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Histomorphology of the oviduct epithelium in the Angora rabbit

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Abstract: The present study was undertaken to investigate cyclic changes in the structure of oviduct epithelial cells, and the content of oviductal mucus in the Angora rabbit using light and electron microscopy. Ten female Angora rabbits, 5 of which were in the estrual stage and the other 5 were in the luteal stage of the estrous cycle, were used in the study. Tissue samples taken from the fimbria, ampulla and isthmus of the oviduct were examined under light and electron microscope. Ciliated cells were demonstrated to be predominant in the fimbria and ampulla, whereas secretory cells were determined to be most numerous in the isthmus. The estrual stage was characterized with greater cell heights, and increased numbers of ciliated cells and secretion. Both secretion and cilia were determined to decrease evidently in the luteal stage. Neutral and acidic mucosubstances were found to be present in the ampulla and isthmus. In the isthmus, in which secretion was dense, acidic mucosubstance was determined to contain sulphate and carboxyl groups by means of combined Aldehyde fuchsin/Alcian blue (AF/Ab) staining. Electron microscopic examination revealed the presence of electron-dense and electron-light secretion granules in secretory cells. In all cyclic stages, electron-dense foci were observed in the electron-light secretion granules of some secretory cells.

Key words: Angora rabbit, oviduct, histomorphology

Ankara tavşanında ovidukt epitelinin histomorfolojisi

Özet: Bu çalışmada, Ankara tavşanında ovidukt epiteli hücrelerinin yapısındaki siklik değişimler ve ovidukt mukus içeriği ışık ve elektron mikroskopik olarak incelendi. Çalışmada 5 adet östrual dönemde, 5 adet luteal dönemde olmak üzere 10 adet dişi Ankara tavşanı kullanıldı. Oviduktun fimbriya, ampulla ve isthmus bölgelerinden alınan doku örnekleri, ışık ve elektron mikroskopta incelendi. Fimbriya ve ampullada silyumlu hücrelerin, istmusda ise sekretorik hücrelerin daha çok olduğu dikkati çekti. Östrual dönemde hücre boylarının yüksek olduğu, silyumlu hücrelerin sayısının ve salgının arttığı görüldü. Luteal dönemde ise salgı ve silyumlar belirgin olarak azalmıştı. Nötral mukosubstansın ampullada, asidik mukosubstansın isthmusta lokalize olduğu belirlendi. Salgının yoğun olduğu isthmusda asidik mukosubstansın sülfatlı ve karboksilli gruplardan oluştuğu Aldehit fuksin/Alcian blue (AF/Ab) boyası ile ayırt edildi. Elektron mikroskopik incelemelerde sekretorik hücrelerde elektron koyu ve elektron açık salgı granüllerine rastlandı. Tüm siklus dönemlerinde, bazı salgı hücrelerinin elektron açık salgı granülleri içinde elektron koyu odaklara rastlandı.

Anahtar sözcükler: Ankara tavşanı, ovidukt, histomorfoloji

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Introduction

The mammalian oviduct provides a suitable environment for oocyte maturation, sperm capacitation, and early embryonic development (1-3). The oviduct epithelium plays a major role in the fulfillment of these functions (1,2).

The oviduct epithelium is composed of 2 types of cells, namely, ciliated cells and secretory cells (2,4). The mucus secreted by secretory cells is propelled onto the surface of the oviduct epithelium by ciliary movement, and thereby facilitates the transport of the gametes and zygote. Ciliated cells are known to predominate in the fimbria region, and the number of secretory cells gradually increases towards the isthmus (2,4). Previous studies (5,6) also indicate the presence of a cell type named as "basal", "reserve" or "indifferent cell" in the oviduct epithelium. Localized in the basal layer of the epithelial lineage, these cells, which are small and either round or oval-shaped and possess a heterochromatic nucleus, are claimed to be undifferentiated cells that transform into secretory and ciliated cells (7).

