Haryana Vet. **49** (December, 2010), pp 11-14

Research Article

ULTRASTRUCTURE OF FIMBRIA AND AMPULLA OF BUFFALO OVIDUCT DURING FOLLICULAR AND LUTEAL PHASES

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ABSTRACT

The fimbria and the ampulla of buffalo oviduct during the follicular phase exhibited mainly the ciliated cells. In between the cilia, microvillous processes were also seen. In the ciliated cells, mitochondria were irregularly shaped and mainly located in the supranuclear zone. 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 ciliated cells were reduced, however, the basal plates were clearly evident. The non-ciliated cells were of light and dark in nature. The light cells were more in number and bigger in size, however, the cytoplamic organelles and the granules were less. The dark cells were elongated and their granules were pleomorphic. In the ampulla, there was reduction in the size of cilia and their number, however, the presence of granules was more evident in the luteal phase.

Key words: TEM, fimbria, ampulla, follicular, luteal phase

Oviduct is a tubular conduit in which maturation of gametes, fertilization and early embryonic development takes place. The success rate of fertilization and early embryonic development is dependent upon the oviduct providing a satisfactory microenvironment for various events to take place. Keeping in view the importance of reproductive tract in the buffalo, the present study has been envisaged.

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 fimbria and ampulla of the oviduct. These tissues were thoroughly washed in phosphate buffer saline (pH 7.4) solution and fixed for 2hr in 2.5% glutaraldehdye and then secondary fixation was done for 2hr in 2% osmium tetraoxide. The tissue samples were then subjected to dehydration in ascending grades of acetone at room temperature. The clearing of the samples was accomplished by treatment with toluene. Subsequently infiltration and embedding were 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 (Reichert TM 60) and semi-thin sections were cut to scan the tissues under optical microscope for selection of are for ultra-thin sectioning. After scanning, the ultrathin sections (70-90 nm) were cut, lifted on copper grids (100 mesh size) and stabilized by coating with carbon film of 50Å thickness. The grids were then stained with uranyl acetate (15 min) followed by lead citrate (10min). The grids thus prepared were examined under TEM (CM-10 Philips) for detailed study.

RESULTS AND DISCUSSION

Fimbria

Follicular phase: The lining epithelium consisted more of ciliated as compared to non-ciliated cells (Fig. 1). The cilia were better developed and were more elongated as compared to those seen in the luteal phase. The ciliated cells also exhibited microvillous processes in between cilia which were short and thick. The cytoplasm of the ciliated cells contained large number of elongated, irregularly shaped mitochondria in the surpra nuclear zone (Fig. 2). The superficial cells formed large wavy intercellular junctions (Fig. 2) which formed a wavy band with comparatively wider intercellular spaces. The basal plates (Fig. 2) and the ciliary rootlets were also evident. The number of mitochondria was comparatively much more during this phase. Very few non-ciliated cells which were usually

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placed at a lower level were observed. These cells were large and darkly stained and the nuclei of these cells were arranged along the long axis of the cells. These cells had short and thick microvillous process. Few free floating protrusions were observed and these protrusions also had microvillous processes on their surface (Fig. 1). The protrusions also contained elongated mitochondria but in addition had few cytoplamic granules which were darkly stained. Some of the protrusions had large number of cytoplasmic processes along with the nucleus, granules and mitochondria as if these were the complete cells which have been ejected from the lining epithelium (Fig. 3).

Luteal phase: The epithelial lining was mainly composed of ciliated cells with tightly packed cilia and very few non-ciliated cells (Fig. 4). The cells underlying the ciliated cells could be identified as light and dark cells (Fig. 4). The cytoplasm as well as the nucleus was lightly stained in lighter cells. The nuclei of these cells although circular had indentions on their surface (Fig. 4). These light cells were apparently more in number and bigger in size as compared to dark cells (Fig. 4). The cytoplasm of light cells had less cytoplasm and very less cytoplasmic organelle; however, few scattered cisterns of rough endoplasmic reticulum (ER) were seen. The granules in such type of cells were fewer, smaller in size but electron dense in nature (Fig. 4). In the light cells, the clumps of dense chromatin were much less and were mainly close to the nuclear membrane. The darkly stained cells were wedged in between the light cells and were mostly elongated in outline. Their nuclei were small but elongated and the chromatin material was darkly stained having large clumps of condensed chromatin (Fig. 4). The chromatin material gave a striated appearance to the nucleus. The secretory granules present in these types of cells which were placed towards the luminal surface were pleomorphic and were uniformly stained (Fig. 5). The cytoplasm of ciliated cells which were towards the luminal surface was also darkly stained and large numbers of cilia were seen originating from apical surface of cells. The basal plates were clearly seen; however, the ciliary rootlets were not clearly evident as compared to follicular. The junctional complexes were not as wavy as in follicular phase and the intercellular spaces were narrow (Fig. 5). Very few small size protrusions were observed in the lumen which were irregularly shaped (Fig. 4). Few cells having microvillous process were usually present at a lower level as compared to ciliated cells (Fig. 4).

