Ultrastructural features of goat oviductal secretory cells at follicular and luteal phases of the oestrous cycle

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ABSTRACT

The aim of the present study was to investigate the ultrastructure of secretory cells in the various regions of the goat oviduct during the follicular and luteal phases of the oestrous cycle. During the follicular phase in the fimbriae, the secretory cells contained small secretory granules with electron-dense matrices. In the luteal phase, the secretory granules disappeared and cytoplasmic protrusions, extending beyond the luminal border of the ciliated cells and often containing the nucleus, were predominant. During the follicular phase in ampullary secretory cells, numerous secretory granules with moderately electron-dense matrices were present in the supranuclear cytoplasm and exocytosis of secretory granules was observed. The number of secretory granules was dramatically reduced in the ampullary secretory cells at the luteal phase. Conspicuous cytoplasmic protrusions of secretory cells were observed similar to those of the fimbrial epithelium. Isthmic cells were almost free of secretory granules and lysosome-like bodies were found both at the follicular and luteal phases. In conclusion, our ultrastructural observations of goat oviduct revealed marked cyclic changes in the ultrastructural features of secretory cells and the ultrastructural features and the numbers of secretory granules were distinctive for each particular segment.

Key words: Fine structure; secretion; cyclic change; reproduction.

INTRODUCTION

The oviductal epithelium consists of 2 morphologically distinct types of cells, ciliated and nonciliated. The nonciliated cells synthesise and release secretory materials (Bjorkman & Fredricsson, 1960). Secretory products (glycoproteins) originating from the oviductal epithelial cells have been identified and characterised in several mammalian species (Abe, 1996). Some of these oviduct-specific glycoproteins are associated with the zona pellucida of ova and/or the surface of spermatozoa and may play important roles in fertilisation, early embryonic development, and functions of spermatozoa (Hunter, 1994; King et al. 1994; Abe et al. 1995*b*; Gandolfi, 1995). Moreover, it has been shown that embryonic development in vitro is improved by using coculture with oviductal epithelial cells (Gandolfi & Moor, 1987). Thus it is tempting to speculate that oviductal secretions create an important microenvironment for fertilisation and early embryonic development.

A previous study revealed that the oviductal epithelial cells of goats secreted an oviduct-specific glycoprotein (Abe et al. 1995*b*). In addition, coculture with the goat oviductal epithelial cells has definite beneficial effects on embryo development in vitro (Prichard et al. 1992). In spite of the importance of these findings, available information on the secretory activity of epithelial cells of the goat oviduct is still limited. The condition and region of the oviduct for providing epithelial cells suitable for supporting embryo development has not been determined. The oviductal epithelial cells with high potential for secretion may be useful for improving in vitro

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fertilisation and embryo development. The objective of the current study was to examine the ultrastructural features of secretory cells in various regions of the goat oviduct during the follicular and luteal phases of the oestrous cycle in order to clarify the relationship between structure and secretory activity. On the basis of these observations, it is suggested that there are regional differences in the secretory activities of oviductal secretory cells in the goat which may have physiological ramifications.

MATERIALS AND METHODS

Goats were observed at least twice per day for evidence of oestrus before they were placed in the experiment. Four animals were killed within 24 h after the initiation of oestrus (d 1) during the follicular phase and 3 were used at d 11–14 of the oestrous cycle, which corresponds to the luteal phase.

Oviductal segments were processed for transmission electron microscopy according to our previous study (Abe et al. 1993*a*). The fimbriae, ampulla, and isthmus (caudal region) were cut apart and these tissue segments were fixed by immersion in 2% paraformaldehyde-2.5% glutaraldehyde and postfixed with 1% osmium tetroxide. After fixation, the oviducts were embedded in Epon 812 and ultrathin sections cut with an ultramicrotome. The sections were contrasted with uranyl acetate and lead citrate and examined with a transmission electron microscope (JEM-100SX, JEOL, Tokyo, Japan).

To obtain quantitative data on the percentage of ciliated cells and nonciliated secretory cells, 1.5 μm semithin sections were examined by light microscopy as described previously (Abe & Oikawa, 1993 *b*). The percentages of ciliated and secretory cells were determined by scanning 200–500 cells per block from at least 3 different blocks of fimbriae, ampulla, and isthmus per animal. All the epithelial cells that extended as far as the luminal surface were counted, while those in very obliquely cut areas were excluded. The height of epithelial cells was measured with an ocular micrometer. For these measurements we selected, in each region, 32–50 ciliated and secretory cells in which the plane of the section clearly passed through the cell nucleus, parallel to the longitudinal axis of the cell.

