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Ultrastructural Features of Secretion by Murine Oviductal Epithelium

Maki Morita, Miki Sugimoto, Hajime Miyamoto and Noboru Manabe*

Unit of Anatomy and Cell Biology, Department of Animal Sciences, Kyoto University, Kyoto 606-01, Japan

Abstract: *We examined the ultrastructural features of secretion by the murine oviductal epithelia. In ampullae, mucus material accumulated in the apical parts of the secretory cells in proestrus, estrus and metestrus. In estrus and metestrus, numerous secretory granules of various sizes were seen. Fusion of secretory granules and the plasma membrane, i.e. exocytosis, was observed in metestrus. In diestrus, however, the secretory cells were atrophied. Secretory products from the ampullar epithelium may support fertilization and embryonic development in early stages. At the isthmuses, largely-expanded rough endoplasmic reticulum was seen in metestrus. Secretory granules were observed in proestrus and estrus, and these were released by exocytosis especially in proestrus. The material contained in these granules may play an important role in sperm capacitation. The present results indicate that the secretory cells of ampullae and isthmuses are regulated differentially by the estrous cycle dependent factors in mice.*

Key words: *Oviductal epithelium, Secretory cell, Estrous cycle, Ultrastructure, Mouse.*

The oviductal epithelium is comprised of two major cell populations, ciliated cells and secretory cells. Ciliated cells are supposed to contribute to the transport of embryos by movement of cilia, and secretory cells are thought to support fertilization and early embryo development by secretion of nutrients and growth factors. These cells show morphological and functional changes during the estrous cycle and pregnancy [1–6]. In addition, oviduct fluid secreted by the mammalian oviductal epithelium provides an environment that is suitable for the gametes and supports sperm capacitation, fertilization and early embryonic development [7]. One-cell embryos of mouse [8], golden hamster [9], pig [10], cattle [11] and sheep [12], cultured in conventional cell-culture media, are known to have no capacity to grow because of the 2-cell block. The development of the bovine embryos [13, 14] is also arrested between the 8-cell and 16-cell stages *in vitro*. Co-culture with oviductal cells can support the development of embryos through these blocks [12, 15–19]. The physiological functions of the fluid and the mechanism of its secretion, however, are not fully understood. In the present study, we examined the ultrastructure of the murine oviductal epithelia and assessed the secretory activity during the estrous cycle.

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*To whom correspondence should be addressed.

Materials and Methods

Animals and tissue collection: Jcl:ICR mice used in this study were purchased from Japan Clea (Osaka, Japan). Mature female mice (15 ± 3 weeks old) were caged with adult males separated by steel mesh to stimulate cycling, and kept at 23 ± 2°C with intervals of 14 h light (5:00–19:00) and 10 h darkness. All animal experiments conformed to the Guide for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee according to NIH #86-23; revised 1985). Vaginal smears were taken daily and mice which showed at least two successive normal estrous cycles were used. Oviducts were removed at 13:00 on each day of the estrous cycle and small tissues from the ampullae and the isthmuses were cut under a stereomicroscope by dissecting the mesosalpinx for identification.

Morphological techniques: The ampullae and isthmus tissues prepared from the female mice were cut into small pieces, fixed in 2.5% glutaraldehyde (Nacalai Tesque, Kyoto, Japan) in 0.1 M phosphate buffer (PB; pH 7.4) at 4°C for 2.5 h, and then post-fixed in 1% osmium tetroxide (Wako Pure Chemical, Osaka, Japan) in PB at 4°C for 2 h. Oviducts were dehydrated through a graded ethanol series and embedded in Epoxy resin (Luveak resin; Nacalai). Semithin sections (0.8 μm in

thickness) were cut on an ultramicrotome (Reihert Ultracuts, Reichert Jung, Nussloch, Germany), stained with Giemsa and basic fuchsine staining solutions (Wako), and used for histological observation under an optical microscope. For electron microscopic observation, silver ultrathin sections (approximately 70 nm in thickness) were cut, mounted on copper grids, double stained with uranyl acetate (Merk, Darmstadt, Germany) and lead citrate (Wako), and examined with a transmission electron microscope (Hitachi H-7100FA, Hitachi, Japan) at 75 kV.

