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Scanning electron microscopic studies on uterus of sheep in follicular and luteal phases of estrous cycle

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ABSTRACT

The tissue samples from body, horn and cervix of uterus of 5 sheep each during follicular and luteal phases were fixed in Karnovsky's fixative and processed for scanning electron microscopic study. The endometrium showed long ridges and folds. Endometrial surface of body was less folded as compared to the horns. The apical part of luminal epithelial cells of corpus uteri was flat and the cells often formed a pattern of hexagonal structure. The epithelium was composed of prociliated, ciliated and secretory cells. The body contained more ciliated cells than the horn of the uterus. Ciliated and prociliated cells were more in follicular phase as compared to the luteal phase. The secretory cells had microvillous processes and apical blebs. Cervix uteri had complex, deep, narrow folds with small crypts, which were more complex near the internal os. It was also lined with ciliated and secretory cells. The internal os area contained more ciliated cells than the external os area. In the follicular phase the cervix had more of ciliated as compared to that of luteal phase.

Key words: Follicular phase, Luteal phase, Scanning, Sheep, Uterus

Scanning electron microscopic studies on the uterus have been made by Kanagawa *et al.* (1972) in the rabbits, Cruchten *et al.* (2002) in canines, Samuel *et al.* (1979) in mare and Hafez and Kanagawa (1972b) in bovines but little work has been done on the sheep uterus. The present work was intended to throw light on scanning electron microscopic studies on uterus and cervix uteri of sheep in follicular and luteal phases of estrous cycle.

MATERIALS AND METHODS

The present study was conducted on horn, body and cervix of uterus from 5 sheep each during follicular and luteal phases of estrous cycle. The tissue samples from cornua (horn), corpus (body) and cervix of uterus were collected and washed in chilled 0.1 M phosphate buffer (pH 7.2) and were subjected for fixation in Karnovsky's fixative (2.5% gluteraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer solution at pH 7.2 for 4–6 h. Fixed samples were washed in 0.1 M phosphate buffer with 3 changes of 15 min each at 4°C. 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 4°C.

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 (31.5°C) at 1100 p.s.i. Thereafter the tissues were placed in a desicator. The specimens were mounted on aluminium stubs and sputter coated with 35nm thick layer of gold. The processed tissue samples were viewed under LEO 435 VP scanning electron microscope and photographs were taken at AIIMS, New Delhi.

RESULTS AND DISCUSSION

The endometrium was found folded into long ridges and was lined with ciliated and secretory cells. The number and distribution of these cells vary in body and horn of uterus during different reproductive phases.

Cornua uteri

The endometrium of horn of uterus was less folded (Fig.1) whereas Kanagawa *et al.* (1972) reported 2 different types of fold formations, mosaic like pattern in the body of uterus while wave like in the horn of uterus.

The horn of uterus was lined with ciliated and non-ciliated cells and this region had less number of ciliated cells as compared to that of body of uterus. The apical part of luminal

