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

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ABSTRACT

The present investigation was conducted on different segments of oviduct of six buffaloes each during follicular and luteal phases of estrous cycle. The mucosa of different segments of oviduct was thrown into folds which were oriented as primary and secondary folds. The lamina epithelialis was lined with ciliated and non-ciliated cells. Ciliated cells had many kinocilia during the follicular phase. The non-ciliated cells were of two types, viz. secretory and non-secretory type. The infundibulum during the follicular phase had large number of ciliated cells as compared to that of luteal phase. The secretory cells had short, thick and stubby microvillous processes. The ampulla had almost similar ciliation as that of infundibulum. The luminal surface of secretory cells had secretory blebs and secretory material over it. The extent of ciliation decreased towards the uterotubal junction. The ciliated cells were more during the follicular phase as compared to that of the luteal phase.

Key words: Buffalo, Estrous cycle, Follicular phase, Luteal phase, Oviduct, Scanning electron microscopy

The oviduct is a secretory organ that maintains and modulates a dynamic fluid filled milieu in which maturation of gametes is completed, fertilization occurs and early embryonic development begins (Kapur and Johnson 1988).The scanning electron microscopy of oviduct during different phases of estrous cycle has been described by Abe *et al.* (1993) in goat oviduct, Abe and Oikawa (1993) in cow, Kamaci *et al.* (1999) in human and Yaniz *et al.* (2006) in porcine oviduct but scanty literature is available regarding scanning electron microscopy of oviduct of buffalo. Therefore, the present work was undertaken to elucidate the scanning electron microscopic studies on oviduct during follicular and luteal phases of estrous cycle.

MATERIALS AND METHODS

The present study was conducted on different segments of oviduct of six buffaloes each during follicular and luteal phases of estrous cycle. The tissue samples from infundibulum, ampulla, isthmus and uterotubal junction 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 min 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 p.s.i. Thereafter 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.

RESULTS AND DISCUSSION

The mucosa of different segments of oviduct was thrown into large number of longitudinal folds with mucosal crypts in between them. The mucosal folds were branched into primary, secondary and tertiary branches (Fig. 1). The branching was more pronounced during follicular phase as compared to that of luteal phase and was more extensive in infundibulum which reduced towards the uterotubal junction. Similarly, Myers *et al.* (1984) in canine uterine tube observed irregular mucosal folds with diverticulum in between the folds and Nayak (1977) noticed randomly oriented folds and mucosal crypts in camel uterine tube. The folds were lined with two types of cells viz. ciliated and non-ciliated cells. The number and distribution of ciliated and non-ciliated cells varied during the two phases of estrous cycle and between different segments of uterus.

The epithelial lining of infundibulum appeared to be densely ciliated with few non-ciliated cells during follicular phase (Fig. 2). The ciliated cells had bunch of cilia over

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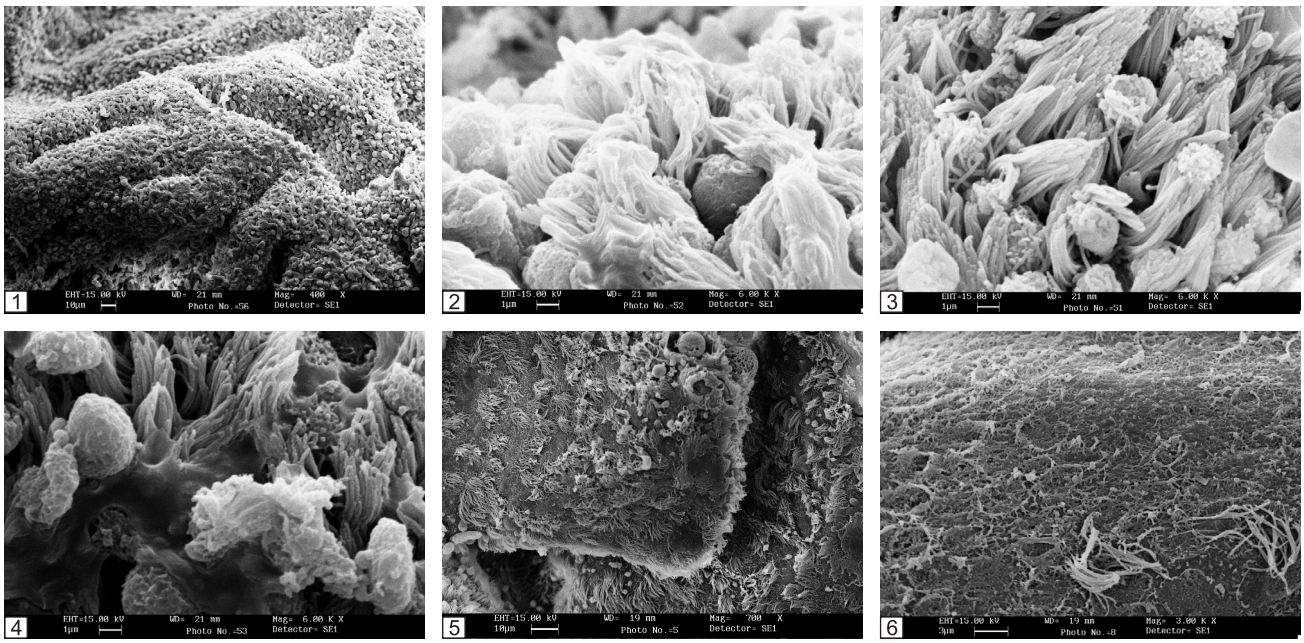
their surface. Cilia were lengthy and covered the neighbouring non-ciliated cells. The non-ciliated cells had convex surface which bore on them microvillous processes. These observations were in consonance with the findings of Abe and Oikawa (1992) in oviduct of prolific Chinese Meishan pig and Kanagawa *et al.* (1972) in the rabbit oviduct. Contrary to our findings, Stalheim *et al.* (1975) reported that luminal surface of oviducts of cow, mare, sow and doe contained clusters of ciliated and non-ciliated cells in approximately equal numbers in the infundibulum. The non-ciliated cells were of two types, viz. secretory and non-secretory cells. The secretory cells had small secretory materials over their apical surface. During the luteal phase, the number of ciliated cells reduced as compared to that of follicular phase but still they out numbered the non-ciliated cells. More number of secretory cells were observed during the luteal phase. Contrary to present findings, Nayak (1977) studied the camel uterine tube during estrous cycle and observed no pronounced alterations in the three dimensional surface features were apparent. The cilia of the infundibulum are considered to be primarily responsible for the pick up and first phase of transport of ovulated eggs. The richly ciliated epithelium of infundibulum at ovulation is important (Odor and Blandau 1973).