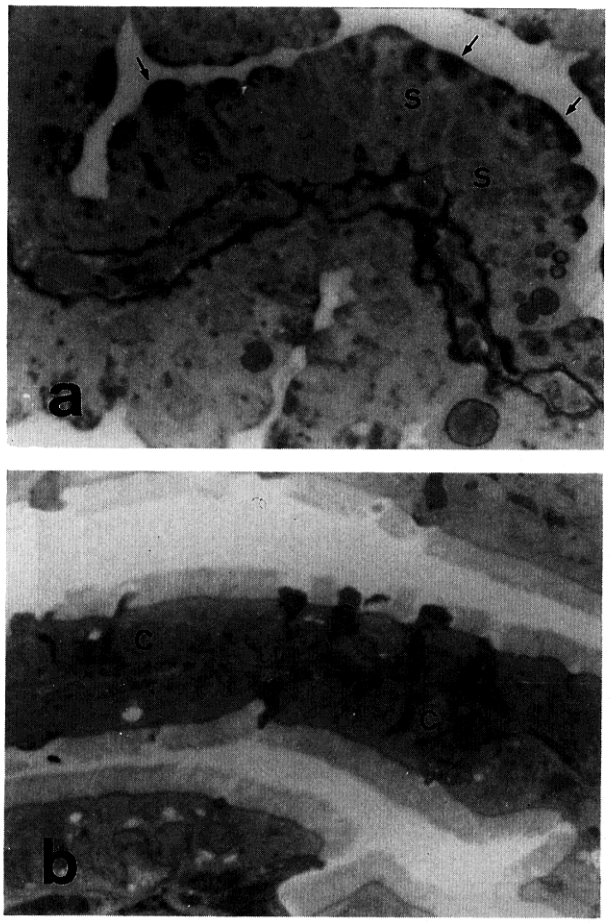

Fig. 1. Semithin sections of mouse ampullar epithelia in metestrus (a) and diestrus (b). They were double stained with Giemsa and basic fuchsine. a) The secretory cells were dominant, and their tips were intensively stained (arrows) with basic fuchsine in metestrus. b) The secretory cells were atrophied, and many ciliated cells were seen in diestrus. S: Secretory cell. C: Ciliated cell. Original magnification, × 200.

Results

Oviductal epithelia of mice consisted of two cell types, i.e. ciliated and secretory cells. These cell types showed periods of hypertrophy and of atrophy during the estrous cycle.

Ampulla: In estrus, most of the epithelium was comprised of secretory cells. Small secretory granules were seen in the apical parts of these cells. Well-developed rough endoplasmic reticulum (rER) and extended Golgi apparatus were observed in the cytoplasm of the secretory cells. In metestrus, the cytoplasm of the secretory cells facing the oviductal lumen was intensely stained with basic fuchsine (Fig. 1a). Electron microscopic observation showed many electron lucent granules accumulated just beneath the apical plasma membrane of the secretory cells. Fusion of the boundary mem-