Statistical analysis was carried out by one-way analysis of variance (ANOVA, Abacus Concepts, StatView, Berkeley, CA) and Fisher's protected least significant differences test.

RESULTS

Cytomorphometric study

Figure 1 shows the morphology of the mucosa in the 3 regions of the goat oviduct during both the follicular and luteal phases of the oestrous cycle. In the epithelium of all regions, 2 distinct cell types, ciliated and nonciliated (secretory) cells were distinguished. Basophilic granules were present in the apical cytoplasm of nonciliated cells in the fimbriae and ampulla at the follicular phase (Fig. 1*a*, *c*), while at the luteal phase, the nonciliated secretory cells in both the fimbrial and ampullary regions, displayed a characteristic slender shape (Fig. 1*b*, *d*). The apices of these cells showed various degrees of protrusion. The isthmic epithelium was composed of cells with cytoplasm which stained with various intensities, but no difference in the morphology of epithelial cells was observed between the follicular and luteal phases (Fig. 1*e*, *f*).

Table 1 shows the mean percentage and cell height of ciliated and secretory cells in the oviductal epithelium of the fimbriae, ampulla, and isthmus during the follicular and luteal phases of the oestrous cycle. In the fimbrial and ampullary epithelium, the ciliated cells were more abundant than those in the isthmic region. In the epithelium of all regions, no marked differences in the proportions of ciliated and

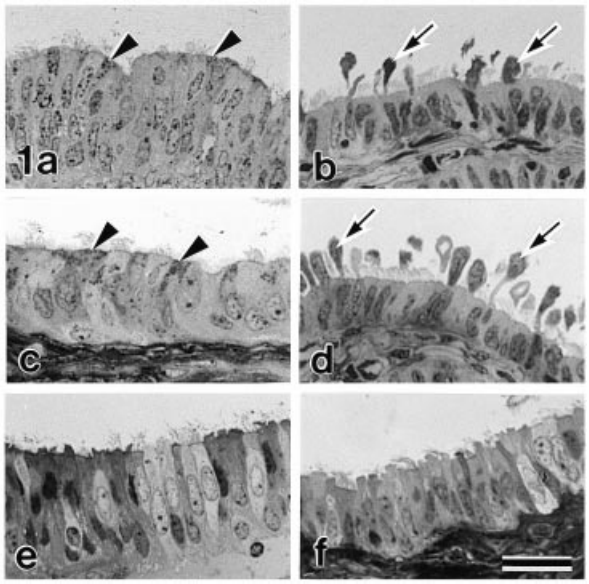

Fig. 1. Light micrographs of the goat oviduct in cross-section showing the fimbriae (*a*, *b*), ampulla (*c*, *d*), and isthmus (*e*, *f*). (*a*, *c*, *e*) Follicular phase. (*b*, *d*, *f*) Luteal phase. Basophilic granules (arrowheads) are found in nonciliated cells in the fimbriae (*a*) and ampulla (*c*) at the follicular phase. Cells are extruded from the epithelium (arrows in *b*, *d*) in the luteal phase. Bar, 20 μm.

Table. *Cyclic and segmental variations in proportion and cell height of ciliated and secretory cells in the goat oviduct epithelium*

	Proportion (% ± S.E.M.)			Cell height (μm ± S.E.M.)		
	F	A	I	F	A	I
Ciliated cells						
F.P.	48.1 ± 1.3	38.4 ± 1.0	24.9 ± 1.4	31.4 ± 0.4[a]	36.2 ± 0.8[a]	31.8 ± 0.4[a]
L.P.	50.7 ± 0.8	39.4 ± 0.9	26.4 ± 1.2	17.9 ± 0.3[b]	16.8 ± 0.4[b]	24.9 ± 0.4[b]
Secretory cells						
F.P.	51.9 ± 1.3	61.6 ± 1.0	75.1 ± 1.4	32.4 ± 0.4[a]	35.2 ± 0.6[a]	32.4 ± 0.5[a]
L.P.	49.3 ± 0.8	60.6 ± 0.9	73.6 ± 1.2	29.9 ± 0.3[c]	31.5 ± 0.6[c]	25.5 ± 0.4[b]

F.P., follicular phase; L.P., luteal phase; F, fimbriae; A, ampulla; I, isthmus.
Values are expressed as means ± S.E.M. (proportion: n = 8–13, cell height: n = 32–50).
Means (cell height) with different subscripts are different ($P < 0.001$).

