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Research Article

ULTRASTRUCTURE OF ISTHMUS AND UTEROTUBAL JUNCTION OF BUFFALO OVIDUCT DURING FOLLICULAR AND LUTEAL PHASES

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ABSTRACT

The isthmus and the uterotubal junction of oviduct during the follicular phase were lined with more of non-ciliated cells as compared to ciliated cells. The ciliated cells were less and so was the number and height of the cilia. In the ciliated cells, the mitochondria were mainly located in the supranuclear zone and were irregularly shaped. The non-ciliated cells were darkly stained and had short and thick microvillous processes. The intercellular junctions were large and wavy. Free floating protrusions having mitochondria and cytoplasmic granules were also observed. In the luteal phase, the number of ciliated cells were reduced, however, the non-ciliated cells contained large number of granules.

Key words: TEM, isthmus, uterotubal junction, follicular, luteal phase, buffalo

Epithelial lining of the different segments of oviduct is of great significance as these cells play a pivotal role during different reproductive phases. The present study was a step forward in recording the structure of these cells during follicular and luteal phases in buffalo.

MATERIALS AND METHODS

The present study was conducted on oviducts of five buffaloes during follicular and luteal phases of estrous cycle. The oviducts were collected from a slaughter house after examining the status of ovaries. Tissue samples were collected from the isthmus and uterotubal junction of oviduct. These tissues were thoroughly washed in phosphate buffer saline (pH 7.4) solution and subsequently trimmed. The tissue samples for transmission electron microscopy were of 1 mm3 in size. These samples were fixed for 2 hrs in 2.5% glutaraldehdye and then secondary fixation was done for 2 hrs in 2% osmium tetraoxide. The tissue samples were then subjected to dehydration in ascending grades of acetone at room temperature, clearing with toluene followed by infiltration and embedding.

Semi thin sections of the blocks were cut to scan the tissues under optical microscope for selection of area for ultrathin sectioning. After scanning, the ultrathin sections (70-90 nm) were cut and lifted on copper grids (100 mesh size) and stabilized by coating with carbon film of 50Å thickness. The grids were then stained with urayl acetate (15 min) followed by lead citrate (10 min) and were examined under TEM (CM-10 Philips) for detailed study.

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RESULTS AND DISCUSSION

Isthmus

Follicular phase: The lining epithelium consisted of ciliated and non-ciliated cells (Fig. 1); however, the number of ciliated cells was less. The ciliated cells usually occurred in groups and the cilia were quite long, however, solitary ciliated cells lying between the non-ciliated cells were also observed. Large numbers of free floating protrusions were seen trapped between the cilia (Fig. 1). These protrusions had large number of pleomorphic electron dense granules and some of these had lamellated endoplasmic reticulum (ER). Cells in the process of being released had a bulging apex with large number of granules arranged along their periphery (Fig. 2). The protruding masses had less microvillous processes and appeared smoother. In the ciliated cells, the mitochondria were more in number and were placed in the supranuclear zone, however, the cisternae of ER were arranged in the form of bands with very wide spaces (inset of Fig. 1). This arrangement was evident in many cells and was a peculiar phenomenon to be seen in this region and in many cells, it occupied a considerable space. Close to these structures large

number of mitochondria of different shapes and sizes were also observed.

Luteal phase: The cells were more elongated and their nuclei were placed along the long axis of cells. Light and dark cells were observed in the lining epithelium (Fig. 3). The lighter cells on their apical surface had cilia which were comparatively very short. The large numbers of microvillous processes were also placed between the cilia (Fig. 3). These cells contained elongated mitochondria in the supranuclear zone and few cisternae of smooth ER. The mitochondria were comparatively less in number, however, more elongated ones were uniformly stained and were placed close to the basal plate but the mitochondria placed close to the granules had well defined cristae within them. The darker cells were characterized by presence of large number of globular secretory granules of different sizes and different densities (Fig. 3). The secretory cells shaped like goblet cells were separated from other cells by large inter cellular spaces indicating that these were in the processes of being expelled/discharged. The ER was in the form of small straight cisterns arranged along long axis of the cells (Fig. 3). Few cisterns were also observed in between the granules. The apical surface was mostly studded with microvillous processes. Smooth ER cisterns which were having a wider lumen were observed in between the secretory granules and few strands of rough ER (inset of Fig. 3) were also observed. In some of the cells multivesicular bodies were also observed (Fig. 4). Inter cellular junctions at places had wavy appearance.