Secretory cells synthesize and secrete into the lumen fluid some specific proteins and glycoproteins that are involved in ovulation and the early embryonic development (1,2,8,9). Thereby, the oviduct secretion regulates various physiological functions (2,8). Sperm cells have been observed to attain motility and fertilizing capability in the oviduct (3). The energy required for early embryonic cleavage during the passage of the embryo through the oviduct is reported to be supplied by glucose (10).

In a study previously carried out in the New Zealand rabbit (9), secretory activity was reported to be more intense in the isthmus when compared to the ampulla. While neutral mucosubstance was abundant in the ampulla, carboxylated and sulphated glucosaminoglycans were found only in the isthmus.

In rats, secretory cells are reported to be present in large numbers, and ciliated cells are reported to have less and shorter cilia in the ampulla during estrus and metestrus (11). During estrus, the secretion of secretory cells is indicated to accumulate in the apical region of the cells, and upon increase in the amount, this secretion is reported to cause the apical region of the cell to protrude towards the lumen during metestrus. The number of ciliated cells is reported to increase during diestrus (11).

The present study was undertaken to determine cyclic changes in the structure of oviduct epithelial cells and to investigate the histochemical localization of oviductal mucus in the Angora rabbit.

Materials and methods

Tissue samples taken from the oviduct of 10 healthy Angora rabbits, which were obtained from private breeders, constituted the material of the study. The animals were anesthetized, their abdominal cavity was incised, and the excised oviducts were examined under light and electron microscope. Subsequently, the animals were euthanized under high-dose ether anesthesia.

A total of 10 sexually mature female Angora rabbits, 5 in the estrual stage and 5 in the luteal stage, were used in the present study. The cycle stages of the animals were determined by evaluating the macroscopic appearance of the ovaria and genital organs (12).

After being fixed in 10% buffered formalin and rinsed, tissue samples taken from the fimbria, ampulla, and isthmus of the oviduct for light microscopic examination were dehydrated through an alcohol series, treated with methyl benzoate and benzol, and embedded in paraplast. For the purpose of general histological examination, the 6-micron-thick sections obtained from the blocks were applied the Mallory's triple stain modified by Crossmon (13), the periodic acid-Schiff (PAS) reaction (13) for neutral mucosubstance, the Alcian blue (Ab) pH 2.5 method (13) for acidic mucosubstance, the PAS/Ab reaction (13) for the joint evaluation of neutral and acidic mucosubstances, and the combined Aldehyde fuchsin/Alcian blue (AF/Ab) staining method (14) for the examination of acidic mucosubstance.

Tissue samples taken from the 3 regions of the oviduct for electron microscopic examination were prefixed in glutaraldehyde paraformaldehyde (pH 7.4) for 24 h, as described by Karnovsky (15), and after being rinsed in cacodylate buffer for 3 h, they were refixed in 1% osmic acid for 2 h. Subsequently, they were kept in 0.5% uranyl acetate for 2 h, dehydrated

through an alcohol series, treated with propylene oxide, and embedded in araldite M. Then, 300-400 Å-thick sections obtained from these blocks were contrasted as described by Venable and Coggeshall (16), and examined under a Carl Zeiss EM 9S-2 model transmission electron microscope.

Results

The oviduct epithelium was determined to be composed of simple columnar epithelial cells. Two types of epithelial cells were distinguished. Ciliated cells were determined to predominate in the fimbria and ampulla, whereas secretory cells were demonstrated to be most numerous in the isthmus. Both cell height and the number of ciliated cells were determined to increase during the estrual stage (Figure 1A). The amount of secretion and the number of cilia were observed to decrease evidently in the luteal stage (Figure 1B).

The periodic acid-Schiff reaction revealed neutral mucosubstance to be abundant in the ampulla (Figure 2A), and to diminish towards the isthmus (Figure 2B). Cells containing neutral and acidic mucosubstances were differentiated using the combined PAS/Ab staining method. The few secretory cells present in the fimbria were determined to include PAS (+) and Ab (+) cells. The PAS reaction's being stronger than the Ab reaction in the ampulla segment (Figure 3A) and the isthmus' displaying only an Ab (+) reaction were noteworthy (Figure 3B). Secretion was determined to reach the maximum level during metestrus. The intense staining of the isthmus with the Ab pH 2.5 stain is an indicator of the large amount of acidic mucosubstance localized in this region. Properties of this acidic mucosubstance were studied using the AF/Ab technique. The acidic mucosubstance containing sulphates was observed to produce a purple color with aldehyde fuchsin, whereas cells containing carboxyl groups were observed to stain blue. Cells containing both mucosubstances were determined to stain mixed colors (Figure 4).