Nayak and Wu (1975) in guine pig, cattle, sheep and swine and Nayak *et al*. (1976) in ewe reported that true degeneration of cilia was not evident in the fimbria epithelium during luteal phase. They further reported that ciliary rootlets were present in variable number in the ciliated cells during follicular as well as luteal phases which were, however, not so prominent in the present study. The fibrous granules were seen to be closely associated with the basal bodies during the luteal phase in the present study indicating their role in the development of cilia and rootlets. Nayak *et al.* (1976) also observed that maximum secretory cell differentiation was apparent during the follicular phase in which the cells were characterized by well developed rough endoplasmic reticulum, numerous ribosomes and secretory granules of varied size, shape, and density. Typical extrusion of secretory granules into the tubal lumen was apparent during the follicular and luteal phases, which however, in the present study was more in the follicular phase. Abe *et al.* (1999) in their description of the fimbria in the follicular phase pointed that ciliated cells had low electron dense cytoplasm while the secretory cells had a characteristics slender shape and cytoplasm was usually stained more densely as has also been observed in this study. Further in consanance with the present study, they also observed that the secretory granules were fewer in number in the luteal phase.

Ampulla

Follicular phase: The linning epithelium consisted of ciliated and non-ciliated cells. Cilia were comparably lesser in height (Fig. 6); however, the ciliated cells present in and around the pits had large number of elongated cilia. On the apical surface of lining epithelium, quite a few number of protrusions of varying height were observed and some of these were seen extending beyond the height of cilia (Fig. 6). The protrusions had large number of microvillous processes and in addition few short but wider projections were also observed. These projections contained large number of empty vesicles (Fig. 6). Some of the protrusions were seen arising out of the cells which were slightly darker as compared to rest of the cells and they contained groups of membrane bound granules which were fairly uniformly stained (Fig. 6). The apical surface of few cells had a brush border like appearance (Fig. 6) and

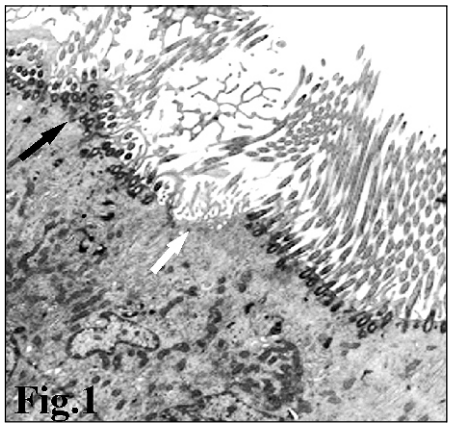

Fig 1. Section of fimbria during follicular phase showing ciliated cell (black arrow), non-ciliated cell (white arrow) and free floating protrusion (arrow head). (x 1150)

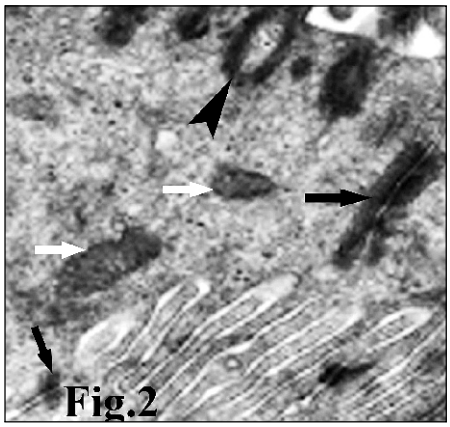

Fig 2. Section of fimbria during follicular phase showing mitochondria (white arrows), intercellular junctions (black arrows) and basal plate (arrow head). (x 8400)

