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

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ABSTARCT

The present investigation was conducted on different segments of both the oviducts from buffaloes (12) during follicular and luteal phases of estrous cycle. It was lined by two distinct types of columnar cells, viz. ciliated and secretory cells. The numbers of ciliated and secretory cells varied in different segments and phases of estrous cycle. Ciliated cells were more observed in infundibulum and ampulla as compared to isthmus. The nuclei of these cells were elongated and usually centrally or basally located. Secretory cells with the cytoplasmic protrusions extending beyond the luminal border of the secretory cells were observed in higher number in the follicular phase of estrous cycle. In the secretory cells, some secretory granules were present in the supranuclear cytoplasm. The secretory granules had homogenous electron-dense matrices. The non-ciliated secretory cells of the isthmus had few secretory granules, but contained many lysosome like bodies. Secretory cells at follicular phase were characterized by numerous secretory granules showing maximum differentiation and a well developed rough endoplasmic reticulum with dilated cistern. During the luteal phase the ciliation was less and amount of secretory activity also reduced as compared to that of follicular phase.

Key words: Buffalo, Follicular phase, Luteal phase, Oviduct, Transmission electron microscopy

The ciliated cells of oviduct play an important role in the transport of oocytes into the oviduct (Odor and Blandau 1973). On the other hand the secretory cells synthesize and release specific glycoproteins (Oliphant 1986). Transmission electron microscopy of oviduct was studied by various authors in bovines (Nayak and Ellington 1977), ewes (Hollis *et al.*1984), hamster (El-Mestrah and Kan 1999), goat (Abe *et al.*1999), mouse (Lauschova 2003) and pigs (Abe and Hoshi 2008), but scanty of literature is available regarding transmission electron microscopy of oviduct of buffalo. Therefore the present work was intended to throw light on transmission electron microscopic study of oviduct in follicular phase of estrous cycle.

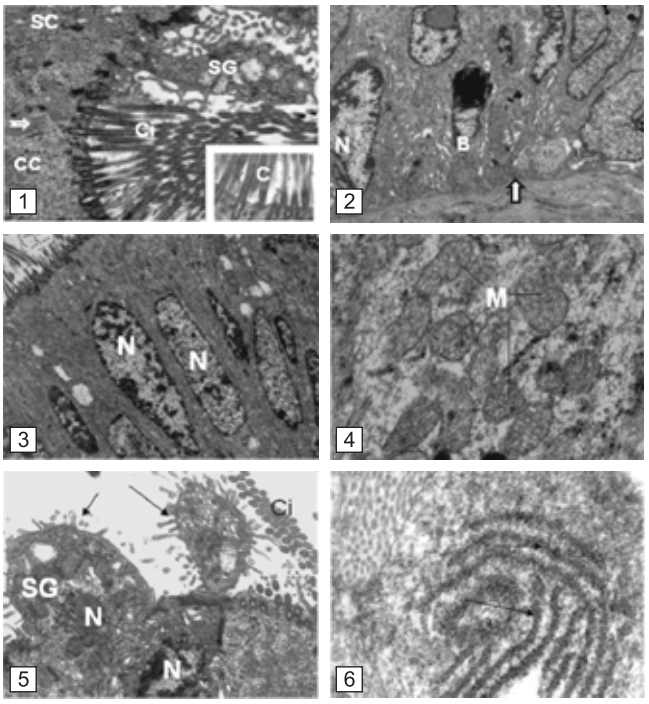
MATERIALS AND METHODS

The present study was conducted on oviducts of 12 buffaloes during follicular and luteal phases of estrous cycle. Both the left and right oviducts were collected from slaughter house after examining the status of ovaries. Tissue samples were collected from the infundibulum, ampulla and isthmus parts of oviduct. These tissues were thoroughly washed in phosphate buffer saline (pH 7.4) solution and subsequently trimmed of 1 mm^3 size. These samples were fixed for 2 h in Karnovsky's fixative and then secondary fixation was done for 2 h in 2% OsO_4. 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. Subsequently, polymerization was undertaken. The blocks thus 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, the ultrathin sections (70–90nm) were cut and lifted on copper grids (100 mesh size) and stabilized by coating with carbon film of 50 Å thickness. The grids were then stained with uranyl acetate (15 min) followed by lead citrate (10 min). The grids thus prepared were examined under TEM for detailed study and required photographs were taken.