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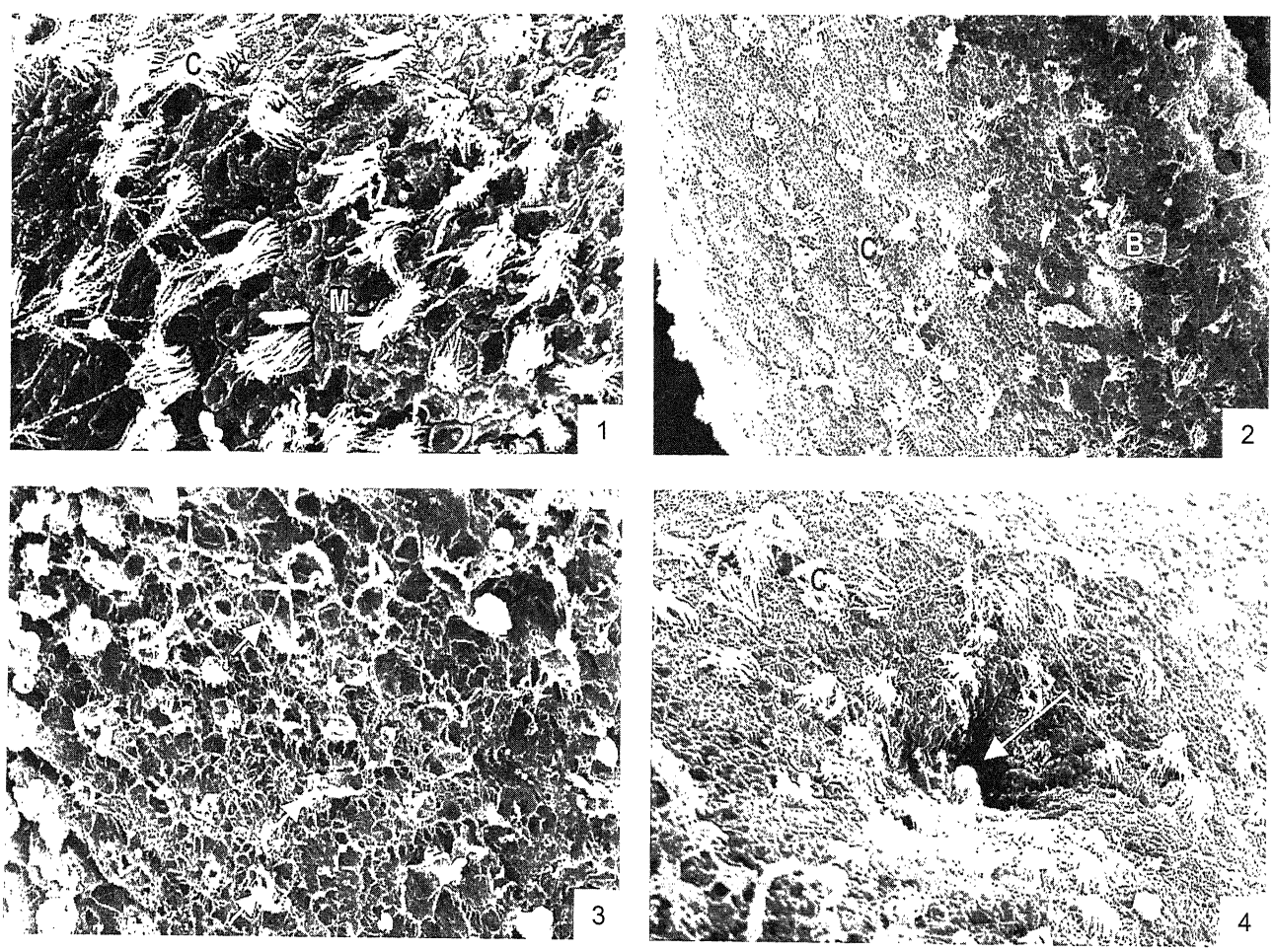
epithelial cells was flat and the cells often formed a pattern of hexagonal structure. Majority of these cells were non-ciliated cells though a small number of ciliated cells were also found (Fig. 2). Further horn of uterus had more number of ciliated cells in follicular phase and cilia were well developed and erect from the apical cell surface as compared to that of luteal phase (Fig. 3). These support earlier findings of Samuel *et al.* (1979) in mare.