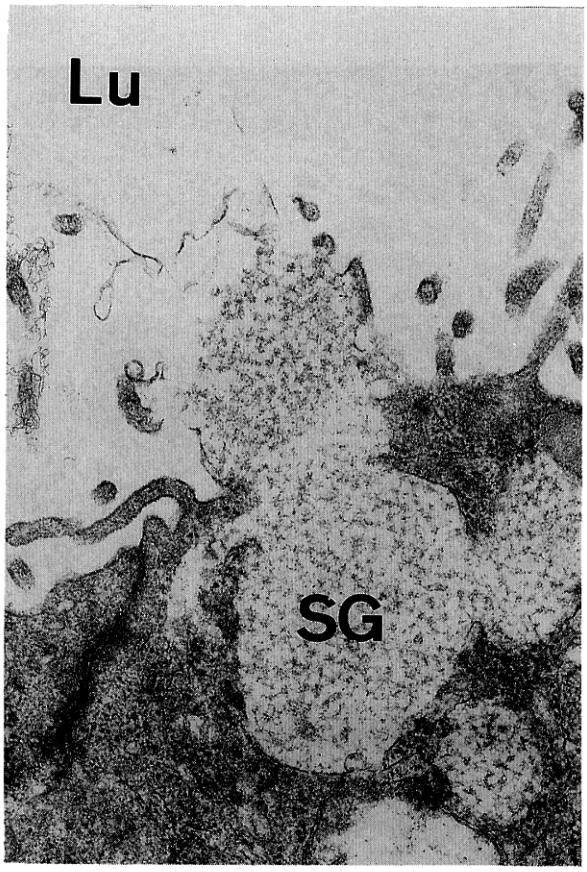

Fig. 2. Electron micrography of ampullar secretory cell in metestrus. The secretory granules (SG) of the secretory cell can be seen to be releasing their contents into the oviductal lumen (Lu). × 30,000.

branes of these granules with the apical plasma membrane was detected as shown in Fig. 2. In diestrus, the ciliated cells were dominant among the epithelial cells, and the secretory cells were darkly stained with Giemsa (Fig. 1b). Most of the secretory cells shrank and protruded into the lumen (Fig. 3). In proestrus, the apical tips of the secretory cells were lightly stained with basic fuchsine. Electron microscopical observation revealed small secretory granules located in the apical cytoplasm and a parallel arrangement of flattened Golgi sacculus located in the supranuclear region in the secretory cells.

Isthmus: Secretory cells were the predominant epithelial cell type in the isthmuses throughout the estrous cycle. In estrus, most secretory cells contained numerous secretory granules which varied widely in size and were stained with basic fuchsine. Electron microscopic observation demonstrated that many large secretory granules were accumulated and made contacted with each other in the cytoplasm of the secretory cells. In metestrus, few secretory cells were stained with basic fuchsine (Fig. 4a). Many secretory cells with vesicles were observed by light microscopy. Electron microscopic observation revealed that the highly distended rER was distributed throughout the cytoplasm of the secretory cells which contained electron-lucent materials (Fig. 5), and that large and spherical mitochondria were scattered in the cytoplasm of the secretory cells which contained few secretory granules. In diestrus, a large percentage of secretory cells were stained with basic fuchsine. Electron microscopy showed that the supranuclear cytoplasm of the secretory cells contained small secretory granules, and rER filled with homogeneous electron-dense materials was observed. These homogeneous electron-dense materials were also detected in the large secretory granules in the same secretory cells. In proestrus, many secretory cells were stained with basic fuchsine (Fig. 4b) similarly to those during estrus. Large secretory granules were accumulated in the apical regions of the secretory cells (Fig. 6) and were frequently released into the oviductal lumen.