Uterotubal junction

Follicular phase: The lining epithelium consisted of ciliated and non-ciliated cells. The non-ciliated cells were much more in number as compared to ciliated cells (Fig. 5). The non-ciliated cells had large number of small, thick or thin microvillous processes. The height of microvillous process appeared to be uniform, however, their distribution per cell varied. The nuclei in these cells were elongated and were arranged along the long axis of cells. The nuclei were comparatively (to cytoplasm) lightly stained and few plaques of dense chromatin were placed here and there. The cells as a whole were elongated and were seen to be wide above and narrow below (Fig. 5). The basal plates in the ciliated cells were clearly seen and glycogen particles, occcuring singly or in groups were also seen (Fig. 6). The ciliary rootlets were, however, less and smaller in size. In the ciliated cells, mitochondria were elongated and mainly confined to the apical portion of the cell (Fig. 6). In between the cilia, large number of varied sized microvillous process were also observed. The cell boundaries were almost straight and the cell junctions were evident. These junctions were quite thick and straight towards the apical part of the cells while lower down these were light, and had irregular outline. The secretory granules in the non-ciliated cells were either homogenously electron lucent or electron dense. The electron dense granules at times were also seen to have electron lucent areas. The electron lucent granules were mainly circular in outlines and uniform in size while the electron dense granules were of different shape and sizes. Interspersed between the granules were large number of glycogen particles and also small sized cisterns of rough ER distributed irregularly.

In some of the cells, spherical bodies consisting of large number of small sized vesicles were observed and such bodies gave flocculent appearance (Fig. 7). Close to such bodies were scattered large numbers of cisterns of ER. These cisterns were either placed singly or in groups and were of different shapes and sizes. Some of the granules were spherical in outline and much larger in size and consisted of clearly demarcable electron lucent and electron dense areas (Fig. 7).

Luteal phase: The lining epithelium mainly comprised of non-ciliated cells (Fig. 8). Occasionally a single isolated ciliated cell was observed. The ciliated cells had very small, thick cilia which did not even extent beyond the height of non-ciliated cells. Ciliary rootlets were not discernible and the glycogen particles were very few. The nuclei were mainly towards the base and irregular in outline. Supranuclear area of the cells was much wider but darkly and homogenously stained. The intercellular junctions were mainly confined in the apical part and appeared thick and dark (Fig. 8). Cytoplasmic granules were either homogenously stained or had a central core of electron dense material (Fig. 9). Close to these granules, small and irregularly placed cisterns of rough and smooth ER were also observed.

Hollis *et al.* (1984) observed that isthmic secretory cells contained granules but no evidence of granule release was observed in either of the phases in Merino ewes. Abe *et al.* (1999) reported in goat that secretory cells showed little difference in ultrastrucutural features between the follicular and luteal phases.

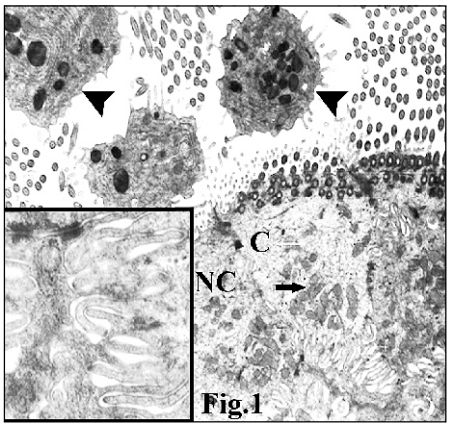

Fig 1. Section of isthmus during follicular phase showing ciliated cell (C), non-ciliated cell (NC), protrusions having granules and lamellated endoplasmic reticulum (arrow heads), mitochondria (arrows), and inset showing cell junction and cisterns of endoplasmic reticulum. (x 1950)