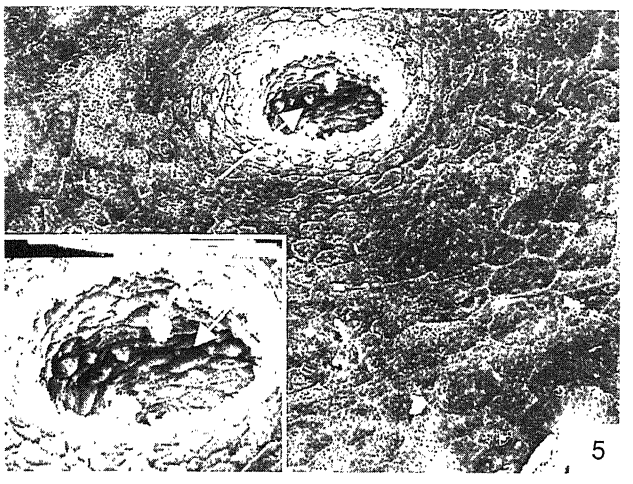
Corpus uteri

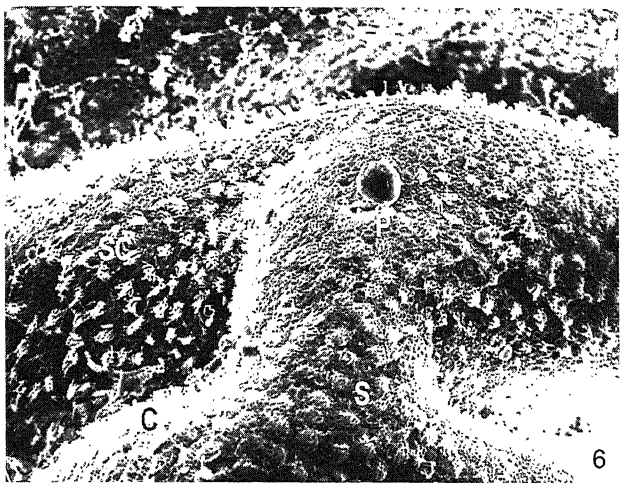
The endometrium in this region was folded into wide folds (Fig.4) and endometrium was found lined with prociliated, ciliated and secretory cells. Prociliated cells had stubby surface projections that can be tentatively identified as cilia in early stages of development. Similar reports have been made by Samuel *et al.* (1979) in the mare endometrium. However prociliated cells have not been identified in the uterus of canine by Cruchten *et al.* (2003), and rabbit by Kanagawa *et al.* (1972). The ciliated cells were more in number in the follicular phase as compared to the luteal phase. Similar reports have been made by Hafez (1972) in rabbit and monkey and by Samuel *et al.* (1979) in mare. The presence of well-developed cilia during the follicular phase and thereafter their degeneration during the luteal phase indicates that the ciliated cells undergo physiologic changes, which can be correlated to the changes in the level of reproductive hormones. It has also been suggested that the cilia of the luminal epithelium are important for the transport of the secretory product. The frequency of ciliated cells next to the gland openings gives support to this hypothesis.

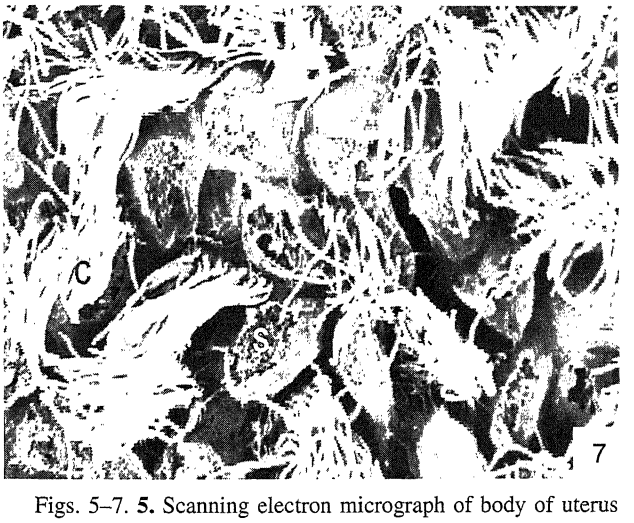
Secretory cells were seen with secretory materials over its surface. Secretory cells were more in number around the openings of the glands and along the ridges formed by the folding of the endometrial surface (Fig. 5). There were more secretory cells with secretions in the late follicular phase and early luteal phase as compared to the late luteal phase. Nilsson (1958) has shown that the secretory activity in the mouse is closely related to the estrogen surge, which



Figs 1–4. **1.** Scanning electron micrograph of horn of uterus showing mucosal folds (arrow). **2.** Scanning electron micrograph of horn of uterus showing hexagonal microvillous cells (M) and few ciliated cells (C). **3.** Scanning electron micrograph of horn of uterus in follicular phase showing hexagonal microvillous cells (M) and few ciliated cells (C). **4.** Scanning electron micrograph of body of uterus showing wide mucosal folds (M) and troughs (arrow).







Figs. 5–7. **5.** Scanning electron micrograph of body of uterus showing secretory cells with secretory blebs (B) in the trough between two mucosal folds (M) **6.** Scanning electron micrograph of horn of uterus in follicular phase showing opening of endometrial gland (arrow) surrounded by ciliated cells (C). **7.** Scanning electron micrograph of cervix uteri (external os) showing mucosal folds, primary fold (P) and secondary fold (SC).

accompanied ovulation in that species. The occurrence of secretory activity during oestrus corresponds closely with that observed in other species during oestrogen dominance in mouse (Nilsson 1958).