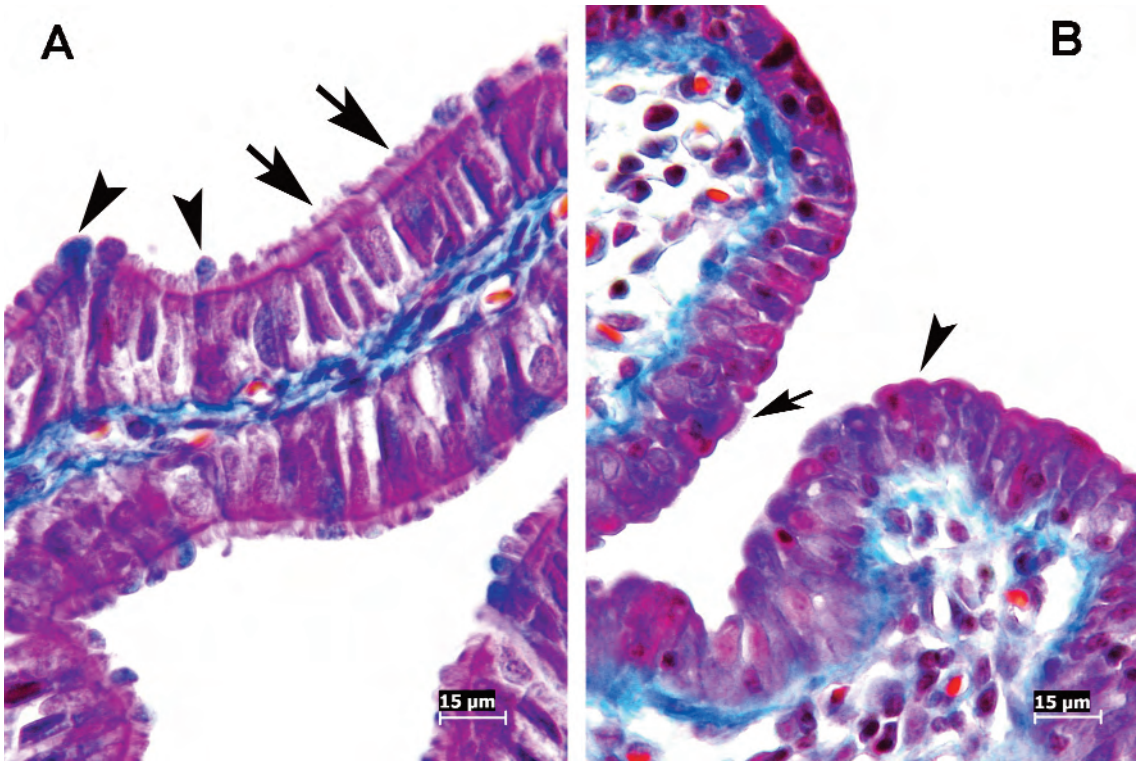

Figure 1. A. Epithelium of the ampulla in the estrual stage, Triple. B. Epithelium of the ampulla in the luteal stage, Ciliated cells (arrows), Secretory cells (arrow heads), Triple. Bar = 15 µm.