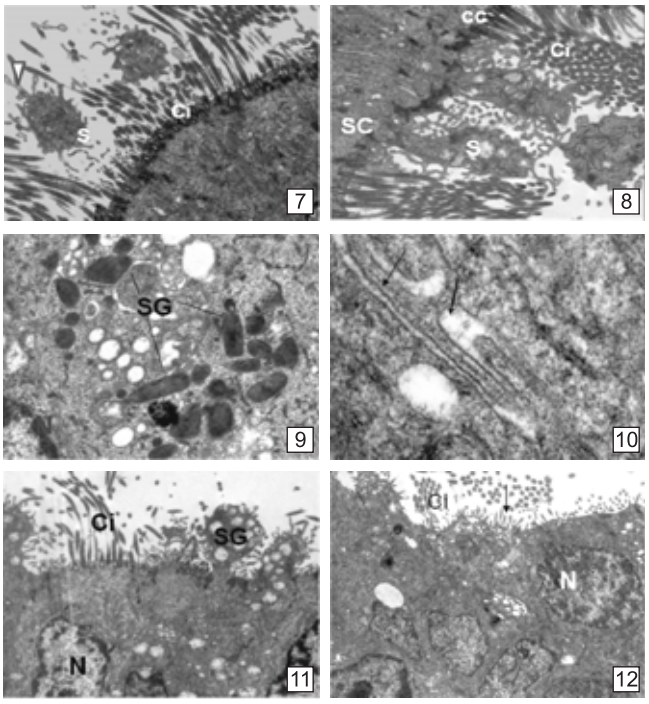
RESULTS AND DISCUSSION

Transmission electron microscopy of the different segments of the buffalo oviduct (infundibulum, ampulla and

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Figs 1–6. **1.** Apical portion of epithelium of infundibulum at follicular phase showing ciliated cell (cc) with many mature cilia (ci) and secretory cells with apical protrusions containing secretory granules (SG). × 1100. Photo inset showing higher magnification of apical portion of ciliated cells with cilia (C) and microvilli (arrow). × 5600. **2.** Epithelium of infundibulum during the follicular phase showing cells with nucleus (N) lying on the basal lamina (arrow) and basal cell (B). × 880. **3.** Apical portion of epithelium of infundibulum at follicular phase showing cells with spindle shaped nucleus. × 880 **4.** Supranuclear zone of a ciliated cell showing mitochondria (M with arrows) with different shapes and sizes. × 5600. **5.** Apical portion of epithelium of Infundibulum at follicular phase showing secretory cells with apical protrusions containing secretory granules (SG), nucleus (N) and microvilli (arrows). × 1800. **6.** Supranuclear zone of a ciliated cell showing rough endoplasmic reticulum (arrows). × 7100.



Figs 7–12. **7.** Apical portion of epithelium of Infundibulum at luteal phase showing ciliated cell (cc) with many mature cilia (ci) and secretory cells with apical protrusions containing secretory granules (SG). × 1400. **8.** Apical portion of epithelium of ampulla at follicular phase showing ciliated cell (cc) with many mature cilia (ci) and secretory cells with apical protrusions containing secretory granules (SG). × 1400. **9.** Supranuclear zone of secretory cells of ampulla at follicular phase showing different sizes of secretory granules (SG with arrows). × 2800. **10.** Supranuclear zone of secretory cells of ampulla at follicular phase showing dilated cisterns of golgi complex (arrows). × 11000. **11.** Apical portion of epithelium of Isthmus at follicular phase showing ciliated cell (cc) with cilia (ci) and nucleus (N) and secretory cells with apical protrusions containing secretory granules (SG). × 880. **12.** Epithelium of Isthmus at luteal phase showing ciliated cell (cc) with few cilia (ci) and with microvilli (arrow) and secretory cell (S) × 1100.

isthmus) revealed that the mucosa was lined with 2 types of columnar cells, viz. ciliated and non-ciliated secretory cells. Regional differences between different segments of oviduct and differences during the two phases of the estrous cycle were observed.

Infundibulum: The infundibulum was lined with ciliated and the non-ciliated secretory cells. During the follicular phase in the infundibulum the majority of cells were the ciliated cells. The apical border of these cells had large number of long-kino-cilia. Few microvillous processes were observed interspersed between the cilia (Fig. 1). Both types of cells along with few basal cells were lying on the basement membrane (Fig.2). The nuclei of the cells were mostly elongated in shape and centrally or basally located (Fig. 3). Similar observations were made in the oviduct of the domestic animals (Abe and Oikawa 1991). The ciliated cells generally had low electron dense cytoplasm. The mitochondria were located primarily in the supra-nuclear zone and were of different shapes (Fig. 4). Heavy concentration of the mitochondria could be related to their role in providing energy for beating of cilia which helps in the movement of ova and the luminal secretions. Cilia thought to be primarily responsible for the pickup and transport of ovulated eggs in the infundibulum (Odor and Blandau 1973).