The apical smooth-surfaced blebs of the secretory cells were occasionally pedunculated and apparently, building-off into the uterine lumen. This was interpreted as evidence of apocrine secretion similar to that observed by Samuel *et al.* (1979) in the cells of the uterine glands. It is possible that uterine secretions may be involved in the capacitation of spermatozoa.

The microvilli were found in the ciliated as well as in the non-ciliated cells. Microvilli were numerous and long in follicular phase period while were short in the luteal phase. Similar reports have been made by Cruchten *et al.* (2003) in canine endometrium. Microvilli showed some variation in length and were connected with each other by secretory materials. Cruchten *et al.* (2002) reported that in oestrus period the endometrial cells of canine uterus were convex with fairly long microvilli and cell boundaries that were apparent but situated on a deeper level than the cell surface. While at the beginning of metoestrus the cells became less convex with many but short microvilli and prominent but lower situated cell boundaries.

Endometrial glands

The openings of uterine glands were observed on the uterine epithelium. They were often surrounded by a ring of ciliated cells and were visible as deep depressions in the uterine surface (Fig.6). Secretory materials oozing out of the opening were seen in follicular phase. While in the late luteal phase inactive endometrial glands without any secretions were observed. These observations indicate that the activity of endometrial glands was also under the influence of reproductive hormones. Alkhalaf *et al.* (1992) reported that during estrous, glandular cells increase in characteristic secretory feature, which paralleled the rise of progesterone level in plasma.

Cervix uteri

Complex, very deep, narrow folds with small crypts were observed in cervix uteri. The primary cervical crypts were arranged in parallel rows from the external os to the internal os. Secondary cervical crypts were observed on the longitudinal folds and the cervical canal was very irregular due to the presence of small crypts, which delved into the stroma (Fig. 7). The cervical crypts near internal os were more complex than the external os. Similar observations were made by Hafez and Kanagawa (1972a) in cervix uteri of cattle and Wergin (1979) in the cervix of ewe. Kanagawa *et al.* (1972) has suggested that the surface pattern of mucosal folds possibly act as a sperm passage to the site of fertilization or as a sperm barrier or as a sperm reservoir.

The cells that constitute the columna epithelia were of

two types which are easily distinguished by the structures of their apical membranes. They were ciliated cells having bunch of cilia over their surface and non-ciliated secretory cells. The ciliated cells covered by kinocilia, which overlap the surface of secretory cells. The internal os area contained more ciliated cells than the external os area. Similar to our findings, presence of ciliated cells have also been observed by Wordinger *et al.* (1973) and Hafez and Kanagawa (1972b) in bovines, Riches *et al.* (1975) in rabbit cervix. Contrary to our findings, Wu *et al.* (1976) reported rare presence of cilia in the porcine cervical epithelium while Hafez *et al.* (1971) concluded that there are no ciliated cells in the bovine cervical epithelium.

In the follicular phase the cervix had more of ciliated as compared to that of luteal phase, which can be correlated with its function during the different phases. It has been suggested by Kanagawa *et al.* (1972) in the rabbit endometrium that cilia may facilitate the release of secretory granules adhering to the surface of adjacent secretory cells. Hafez and Kanagawa (1972b) studied the bovine reproductive tract and proposed that kinocilia in the cervix uteri beat rhythmically towards the vagina creating a directional flow of luminal fluids. The cilia may facilitate the release of secretory granules adhering to the surface of adjacent secretory cells. It is possible that the cervix uteri act as a sperm selector to prevent the transport of abnormal and inactive spermatozoa to the uterine cavity.

The non-ciliated secretory cells had a dome-shaped surface and were covered with microvilli. Various sizes of ovoid or spherical protrusions of the cell's apical surface were observed. In late follicular phase secretions were very high and secretory materials formed a layer of mucous covering the ciliated cells and the secretory cells. Similar observations were recorded by Hafez and Kanagawa (1972) in cervix uteri of cattle and Wergin (1979) in the cervix of ewe. Like endometrial glands cervical glands were also seen distributed on the cervical mucosa. The openings of cervical glands were seen surrounded by ciliated cells suggesting the role of cilia in transport of secretions of gland.

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