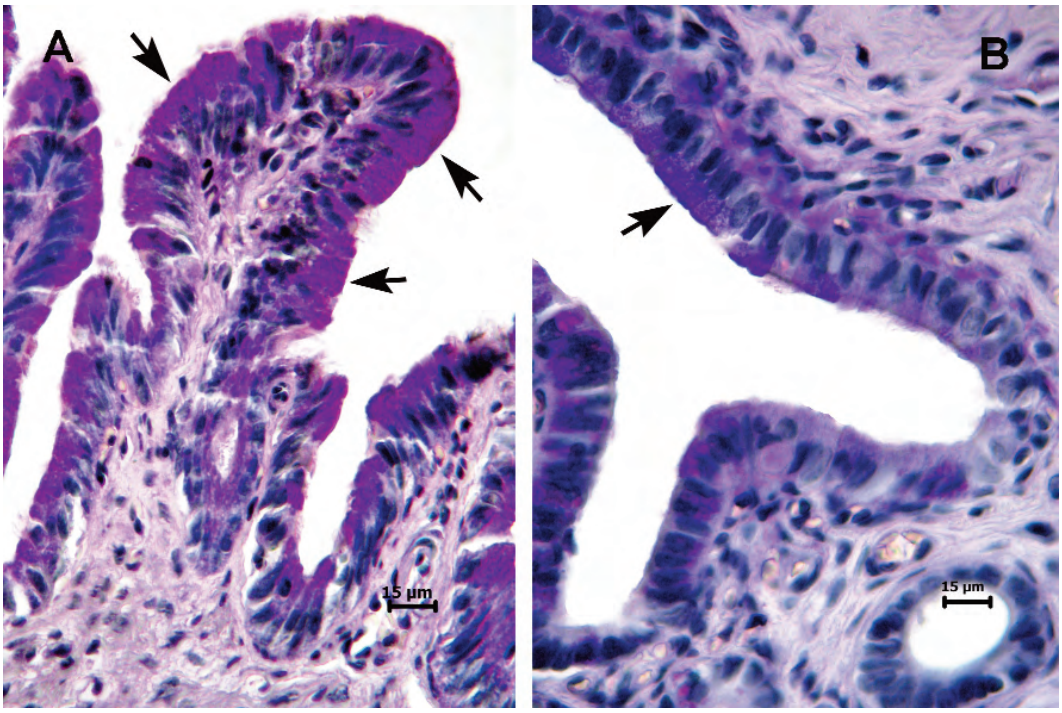

Figure 2. A. PAS reaction in the ampulla in the estrual stage (arrows). B. PAS reaction in the isthmus in the estrual stage (arrow). Bar = 15 µm.