Similar to that of infundibulum, the mucosa of the ampulla was also thrown into longitudinal folds separated by crypts. The lining epithelium in the ampulla region in the follicular phase showed extensive ciliation as reported in cows by Hafez and Kanagawa (1973) and Rumery and Eddy (1973) in the rabbit oviduct. The ampulla had almost similar ciliation as that of infundibulum. The cilia usually extended above the apical surface of the non-ciliated cells. But in some fields slightly more number of non-ciliated cells were observed as compared to that of infundibulum. Microvilli were present on the apical surface of the non ciliated cells (Fig. 3). During the luteal phase, the number of ciliated cells reduced and that of secretory cells increased (Fig. 4). The variable shaped secretory materials were observed on the secretory cells. Contrary to our findings, Stalheim *et al.* (1975) reported that luminal surface of oviduct of cow, mare, sow and doe contained clusters of ciliated and non-ciliated cells in approximately equal number in the ampulla.

Mucosal folds of isthmus were longitudinal but broader, flatter and shorter. These folds lacked secondary and tertiary branches. The crypts were deep and complex. These observations were in consonance with the findings of Myres *et al.* (1984) in canine uterine tubes. During follicular phase, isthmus contained less number of ciliated cells as compared to infundibulum and ampulla as reported by Abe and Oikawa (1993) in the oviduct of cow. The ciliated cells were sparse and were present singly or were in small groups surrounded by many non-ciliated cells (Fig. 5). Increased number of secretory cells were observed. During the luteal



Figs 1–6. **1.** Scanning electron micrograph of infundibulum in the follicular phase showing longitudinal folds of mucosa branched into primary (P), secondary (P), tertiary branches (T) and crypts (arrows). × 400. **2.** Scanning electron micrograph of infundibulum in the follicular phase showing ciliated cells (C), secretory cell with secretion (S) and non-ciliated cells (NC). × 6000. **3.** Scanning electron micrograph of ampulla in the follicular phase showing ciliated cells (C), secretory cell with secretion (S) and microvilli over the non-ciliated cells (M). × 6000. **4.** Scanning electron micrograph of ampulla in the luteal phase showing ciliated cells (C), secretory cell with secretions (S) and mucus secretion accumulated over cells (MC) × 6000. **5.** Scanning electron micrograph of isthmus in the follicular phase showing ciliated cells (C), non-ciliated cells (NC), secretory cell with secretions (S) and cleft between the mucosal fold (arrow head). × 700. **6.** Scanning electron micrograph of uterotubal junction in the follicular phase showing ciliated cells (C) and non-ciliated cells (NC). × 3000.

phase, the epithelium of the isthmus region showed very less number of ciliated cells but more number of secretory cells was observed. The secretory cells had various shapes of secretory materials over their apical surfaces.

The mucosa of uterotubal junction was broad and flat, separated by deep crypts. Very few mucosal folds were observed with few ciliated cells among majority of non ciliated cells. Ciliated cells were usually singular surrounded by many flat non-ciliated cells. The number of cilia emerging from ciliated cells was few (Fig. 6). The non-ciliated cells were flatter as compared to that in other segments of oviduct where it was convex and bulbous. These cells had stubby microvilli. The cyclic changes observed in infundibulum and ampulla were not much evident in this region as also reported by Abe and Oikawa (1993) in cows and Abe *et al*. (1993) in goats.

The cyclic changes observed in the present study may be correlated with the functions of the cilia in the infundibulum and ampulla of buffalo. The reason for the ciliation in the follicular phase might be due to the influence of estrogen and more secretory cells in the luteal phase due to the influence of progesterone. Isthmus might function for the storage of sperm and that its epithelial cells may have the ability to maintain the viability and fertilizing capacity of sperms and to regulate the progression of sperms (Abe *et al.* 1993). It is possible that the regional differences reflect regional variations in sensitivity to circulating ovarian steroid hormone (estrogen). Within the same region but different areas having variation can be due to micro-blood circulation pattern as a result of which the cellular interaction with the circulating hormones is not optimum to show the changes described (Nayak 1977).

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