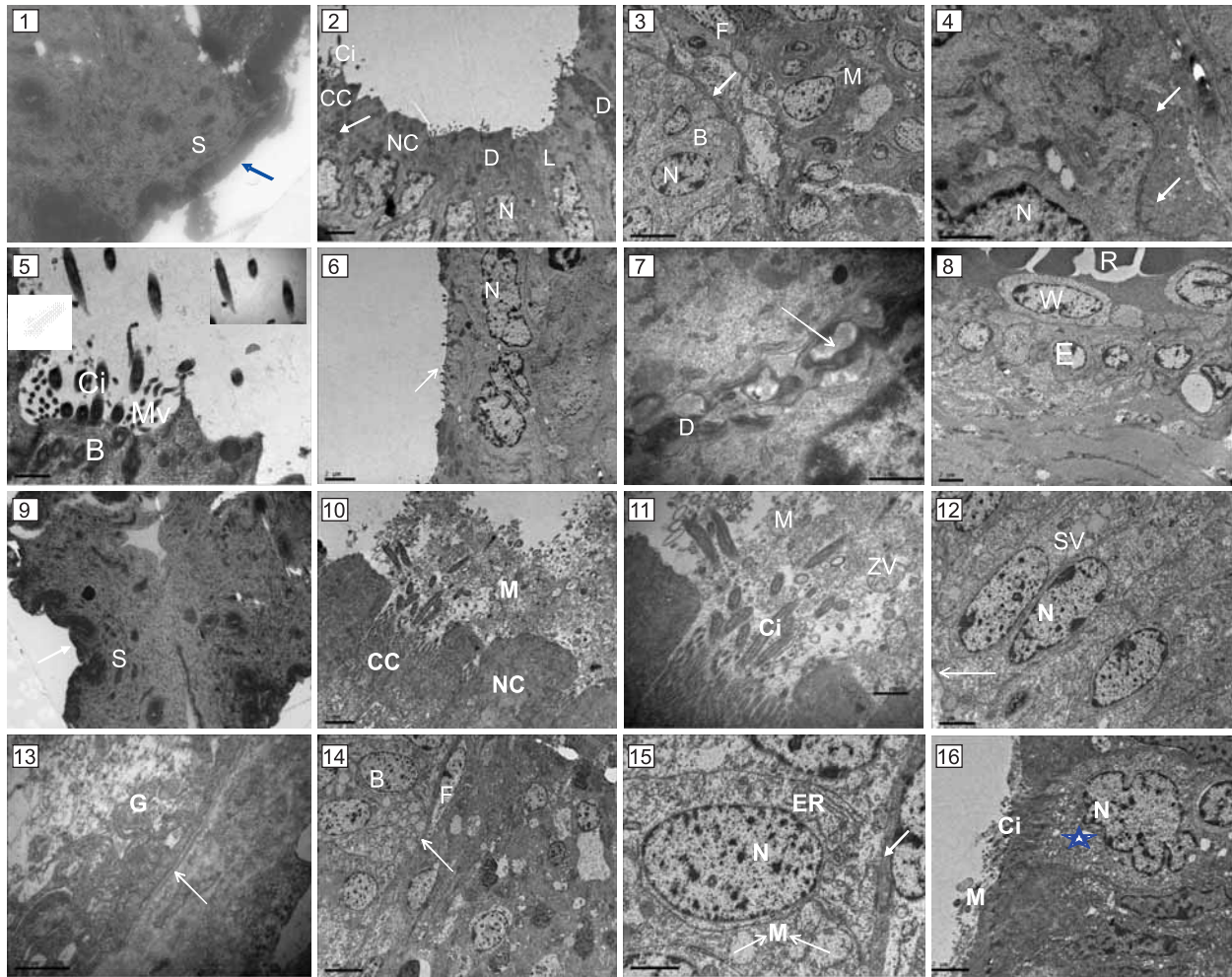
The present study was conducted on uteri of 12 buffaloes during follicular and luteal phases of estrous cycle. Both the left and right cornua uteri, corpus uteri and cervix uteri were collected from slaughter house after examining the status of ovaries. These tissues were thoroughly washed in phosphate buffer saline (pH 7.4) solution and fixed for 2 h in Karnovsky's fixative, then secondary fixation was done for 2 h in 2% OsO₄. Subsequently tissue samples were subjected to dehydration in ascending grades of acetone (30% to absolute). The dehydration in dry acetone was done at room temperature. The clearing of the samples was accomplished by treatment with toluene. Subsequently infiltration was carried out. The tissues were then embedded in pure embedding media using beam capsule and polymerization was undertaken. The blocks prepared were trimmed by block trimmer and semi thin sections (0.5–2.0 µm) were cut to scan the tissues under optical microscope for selection of area for ultrathin sectioning. After scanning, ultrathin sections (70–90nm) were cut and lifted on copper grids (100 mesh size) and stabilized by coating with carbon film of 50Å thickness. The grids were stained with uranyl acetate (15 min) followed by lead citrate (10 min) and examined under TEM for detailed study and required photographs were taken.