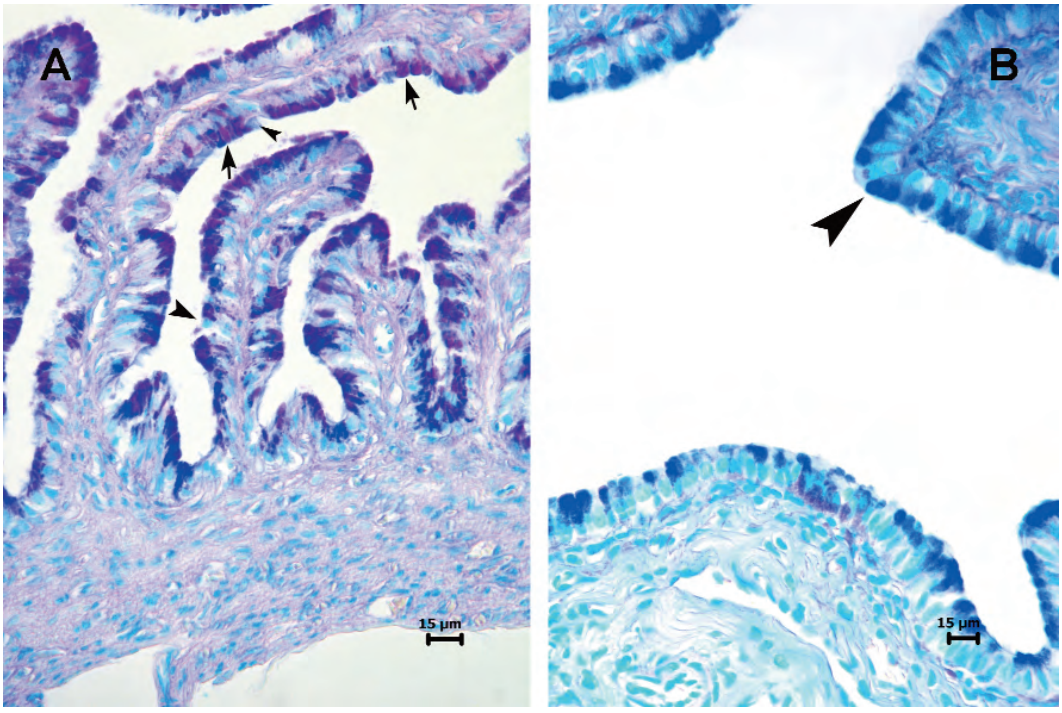

Figure 3. A. PAS / Ab reaction in the ampulla in the estrual stage. PAS reaction (arrows), Ab reaction (arrow head) B. PAS/Ab reaction in the isthmus in the estrual stage. Ab reaction (arrow head). Bar = 15 µm.

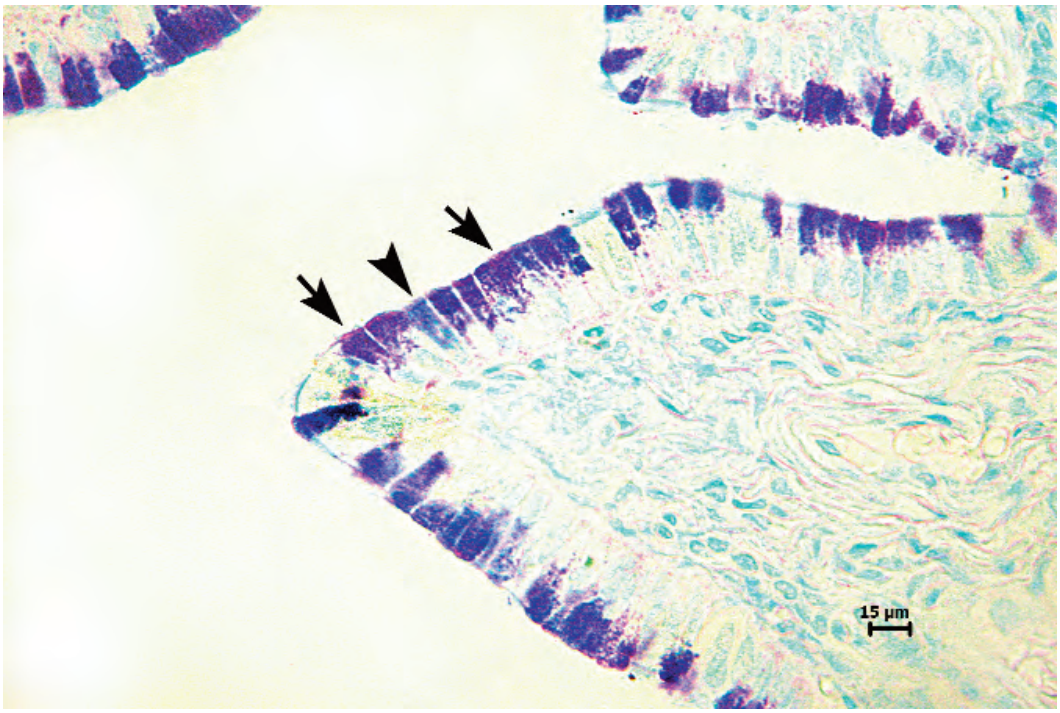

Figure 4. F/Ab reaction in the epithelium of the isthmus during metestrus. AF reaction (arrows), Ab reaction (arrow head). Bar = 15 µm.

Electron microscopic examination revealed the presence of ciliated and secretory cells. In metestrus, the amount of secretion in secretory cells increases (Figure 5). Cilia were determined to have both the so-called "9+2" microtubuli structure and a basal body. Secretory cells were observed to contain electron-dense and electron-light secretion granules (Figure 6). The electron-light secretion granules of some secretory cells were determined to contain secretion granules appearing in the form of electron-dense foci (Figure 7). Secretion was demonstrated to increase particularly in the estrual stage also by electron microscopy (Figure 6).