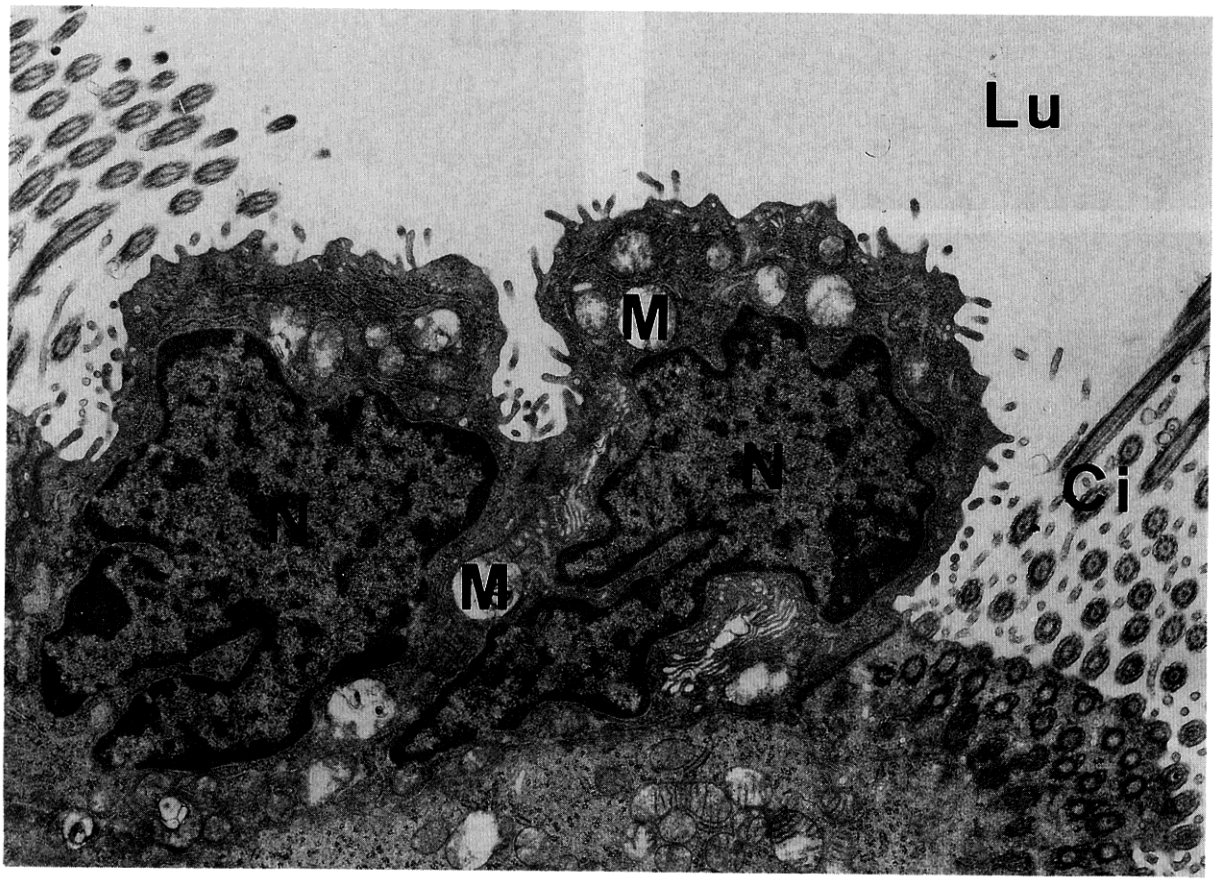

Fig. 3. Electron micrograph of ampullar epithelium in diestrus. The secretory cells with electron-dense cytoplasm shrank and protruded into the oviductal lumen (Lu). N: Nuclei. M: Mitochondria. Ci: Cilia. × 12,500.

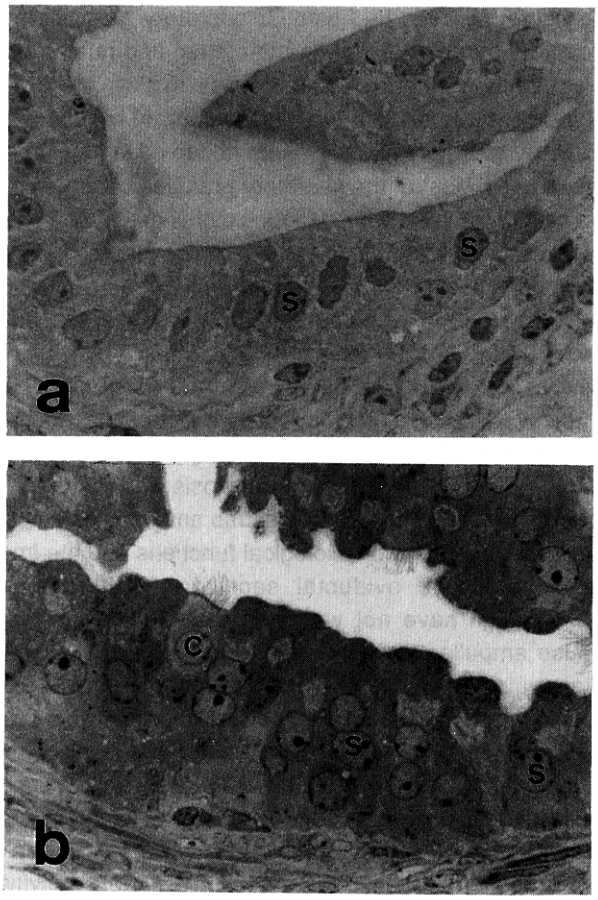

Fig. 4. Semithin sections of mouse isthmic epithelia in metestrus (a) and proestrus (b). The sections were stained with Giemsa and basic fuchsine. a) Most of the secretory cells of the isthmic epithelia in metestrus contained network-like vesicles. b) The secretory cells of isthmic epithelia were heavily stained with fuchsine in proestrus. S: Secretory cell. C: Ciliated cell. Original magnification, × 200.