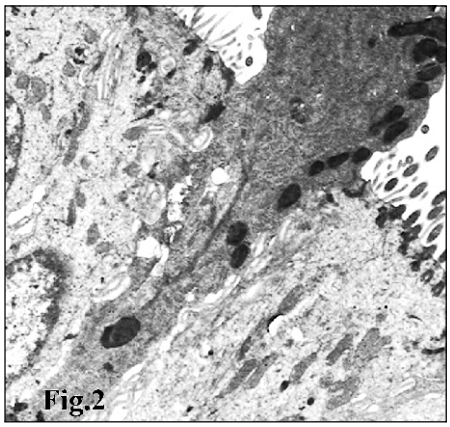

Fig 2. Section of isthmus of oviduct during follicular phase showing a cell in the process of being released with secretory granules arranged along the periphery. (x 1950)

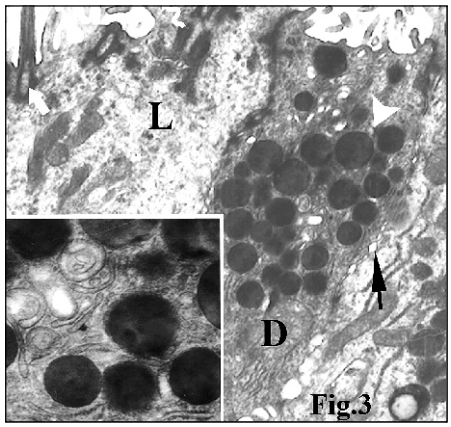

Fig 3. Section of isthmus during luteal phase showing light cell (L), dark cell (D), light having cilia (white arrow) and microvillus processes (curved white arrow), dark cell having electron dense granules (white arrow head), dilated intercellular spaces between adjacent cells (black arrow) and inset shows granules and strands of ER. (x 3400)

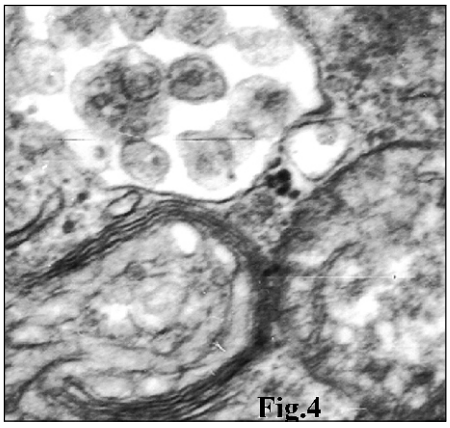

Fig 4. Section of isthmus of oviduct during luteal phase showing multivesicular bodies. (x 11,500)

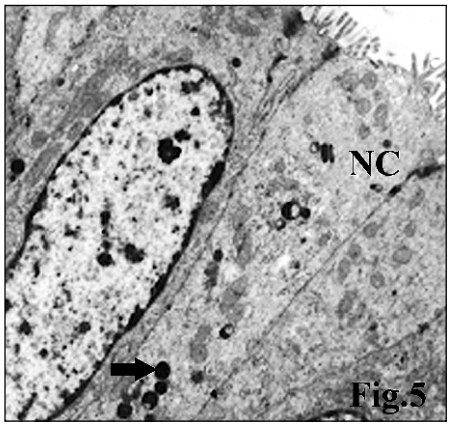

Fig 5. Section of utero tubal junction of oviduct during follicular phase showing ciliated cell (C), non ciliated cell (NC) and granules (arrow). (x 1950)

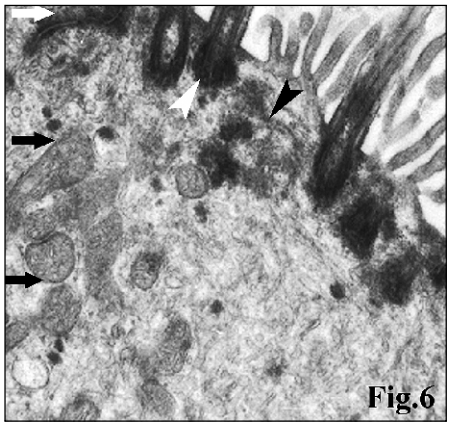

Fig 6. Section of utero tubal junction of oviduct during follicular phase showing basal plate (white arrow head), ciliary rootlets (black arrow head), cell junction (white arrow) and mitochondria (black arrows) (x 6300)