Cells with cytoplasmic extensions, localized on the basal membrane, were observed to be present in the basal region of the oviduct epithelium. Heterochromatin was conspicuous just below the inner nuclear membrane of these cells (Figure 8).

Discussion

The oviduct, due to its functions, is an active organ. In mammals, cells that form the oviduct epithelium and the secretion of these cells play an important role in reproduction and early development (2).

The rate of ciliated and secretory cells in the oviduct varies throughout the estrus cycle. The number of secretory cells increases during estrus, reaches a maximum level during metestrus, and decreases during diestrus. Ciliated cells predominate in the luteal stage (11). However, in a study carried out by Abe (2) in rats, no marked change was observed in the number of ciliated and secretory cells throughout the different stages of the estrus cycle. In the present study, cilia were determined to decrease in the luteal stage, and to increase in the estrual stage. Furthermore, secretory cells were demonstrated to increase evidently during estrus. These findings do not comply with the report of Abe (2) indicating no difference in the number of ciliated and secretory cells throughout the stages of the sexual cycle.

Steroid hormones are reported to have an effect on both ciliated and secretory cells in the oviduct epithelium. Ovariectomized rabbits are indicated to

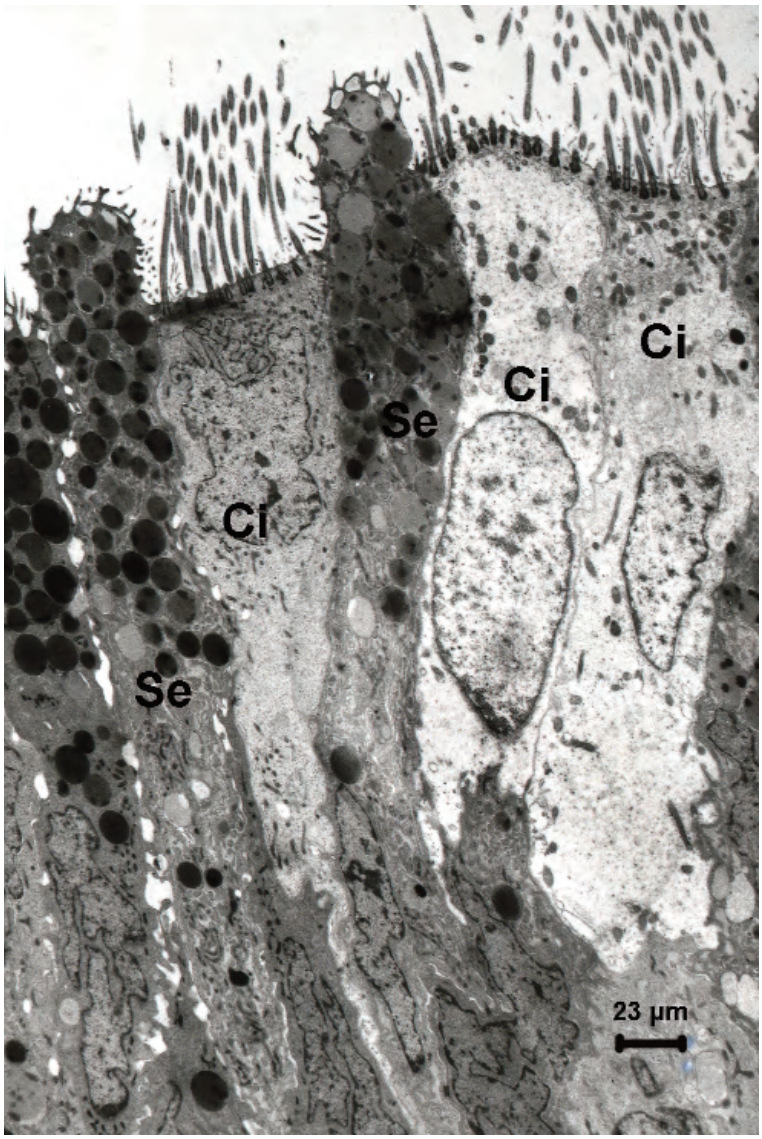

Figure 5. Ciliated (Ci) and secretory (Se) cells under the electron microscope. Metestrus. Bar = 23 µm.