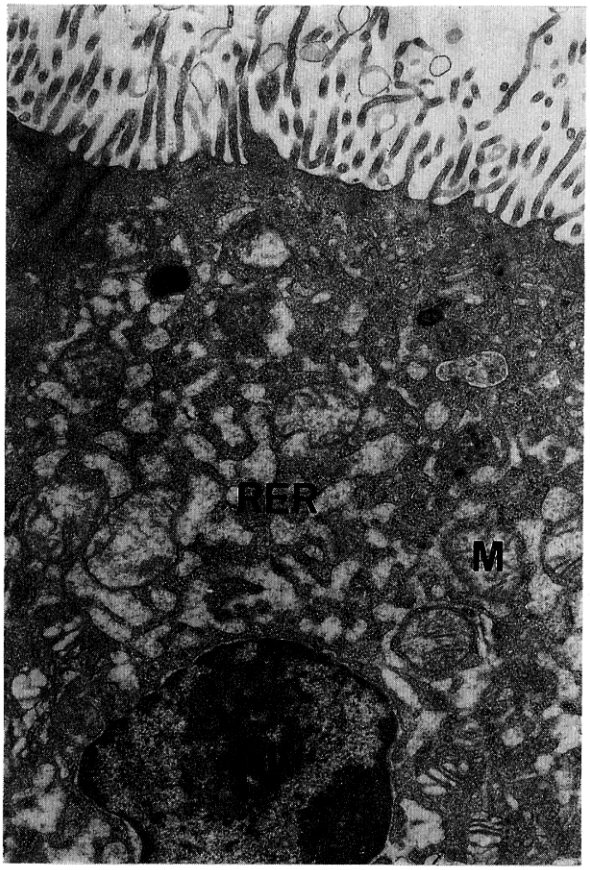

Fig. 5. Electron micrograph of an isthmic secretory cell in metestrus. Largely expanded rough endoplasmic reticulum (RER) with electron-lucent materials filled the cytoplasm of the secretory cells, but few secretory granules were observed. N: Nucleus. M: Mitochondria. × 12,500.

The secretory cells also contained well-developed Golgi apparatus.

Discussion

During the estrous cycle, the ampullar epithelia were reported to undergo estrogen-dependent changes. Estradiol-17β was shown to be responsible for maintaining mature secretory cells in ovariectomized sheep [5], whereas treatment of immature ovariectomized rhesus monkeys with estradiol-17β resulted in various degrees of differentiation of the epithelia [4]. In the present study, secretion by exocytosis from the ampullar secretory cells was shown to occur in estrus and metestrus. These findings indicate that production, maturation and release of secretory granules in the ampullar secretory cells are induced by estradiol-17β. In contrast, progesterone induces atrophy and programmed cell death and apoptosis in the ampullar secretory cells. It has been reported that apoptosis occurred in secretory cells of the oviductal epithelium of cat [6], cynomolgus macaques [1] and the baboon [20], and in the uterine epithelium of the hamster [21] related with the endocrinological status. In these species, increases in the serum progesterone level were coupled with decreases in the serum estradiol-17β level. In cynomolgus macaques [1], macrophages filled with nuclear and cellular fragments were observed during the postovulatory stage. The mouse ampullar secretory cells in diestrus shrank and protruded extensively into the lumen. This observation was the same as that described previously [3, 22–24]. No characteris-