Transmission electron microscopy of cornua, corpus and cervix uteri buffalo revealed that the mucosa was lined with 3 types of columnar cells, viz. ciliated, non-ciliated cells and basal cells.

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Cornua and corpus uteri: Semi thin sections of corpus and cornua uteri revealed that endometrium was lined with pseudostratified epithelium (Fig. 1). Electron microscopy showed that the surface epithelium of the endometrium consisted of a single layer of tall columnar epithelial cells. They were cylindrical in shape with irregular nuclei. Based on the electron density of the cells, two types of cells were observed, viz. dark cells and light cells. Based on the luminal surface features two types of cells were observed, viz. of ciliated cells and non ciliated cells (Fig. 2). At places basal cells were also observed resting on basement membrane giving uterine epithelium a pseudo stratified appearance (Fig. 3). These cells were lying on a distinct basement membrane which was undulating in nature (Fig. 4). The ciliated cells were less common as compared to non ciliated cells both during follicular and luteal phases of estrous cycle. Both ciliated and non ciliated cell type have been described by Larsen (1962) in the uterine epithelium of rabbit. Schmidt (2006) studied uterus of African buffalo uterus and observed that light and dark staining columnar cells resting on undulating basement membrane. He also observed microvilli on the apical surface of the cells and cilia on some lightly stained cells. But contrary to our findings Nilsson (1958) did not report ciliated cells in the uterine epithelium of mouse.

The cytoplasm of ciliated cells was less electron opaque, the nucleus often irregular in shape. The ciliated cells had cilia and microvilli over their cell surface. They were typical motile cilia with nine peripheral and two central microtubules as seen in their cross sections in the luminal surface (Fig. 5 and inset photo). Basal bodies with the ciliary rootlets were located just beneath the cell surface membrane. Cilia of surface epithelium might be playing a role in propulsion of the oocytes. The presence of microvilli and cilia has been reported by Larsen (1962) in rabbit uterine epithelium and Fetaih (1992) in camel endometrium. These microvilli may be associated with the absorption. In the uteri of humans and mouse in the luteal phase, irregular large projections were reported to develop and replace the microvilli on the luminal