display atrophy of the oviduct epithelium and marked decrease in the number of cilia, and cilia are reported to be renewed upon the administration of estrogen (17,18). In studies carried out on the oviduct epithelium during pregnancy (17), the height of epithelial cells in the fimbria, ampulla, and isthmus are reported to decrease remarkably. Bondi et al. (19) reported ultrastructural changes to be observed in the oviduct epithelium of rabbits after hCG (human Chorionic Gonadotropin) treatment. The findings of the present study support the findings of the indicated researchers, particularly due to the secretory cells being full with secretion during the estrual stage and the amount of this secretion reaching a maximum level in metestrus, as well as the presence of active cilia in the estrual stage and the number of cilia decreasing in the luteal stage. However, the decrease in the number of cilia in the luteal stage contradicts the findings reported by Shirley and Reeder (11).

Sulphated glucosaminoglycans were observed in large amounts in the isthmus and ampulla regions of the rabbit oviduct (1). Menghi et al. (9) reported

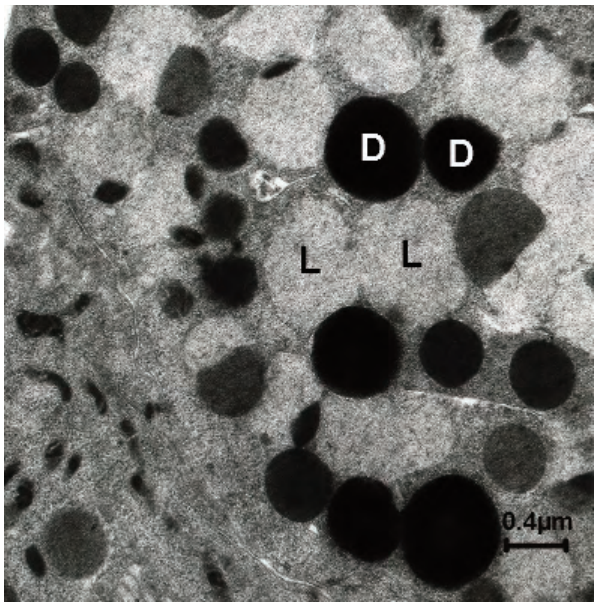

Figure 6. Electron light (L) and electron dense (D) secretion granules in secretory cells of the isthmus epithelium during the estrual stage. Bar = 0.4 µm.

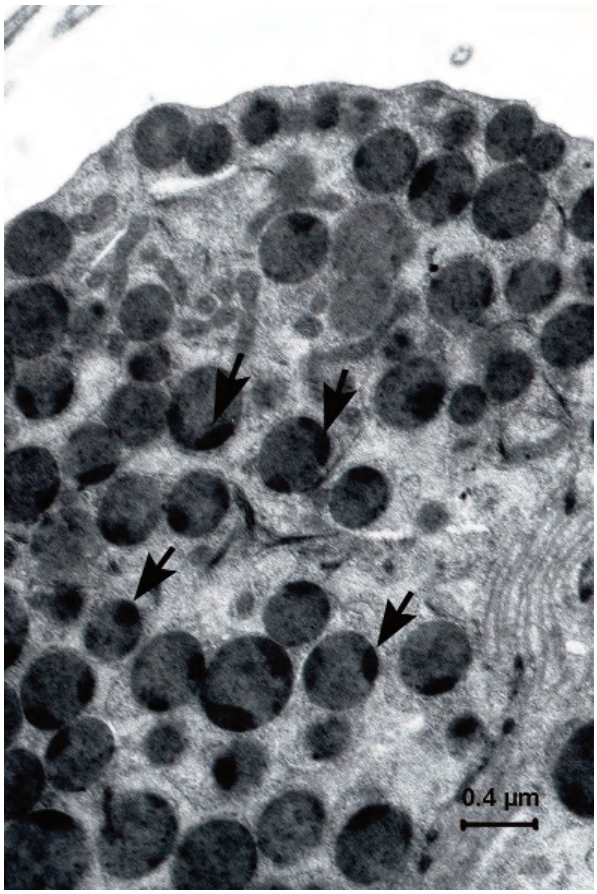

Figure 7. Secretion granules observed as electron dense foci in electron light secretion granules (arrows). Bar = 0.4 µm.