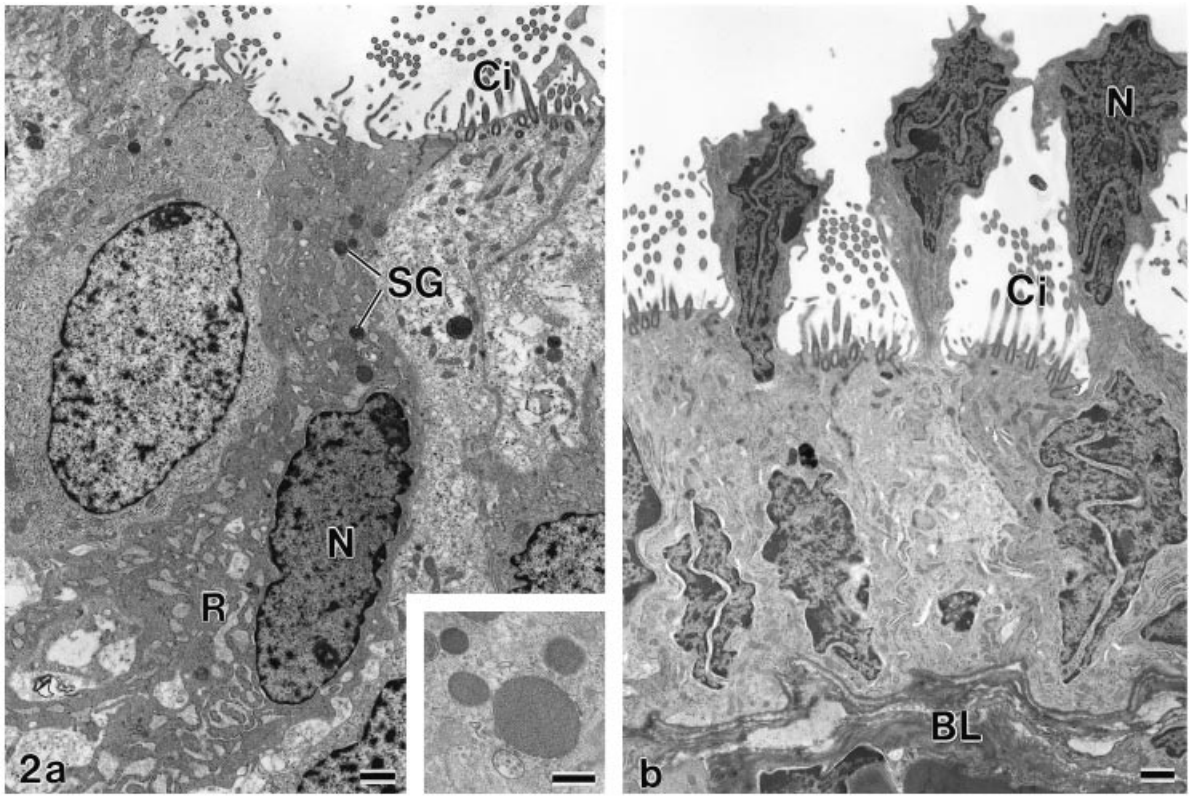

Fig. 2. Fimbrial epithelial cells of goat oviducts at the follicular (*a*) and luteal (*b*) phases of the oestrous cycle. (*a*) The fimbrial secretory cells at the follicular phase have secretory granules (SG) and extensive rough endoplasmic reticulum (R) in their cytoplasm. Inset: Most of the secretory granules are small in size and have an electron-dense homogeneous matrix. (*b*) The nonciliated cells display large cytoplasmic protrusions, usually containing the nucleus (N). BL, basal lamina; Ci, cilia. Bars, 1 μm (in inset, 0.5 μm).

secretory cells were observed in relation to the phase cycle. However, the height of both ciliated and secretory cells in the epithelium of all regions was significantly reduced at the luteal phase when compared with the follicular phase. This reduction in height at the luteal phase was most dramatic in the ciliated cells of the fimbriae and ampulla. In the fimbria and ampulla at the luteal phase, the height of secretory cells was significantly higher than that of the ciliated cells.