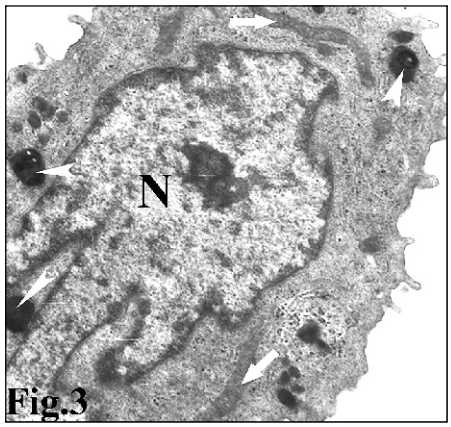

Fig 3. Section of fimbria during follicular phase showing a protrusion with nucleus (N), granules (arrow heads) and elongated mitochondria (arrows). (x 4600)

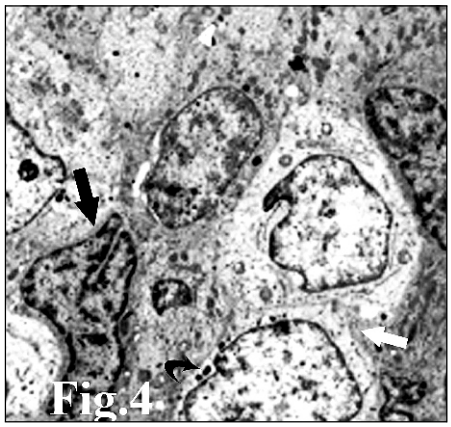

Fig 4. Section of fimbria during luteal phase showing ciliated and non-ciliated cells along with light cells (white arrow), dark cells (black arrow), granules in light cell (curved arrow) and protrusion (arrow head). (x 1150)

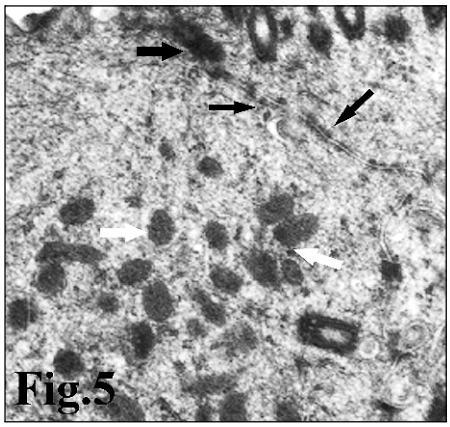

Fig 5. Section of fimbria during luteal phase showing pleomorphic granules (white arrows) and cell junctions (black arrows). (x 4600)

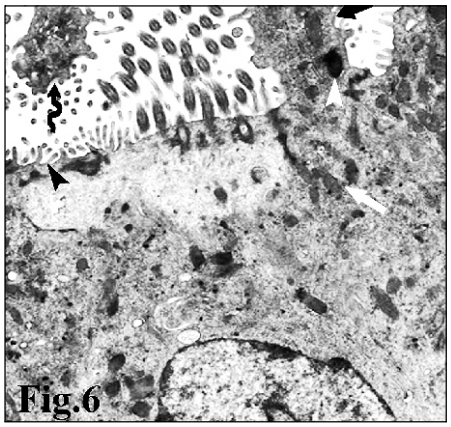

Fig 6. Section of ampulla during follicular phase showing ciliated and non-ciliated cells with brush border (black arrow head), protrusion (black arrow) containing electron dense granule (white arrow head), a second protrusion (curved arrow) with granules and empty vesicles and mitochondria within a cell (white arrow). (x 1870)

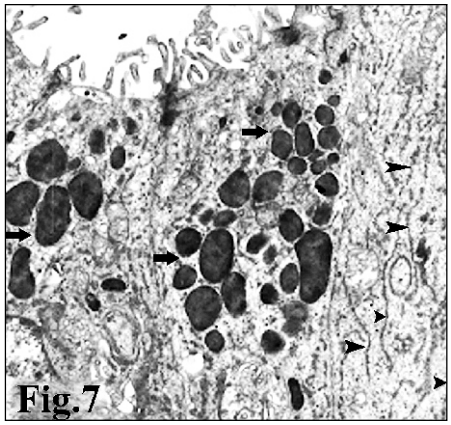

Fig 7. Section of ampulla during luteal phase showing uniformly stained granules (arrows) and parallel rows of endoplasmic reticulum (arrow heads). (x 1950)