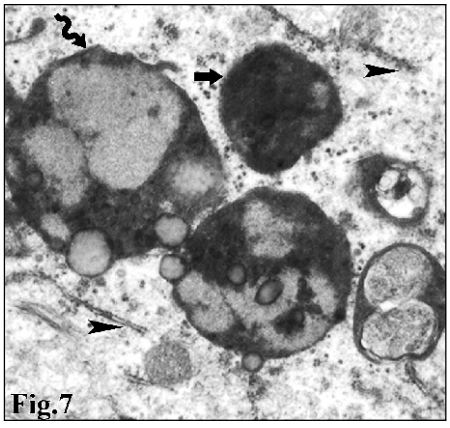

Fig 7. Section of utero tubal junction of oviduct during follicular phase showing endoplasmic reticulum (black arrow head), bodies/granules giving flocculant appearance (curved black arrow) and homogeneous granules (arrow). (x 6300)

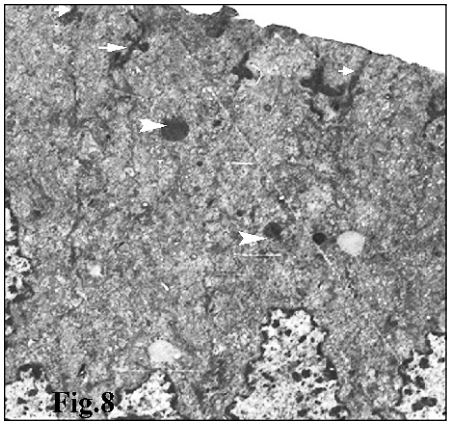

Fig 8. Section of utero tubal junction of oviduct during luteal phase showing junctional complexes (arrows) and uniformly stained granules (arrow heads). (x 1150)

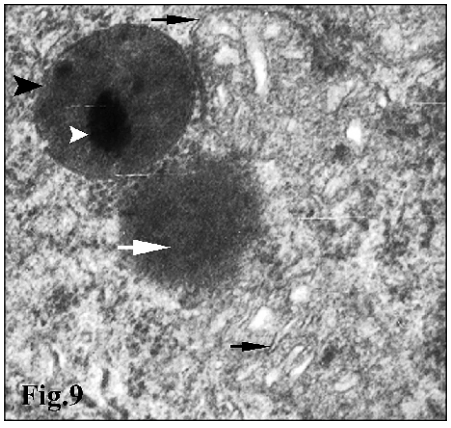

Fig 9. Section of utero tubal junction of oviduct during luteal phase showing cisterns of endoplasmic reticulum (black arrows), uniformly stained granule (white arrow), large granule (black arrow head) with electron dense central area (white arrow head). (x 15,500)

The presence of different types of granules in the different segments of oviduct are indicative of the fact that the contents of these granules are segment specific and have a distinctive localized effect either on gametes or developing embryo. Even Gandolfi and Moor (1987) stated that oviduct cells appear to be required for the acquisition of full embryonic viability. Kapur and Johnson (1988) also stated that the different granules probably relate to the variety of developmental and reproductive events occurring along the length of the oviduct. They further pointed several proteins secreted by the oviduct get sequestered in the perivitelline space and contribute in the formation of specialized environment in which fertilization and important early developmental events take place.

Abe *et al.* (1993) also pointed that monoclonal antibodies bound selectively to putative secretory granules of non-ciliated cells in ampulla and fimbriae but not in isthmus, thereby suggesting that there are cyclic and regional differences in the production of glycoproteins in bovine oviduct. Abe *et al.* (1995) further pointed that antigens for oviductal glycoprotein present in flushings obtained from ampulla in follicular stage of goats were not detected during the luteal phase. It suggests that the granular contents present in the non-ciliated cells during the follicular and luteal phases could be different chemically as well as functionally.

It is further pointed that whether the secretory material from different segments of the oviduct, if not sufficiently synthesized or released may inhibit the fertilization process. Murray (1992) stated that the epithelial cells of the sheep uterus undergo morphological changes in protein synthesizing organelles and apical plasma membrane specializations in the presence of estrogen alone or in conjunction with progesterone. This implies that the appropriate level of circulating hormones is of paramount importance in not only maintaining the proper anatomical structure of oviduct but also making a proper microenvironment required for synthesis of secretory products at different levels of oviduct. Lastly it would be imperative to mention that mid cycle growth of follicles also alters the morphological and structural nature of the lining epithelium of the oviduct.

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