The non-ciliated secretory cells were darkly stained. They had short thick microvillous processes. Typical extrusion of secretory granules into the tubal lumen was apparent during the follicular and luteal phases which were more prominent during the follicular phase (Fig. 5). Apical protrusions were interspersed with ciliated cells. The only connection between the protrusion and the rest of the cell was a narrow strand of cytoplasm. The nuclei varied in shape and the nuclear

membrane formed deep folds. Few secretory granules were observed in the supranuclear cytoplasm and the protrusions of the secretory cells. The secretory granules had homogenous electron-dense matrices. Most secretory cells extended beyond the ciliated cells. Secretory cells at follicular phase were characterized by having numerous secretory granules showing maximum differentiation and a well developed rough endoplasmic reticulum with dilated cistern (Fig. 6). Similar observations were made by Abe and Oikawa (1991) in hamster oviductal epithelium.

In the infundibulum during the luteal phase there was apparently a reduction of the ciliated cells and the number of cilia (Fig.7). Similar to our findings, Abe and Hoshi (2008) observed in pig oviduct that during luteal phase there is reduced number and height of ciliated cells. Ciliogenesis during the follicular phase occurs under the influence of estrogen, while the appearance of progesterone at midcycle suppresses ciliogenesis and leads to deciliation (Fredricsson and Holm 1974). The apical protrusions were few in number indicating that secretion has already taken place in the follicular phase.

Ampulla: Electron microscope examination of the ampullary epithelium showed the presence of two main types of cells, ciliated cells and non-ciliated secretory cells. The ciliated cells possessed long cilia interspersed between microvilli at their apical cell surface (Fig. 8). During follicular phase in the ampulla, it was seen that there was reduction in the number of ciliated cells, but the number of cells having microvillous processes was more as compared to infundibulum. Mitochondria and membrane-bound lysosome-like vesicles were frequently seen in the supranuclear region. Secretory cells could be identified by the absence of cilia and presence of protrusions at their luminal surface cells. The protrusions from the secretory cells had plenty of microviillous processes. The golgi apparatus and rough endoplasmic reticulum were abundant in the secretory cells. Most of the protrusions contained large number of empty vesicles and stained dark but some of the protrusions also contained groups of membrane bound granules. The most conspicuous characteristic of the secretory cells was the presence of secretory granules of variable sizes and distinct staining properties (Fig. 9). Some secretory granules appeared to be released by exocytosis. The intracellular location of granules was typically restricted to the supranuclear and apical cytoplasm although a few isolated granules could be observed elsewhere in the cytoplasm. These cells had more of dilated cisterns of golgi complex (Fig. 10). Similar observations were made by Abe *et al.* (1999) in goat oviduct and Rumery *et al.* (1978) in pig-tailed macaque oviduct.

Isthmus: Like infundibulum and ampulla, isthmus was also lined with ciliated and secretory cells. The ciliated cells had thin population of cilia as compared to other two segments of oviduct both during follicular phase (Fig. 11) and luteal phase (Fig. 12). The ciliated cells of the isthmus showed few changes between the follicular and luteal phases, in contrast to that of infundibulum and ampulla. Very few apical protrusions and sparse secretory granules were observed. Microvilli were present on the luminal surface of the ciliated cells between the cilia. The non ciliated secretory cells of the isthmus had few secretory granules but contained many lysosome-like bodies. Similar observations were reported by Hollis *et al.* (1984) in the oviduct of Marino ewes. Hunter *et al.* (1991) suggested that the isthmic epithelial cells act as a sperm reservoir. However, Suarez *et al.* (1991) suggested that isthmic epithelial cells may have the ability to maintain the viability and fertilizing capacity of spermatozoa.

It is well known that the mammalian oviduct is a target organ for the sex-steroid hormones, estrogen and progesterone which cause various morphological changes in the oviductal epithelial cells. In particular, estrogen induces hypertrophy, active ciliation, and formation of secretory granules by atrophied epithelial cells in oviducts (Abe and Oikawa 1993). Fredricsson and Holm (1974) has suggested that oviductal cells show regional differences in their response to steroid hormones.

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