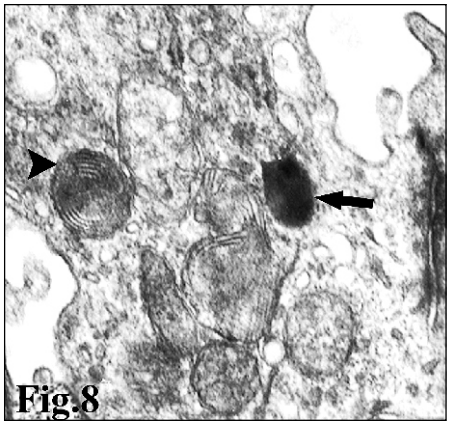

Fig 8. Section of ampulla during luteal phase showing uniformly stained granule (arrow) and a lamellated structure (arrow head). (x 11,500)

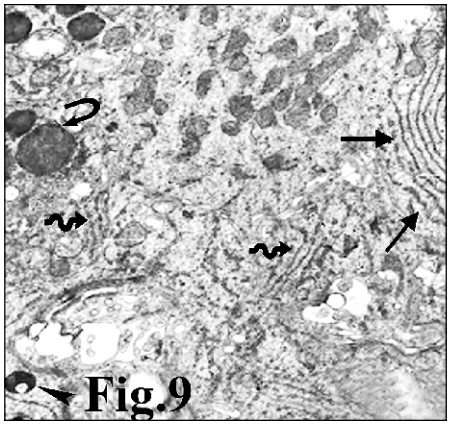

Fig 9. Section of ampulla during follicular phase showing granules (u shaped arrow), isolated cisterns of endoplasmic reticulum (curved arrows), lamellated endoplasmic reticulum (arrows) and granule with electron lucent area (arrow head). (x 2650)

very few light cells were also observed. The lightly stained cells were mainly towards the luminal surface and were small sized. However, large light cells were also observed and the cytoplasm of these cells gave a vacuolated appearance. The intercellular spaces were wider. The cells towards the base were tightly packed and the nuclei of the cells were mostly placed along the long axis of the cells. The number of cells having microvillous process were comparatively more as compared to the fimbria. The mitochondria were elongated but smaller in size as compared to those seen in the fimbria. The mitochondria were mostly seen in supranuclear zone close to the apical surface (Fig. 6). The ciliary rootlets having striated appearance were more clearly seen. All the component of junctional complexes were clearly evident and were mostly located toward the apical border. In between these junctional complexes few wider, inter cellular spaces were also observed which were placed in the form of longitudinally oriented structures. The rough ER was in the form of isolated cisternae and the glycogen droplets were usually present near the basal plates.

Luteal phase: The lining epithelium consisted of ciliated and non-ciliated cells. The non-ciliated cells were comparatively more with larger number of granules in the supra nuclear zone (Fig. 7). Well developed ciliary rootlets with striations were observed in the apical part of the ciliated cells, however, the height of cilia was considerably reduced and large no of apical projections were observed. The apical projection had either vacuolated appearance or contained large number of cytoplasmic granules. Some of the granules were homogenously stained while some of these were in the form of lamelated structure (Fig. 8). Some of these protrusions had small sized cisterns of smooth ER, while others were completely laden with rough ER. The protrusions were also rich in mitochondria which were wider and their cristae were clearly observed as compared to earlier phases. A special arrangement of ER known as Nebenkerne occurred in different forms in the supranuclear zone and was closely associated with cytoplasmic granules. At places, ER had several concentric lamellae which were tightly packed into spiral coils (Fig. 9). The second type of Nebenkerne arrangement of ER consisted of loose irregularly spaced coils having vacuolated structures and granules in the central space (Fig. 9). The granules were of varying sizes but were mainly homogenously stained. Some of them, however, had a central core of electron lucent material. Still another type of arrangement of ER consisted of parallel placed tubules along the long axis of the cells (Fig. 7). The Golgi apparatus was also clearly seen. Secretory cells had large number of granules which were either homogenously stained or had electron dense material in a electron lucent background. The intercellular junctions were not clearly demarkable, were very less and confined only to the apical part of the cell.

Hollis *et al.* (1984) also reported that the presence of secretory granules were a prominent feature of ampullary secretory cells. They further pointed that tight junctions were more evident towards the luminal surface as compared to their lateral surfaces, which was also observed in present study. Abe *et al.* (1999) in goat ampulla during follicular phase observed that secretory cells extended numerous microvilli into the lumen and filamentous material was associated with them while in luteal phase, marked apical protrusions extending beyond the luminal border of ciliated cells were seen. However, the number of secretory granules was dramatically reduced in the cytoplasm of secretory cells in the ampullary epithelium during the luteal phase.

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