Fimbriae

During the follicular phase, the ciliated cells generally had low electron dense cytoplasm. The secretory cells had a characteristic slender shape and cytoplasm that

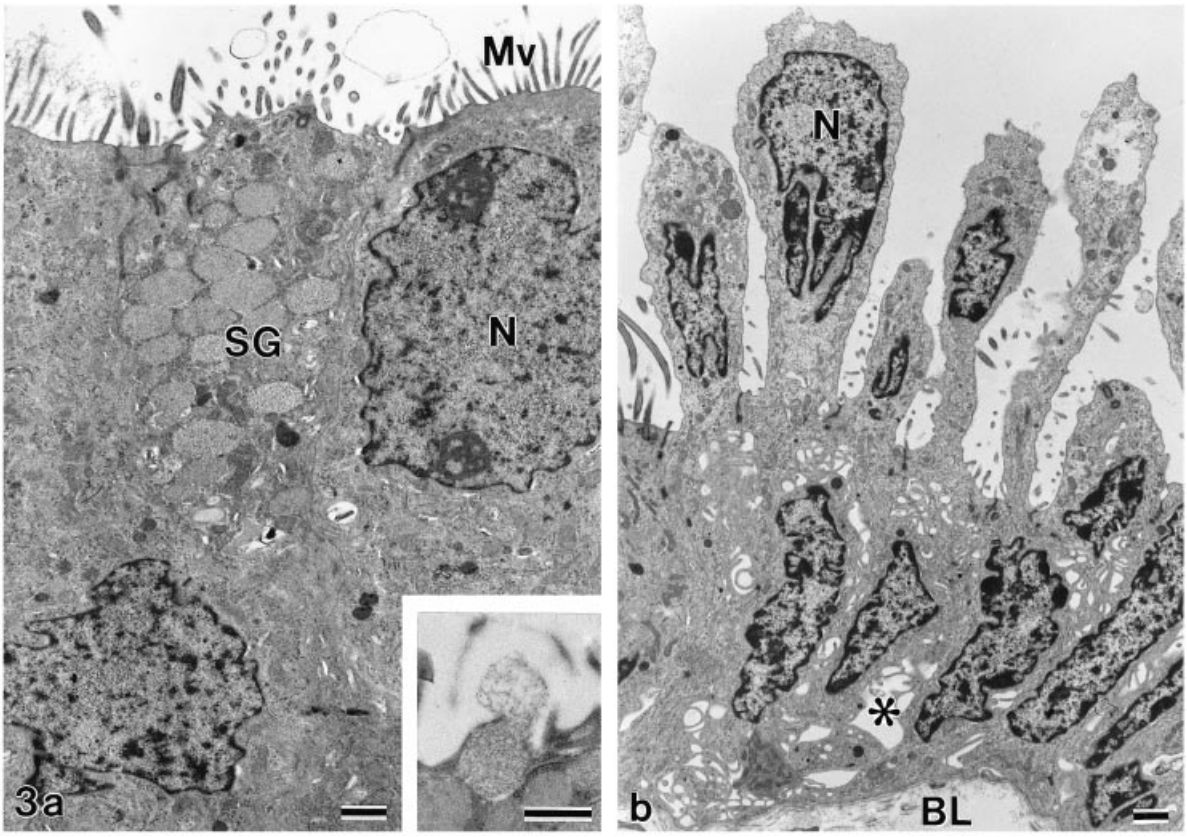

Fig. 3. Ampullary epithelial cells of goat oviducts at the follicular (*a*) and luteal (*b*) phases of the oestrous cycle. (*a*) The ampullary secretory cells at the follicular phase have numerous secretory granules (SG) in the supranuclear cytoplasm. Inset: This figure suggests the exocytosis of a secretory granule. (*b*) The secretory cells display large, long cytoplasmic protrusions, often containing the nucleus and secretory granules. A large intercellular space (asterisk) and interdigitations between the epithelial cells are seen. BL, basal lamina; Mv, microvilli. Bars, 1 μm (in inset, 0.5 μm).

usually stained more densely than that of the ciliated cells (Fig. 2*a*). In the fimbrial secretory cells, some secretory granules were present in the supranuclear cytoplasm. The secretory granules had homogeneous electron-dense matrices (Fig. 2*a*, inset). A well developed Golgi apparatus and extensive rough endoplasmic reticulum were observed in the cytoplasm.