Figs 1–16. **1.** Semi thin section of endometrium of buffalo showing pseudostratified epithelium (arrow) lying over connective tissue stroma (S). Methylene blue $\times 40$. **2.** Transmission electron micrograph (TEM) of apical portion of epithelium of endometrium at follicular phase showing dark cell (D), light cells (L), ciliated cell (cc) with many mature cilia (ci) and non ciliated cells (NC) containing secretory granules (SG). $\times 20000$. **3.** TEM of apical portion of endometrium during the follicular phase showing basal cells (B) with nucleus (N) lying on the basement membrane (arrow) intercellular matrix (M), fibroblast like cell (F). $\times 5000$. **4.** TEM of endometrium showing undulating basement membrane (arrow), nucleus (N). $\times 15000$. **5.** TEM of apical portion of epithelium of endometrium showing ciliated cell with cilia (C) and non ciliated cell (NC), continuity of non ciliated cell with lumen (arrow) and microvilli (Mv). $\times 20000$. **6.** TEM of apical portion of epithelium of endometrium during the luteal phase showing epithelial cells with mainly non ciliated cells with microvilli (arrow) and nuclei (N). $\times 25000$. **7.** TEM of epithelium of endometrium showing inter-cellular membrane and connections as gap junctions (D) and desmosomes (arrow). $\times 30000$. **8.** TEM showing blood capillary in the endometrial stroma with RBCs (R) inside the lumen, WBCs (W) in lumen and one of WBC touching the capillary wall to perform diapedesis, endothelial cells (E). $\times 4000$. **9.** Semi thin section of cervix uteri showing pseudo stratified epithelium (arrows) lying over the connective tissue stroma. Methylene blue. $\times 40$. **10.** TEM of apical portion of epithelium of cervix uterii during follicular phase showing ciliated cell (cc) with many mature cilia (ci), non ciliated cells (NC) and secretory material lying over the ciliated and secretory cells. $\times 10000$. **11.** TEM of apical portion of cervix uterii showing ciliated cells with cilia (C), secreted material on the surface (M) and zeotic vesicle (ZV). $\times 20000$. **12.** TEM of cervix uterii showing columnar cells with cylindrical nuclei (N), presence of mitochondria (M) and secretory vesicles. $\times 6000$. **13.** TEM of cervix uterii showing infra nuclear zone of a secretory cell having many Golgi apparatus (G) and secretory vesicles (arrow). $\times 30000$. **14.** TEM of cervix uterii showing epithelial cells resting on basement membrane (arrow), basal cell (B) stroma of cervix having fibroblast like cells (F), stromal cells (S), and matrix of stroma (Ma) with collagen fibres (arrow). $\times 25000$. **15.** TEM of cervix uterii showing basal cell resting on the basement membrane (arrow). nucleus (N), mitochondria (M), endoplasmic reticulum (ER). $\times 8000$. **16.** TEM of cervix uterii during luteal phase of estrous cycle showing ciliated cell with few cilia (ci), mucoid secretory material (M), nucleus (N), vacuolar cytoplasm in the supra nuclear region of ciliated cell (star). $\times 6000$.

surface of the epithelial cells and they are suggested to be required for the contact with an implanting blastocyst (Nilsson 1962). The mitochondria were smaller and of variable shape. Electron opaque bodies were also present in mitochondria of these cells. The cell surface possessed microvilli as well as cilia containing 2 central and 9 peripheral filaments. The endoplasmic reticulum was poorly developed. During the luteal phase of estrous cycle the ciliated cells were very less and more of microvillous cell type was observed. The nuclei were more flat compared to the follicular phase of estrous cycle (Fig. 6). Similar findings were reported by Schmidt (2006) in African buffalo uterus. The non ciliated cells were tall with an ovoid nucleus and one or two peripheral nucleoli. The luminal surface possessed microvilli. The cell membrane separating two adjacent cells were undulating with frequent desmosomes and gap junctions (Fig. 7). The basement membrane followed the basal cell membrane in its slightly undulating course. The luminal part of cell was occupied by large vacuoles containing filiform or granular material and in some of the cells communication between vacuoles and the surface was observed. Numerous inclusions of different size and density were found. The mitochondria were numerous, elongated and contained one or two electron opaque bodies. Few golgi apparatus were observed near the nucleus. During the luteal phase of estrous cycle, the non ciliated cells lacked vacuoles containing granular material and showed a reduction in the number of microvilli. Inclusions of different size and density were still seen but were fewer than the follicular phase. Number of mitochondria decreased. Smaller vacuoles and elements of Golgi apparatus were observed.