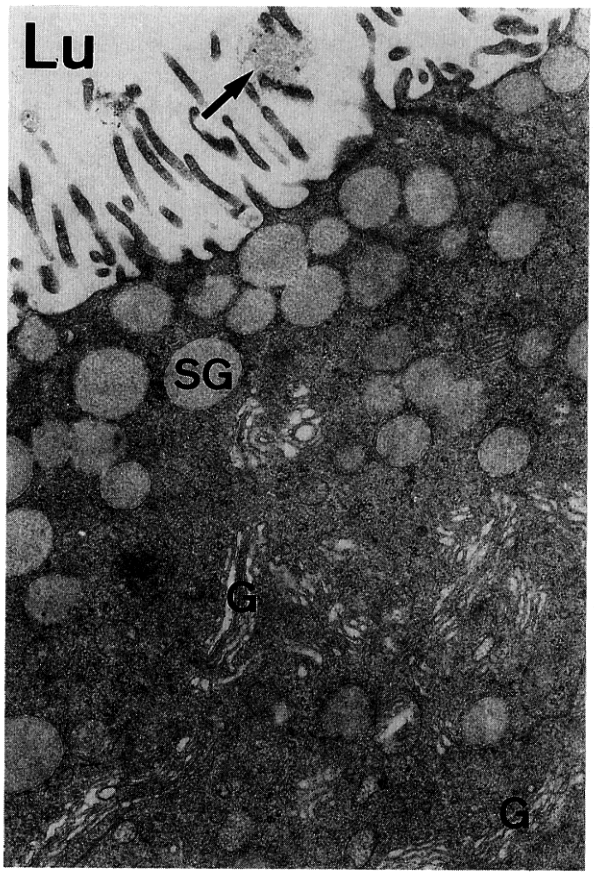

Fig. 6. Electron micrograph of an isthmic secretory cell in proestrus. Large secretory granules (SG) were seen releasing their contents into the oviductal lumen (Lu). These released materials (arrow) were found in the lumen cavity. Well-developed Golgi apparatus (G) was also observed in the cytoplasm of the secretory cells. × 12,500.

tic changes in apoptosis were observed in the ampullar secretory cells. In the small intestine, however, apoptosis occurs in most of the aged epithelia, and then apoptotic cells are shed into the intestinal lumen [25]. This suggests that apoptosis may occur in the murine oviductal epithelia in diestrus.

Homogeneous secretory granules were seen in oviductal epithelia of the rhesus monkey [4], cat [6], goat [24], and dog [26]. Some maturation of secretory materials occurred in the pig-tailed monkey [3], goat [24], sheep [5, 22, 27, 28], and cattle [29]. In addition, the pig-tailed monkey [3] and sheep [5] have matured lamellar structural granules. In the present study, secretory granules that were heavily stained with basic fuchsine were observed in the ampullae and isthmuses, and these may have contained mucus materials. In the ampulla, the secretory cells had smaller secretory granules in proestrus and estrus than in metestrus, and numerous large granules were observed in metestrus. In the isthmus, few secretory granules were seen in metestrus, but many secretory granules were observed in proestrus and estrus. Most of the secretory granules observed in this study contained homogeneous matrix materials. The morphological features of the secretory granules vary among species.

The secretory granules fused to the apical plasma membrane, indicating release of the contents by exocytosis. Exocytosis has been described as a mode of oviductal secretion in cat [6], rabbit [30], goat [24], sheep [28], cattle [29], pig-tailed monkey [3] and the baboon [20]. In the murine oviducts, exocytosis was frequently observed in the ampulla in metestrus and in the isthmus in proestrus, but the physiological functions and the importance of the oviductal secretory products for reproduction have not yet been determined. In the mouse ampulla, maximum secretory activity occurred after ovulation, i.e. in estrus and metestrus. These estrous stages represent the period during which the fertilized gametes remain in the oviductal lumen. The secretory products from the ampullar epithelia must support fertilization and provide nutrients to embryos in the early stages of development. After the rise in the serum progesterone level, the ampullar secretory activity ceased on diestrus. In the isthmus, however, secretion was activated from metestrus through proestrus. Maximum secretion in the isthmus was demonstrated in proestrus. During this period, sperm capacitation occurs in the isthmus. In cattle, the isthmic epithelia show *de novo* protein production and secretion during the coculture with spermatozoa [31]. Regional differences between the ampulla and the isthmus in composition of oviductal secretions were suggested in mice [32]. The secretory products from the isthmic epithelia must provide a favorable microenvironment for sperm capacitation.

In conclusion, the ampullar and isthmic cells are affected differentially by steroid hormones. In mice, secretion by exocytosis mainly occurred in the ampulla in metestrus and in the isthmus in proestrus.

Acknowledgments

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