In the luteal phase, nucleated apical protrusions were interspersed with ciliated cells (Fig. 2*b*). 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 Golgi apparatus and rough endoplasmic reticulum were undeveloped. Bundles of intermediate filaments were prominent in the protrusions of secretory cells.

Ampulla

During the follicular phase, the secretory cells had blunt processes at their apical surfaces. Numerous secretory granules were observed in the supranuclear cytoplasm of the secretory cells (Fig. 3*a*). The secretory granules possessed moderately electron-dense matrices, some of which contained electron-lucent material. Some secretory granules appeared to be released by exocytosis (Fig. 3*a*, inset). The secretory cells were characterised by rough endoplasmic reticulum and well developed Golgi zones. Secretory cells of the ampullary region extended numerous microvilli into the lumen and filamentous materials were associated with these microvilli.

In the luteal phase, marked apical protrusions of the secretory cells occurred in the epithelium of ampullary region (Fig. 3*b*). Cytoplasmic protrusions were numerous, extending beyond the luminal border of ciliated cells similar to those seen in the fimbriae at the luteal phase. The only connection between the protrusion and the rest of the cell was often a narrow cytoplasmic strand. The nucleus, which often showed extreme indentations, was usually located in the mid or basal position, but was often observed in the cytoplasmic protrusions (Fig. 3*b*). The number of

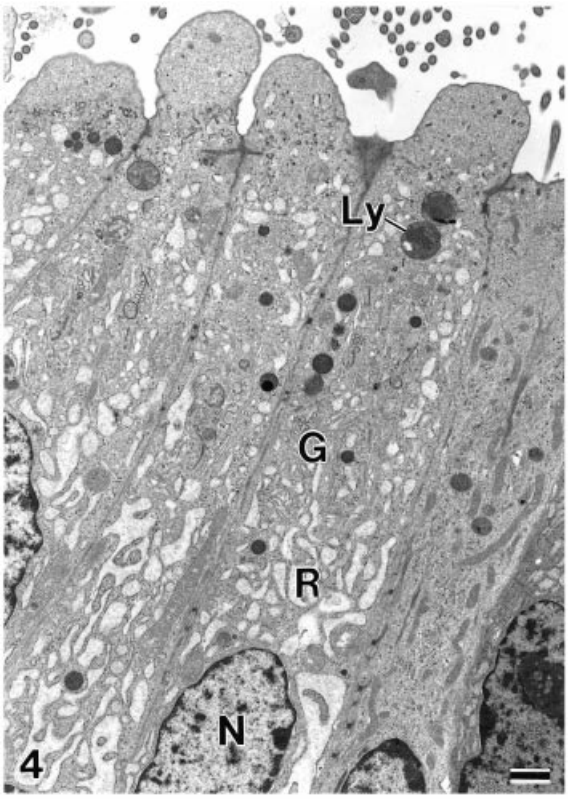

Fig. 4. Isthmic epithelial cells of goat oviducts at the follicular phase of the oestrous cycle. The nonciliated cells have extensive rough endoplasmic reticulum (R) and lysosome-like bodies (Ly). G, Golgi apparatus; N, nucleus. Bar, 1 μm.

secretory granules was dramatically reduced in the cytoplasm of secretory cells in the ampullary epithelium at the luteal phase, and only a few scattered secretory granules were observed in the protrusions. Large intercellular spaces and conspicuous interdigitations were observed between the epithelial cells.

Isthmus

Most of the secretory cells had blunt processes at their apical surfaces and contained only a few secretory granules (Fig. 4). These secretory granules were small and no evidence of granule release was observed. Generally, the isthmic secretory cells showed little difference in ultrastructural features between the follicular and luteal phases. The secretory cells contained extensive rough endoplasmic reticulum and well-developed Golgi apparatus in the cytoplasm and some lysosome-like bodies were observed. Many small dense inclusions were present in the apical cytoplasm. Conspicuous cytoplasmic protrusions were not observed in the isthmic nonciliated cells in either phase of the oestrous cycle, but some protrusions which appeared to possess cilia, occurred in the isthmic ciliated cells (not shown).