The endometrial stroma consisted of stromal cells and blood vessels. The inter cellular matrix was electron lucent or appeared as a finely granular material of medium electron density and of irregular distribution. It contained a moderate amount of collagen fibres. The nuclei of the stromal cells were rounded, oval or elliptical (Fig. 3). The similar observations had been reported by Brinsfield *et al.* (1972) in the endometrium of sheep. During the luteal phase of estrous cycle endometrial stroma became progressively looser through accumulation of extracellular fluid and an increased amount of mucoid matrix. Similar observations were made by Brinsfield (1974). Blood vessels with RBCs and WBCs were observed. Few WBCs were undergoing diapedesis to form part of migratory cells in the stroma (Fig. 8).

Cervix uteri: Semi thin sections of cervix uteri showed broad mucosal folds lined with pseudo stratified columnar epithelium (Fig. 9). Transmission electron microscopy showed that the surface epithelium of the cervix at times consisted of a single layer of tall columnar epithelial cells, at others of more pseudo stratified character because of presence of basal cells. The cells were cylindrical in shape. The nuclei were regular in outline in contrast to the nuclei of endometrial epithelial cells. Based on the surface features of

cervical epithelium two types of cells were observed, viz. ciliated cells and non ciliated cells (Figs 10, 11). The non ciliated cells were prevalent both during follicular and luteal phases of estrous cycle. These cells were lying on a distinct undulating basement membrane (Figs 12, 13). The non ciliated cells were tall with an ovoid nucleus and 1 or 2 peripheral nucleoli. The luminal surface possessed microvilli. The mucoid secretions of different sizes were lying over the luminal surface of both ciliated and secretory cells. These mucoid secretions were observed more during the follicular phase as compared to that of luteal phase. Ciliated cells had cilia and microvilli over their cell surface. They were typical motile cilia with 9 peripheral and 2 central microtubules as seen in their cross sections in the luminal surface. Cilia of surface epithelium play a role in propulsion of the oocytes. At places basal cells were observed giving epithelium a pseudo stratified appearance (Figs 14, 15). Similar to the endometrial stroma, the stroma of cervix uteri consisted of stromal cells and blood vessels. The inter cellular matrix contained a moderate amount of collagen fibres. The nuclei of the stromal cells were rounded, oval or elliptical (Fig. 14). During luteal phase of estrous cycle the epithelium of cervix uteri showed ciliated cell with few cilia. At the luminal surface scanty secretory material and zeotic vesicles were observed. The cytoplasm was more vacuolar in the supra nuclear region of ciliated cell (Fig. 16).

The differentiation of the cervical epithelium attained its maximum development during follicular phase, where secretory cells are fully packed with large secretory granules and ciliated cells are well developed. After extruding their granules, secretory cells may transform into ciliated cells, while ciliated cells show the phenomenon of deciliation in which cilia packets are shed into the cervical lumen. Similar findings have been reported by Wick and Kress (2002) in the cervix of marsupials.

Present study revealed that the cornua, corpus and cervix uteri were lined by ciliated and non ciliated secretory cells. Mucosa of each segment of uterus was more active during the follicular phase as compared to the luteal phase probably because of the influence of estrogen.

SUMMARY

The present investigation was conducted on cornua, corpus and cervix uteri from 12 buffaloes during follicular and luteal phases of estrous cycle. Transmission electron microscopy of endometrium of buffalo revealed that the mucosa was lined with three types of columnar cells, i.e. ciliated, non-ciliated cells and basal cells. Based on the electron density of the cells, 2 types of cells were observed, viz. dark cells and light cells. The ciliated cells were less common as compared to non ciliated cells both during follicular and luteal phases of estrous cycle. Ciliated cells had cilia and microvilli over their cell surface. Cilia were typical motile cilia with nine peripheral and two central microtubules. The non ciliated

cells had microvilli and few secretory vesicles on their surface. The surface epithelium of the cervix at times consisted of a single layer of tall columnar epithelial cells, at others of more pseudo stratified character because of presence of basal cells. The differentiation of the cervical epithelium attained its maximum development during follicular phase, where secretory cells are fully packed with large secretory granules.

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