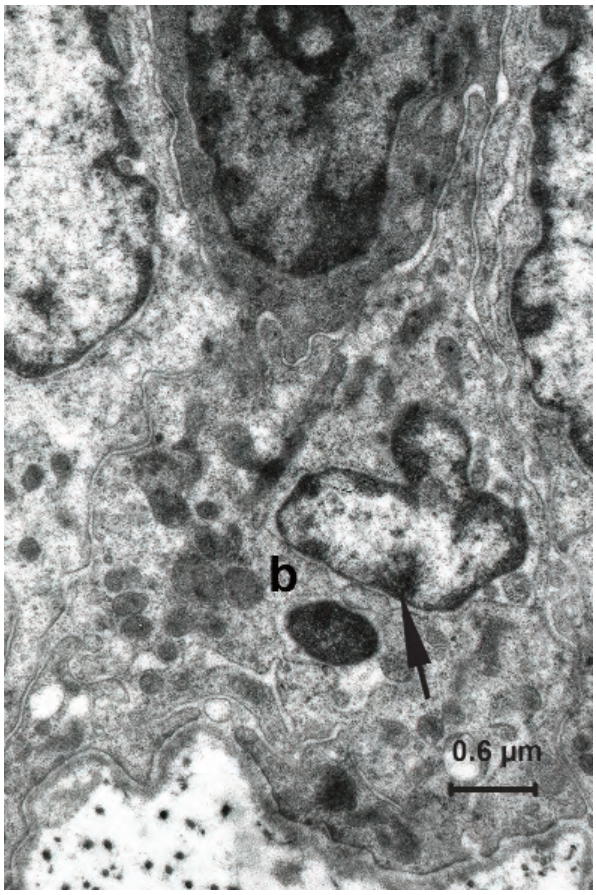

Figure 8. Basal cell (b). Heterochromatin (arrow). Bar = 0.6 µm.

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secretory activity to be higher in the isthmus compared to that in the ampulla; neutral mucosubstance to be found in the ampulla and isthmus, and acidic mucosubstance containing carboxyls and sulphates to be found only in the isthmus in the New Zealand rabbit. Light microscopic findings obtained from the Angora rabbit in the present study are supportive of the findings reported by the the aforementioned researchers.

According to the findings of the present study, the highest secretory activity, observed in the isthmus, contributes to the report of Orihuela et al. (3) indicating spermatozoa to be stored and to attain motility and fertilizing capability in the isthmus.

Electron microscopy revealed the presence of electron-dense and electron-light secretion granules in secretory cells. In some of the electron-light granules, electron-dense foci were observed in certain

regions. Menghi et al. (9) reported these dense foci in secretion granules to be observed during anestrus. The secretion granules detected in the present study were observed not only in anestrus but during all stages of the sexual cycle. These granules are considered to arise from the different chemical properties of the carboxyl and sulphate groups of acidic mucosubstance.

Localized in the basal region of the oviduct epithelium, cells with cytoplasmic extensions not reaching the lumen were observed. Researchers (5,6) have named these cells as basal cells or reserve cells. We agree with the view that supports the oviduct epithelium, which undergoes various changes, degradation and regeneration due to hormones throughout the sexual cycle, to develop from these cells (7).

In conclusion, ciliated and secretory cells of the oviduct were determined to differ in number and histochemical properties in different stages of the sexual cycle in the Angora rabbit. The numbers of secretory and ciliated cells were determined to increase in the estrual stage, whereas the amount of secretion and the number of cilia were demonstrated to decrease evidently in the luteal stage, and acidic mucosubstance found at the highest level in the isthmus was observed to contain sulphate and carboxyl groups.

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