DISCUSSION

In our previous study, scanning electron microscopy revealed that the goat oviduct underwent a cyclic change in ciliation during the oestrous cycle (Abe et al. 1993*b*). In particular, the fimbriae and ampulla were extensively ciliated during the follicular phase, while large numbers of cilia were hidden during the luteal phase. In this study, the proportions of ciliated and secretory cells in the goat oviductal epithelium did not differ significantly between the follicular and luteal phases. However, the cytomorphometric data revealed the height of epithelial cells was reduced in all regions during the luteal phase, and this reduction of height was most dramatic in the ciliated cells in the fimbriae and ampulla. Most secretory cells extended beyond the ciliated cells in the fimbriae and ampulla during the luteal phase. These results suggest that the reduction in the height of ciliated cells is responsible for the reduction in the number of cilia on the surface of the goat oviductal epithelium at the luteal phase. Similar findings have also been observed in the Chinese Meishan pig (Abe & Oikawa, 1992) and cow (Abe & Oikawa, 1993*b*).

The most conspicuous characteristic of the nonciliated cells of the goat oviductal epithelium was the presence of secretory granules. However, the number and distribution varied considerably during the oestrous cycle and between the oviductal segments. The number of secretory granules was highest in the cells of the ampulla at the follicular phase, while the number of granules was less in the fimbriae and isthmic secretory cells were almost free of granules. These results suggest that there are regional and cyclic variations in secretory activity. A previous immunocytochemical study revealed that the immunoreactive goat oviduct-specific glycoprotein (97 kDa) was abundant in the ampullary epithelium at the follicular phase, but it was barely detectable in the isthmus of the goat oviduct (Abe et al. 1995*a*). Although there is no direct evidence that this glycoprotein is localised in the secretory granules in the goat oviduct, these morphological and immunohistochemical findings suggest that the secretory product is mainly produced by the secretory cells in the ampulla.

Transmission electron microscopy revealed that there are marked regional differences in the cyclic changes in secretory cells of the goat oviduct. The reason for the regional differences remains unresolved.

It is well known that the mammalian oviduct is a target organ for the sex steroid hormones, oestrogen and progesterone. These hormones cause various morphological changes in oviductal epithelial cells. In particular, oestrogen induces hypertrophy, active ciliation, and formation of secretory granules by atrophied epithelial cells in oviducts (Verhage & Brenner, 1975; Abe & Oikawa, 1993*a*). It has also been suggested that oviductal epithelial cells show regional differences in their responses to steroid hormones (Fredricsson & Holm, 1974; Bajpai et al. 1977). Although we have not examined segmental differences in the hormonal responses of epithelial cells in the various regions of the goat oviduct, the regional differences may reflect regional variations in sensitivity to circulating ovarian steroid hormones, such as oestrogen and progesterone.

The nonciliated secretory cells of the isthmus (caudal region) had extremely few secretory granules, but contained many lysosome-like bodies. The function of isthmic secretory cells is thought to be something other than the production and release of secretory products. It has been suggested that the isthmic epithelial cells act as a sperm reservoir (Hunter et al. 1991). That study demonstrated specific and active interactions between the tips of the cilia and flagella of bull spermatozoa in the caudal isthmus of the oviduct of mated cows, suggesting that the cilia in the oviductal isthmus may act to regulate the contacts with spermatozoan flagella. In addition, some studies suggest that isthmic epithelial cells may have the ability to maintain the viability and fertilising capacity of spermatozoa (Pollard et al. 1991; Suarez et al. 1991). The main function of isthmic secretory cells of the mammalian oviduct remains unresolved.

In conclusion, the present ultrastructural observations revealed marked regional variations in the epithelial cells of the goat oviduct that are associated with the cyclic changes. The data suggest that the secretory activity of secretory cells is highest in the ampullary region at the follicular phase of the oestrous cycle. Fertilisation occurs in the ampulla of the oviduct at the follicular phase and the embryos migrate to the uterus. The secretory cells of the goat oviduct may provide the suitable fluid milieu for fertilisation and embryonic development through their secretory activity associated with the segmental and cyclic variations. The biological significance of regional differences in the secretory cells of the goat oviduct needs further investigation to improve our understanding of the reproductive process.

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