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MORPHOLOGICAL STUDIES ON THE OVIDUCTAL MUCOSA OF THE MARE

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Introduction

Very few reports are available on the anatomy of the equine oviduct, in spite of the considerable degree of attention that has been focussed on the oviducts in various species where fertilization takes place and zygotes develop. STALHEIM, GALLAGHER and DEYOE (1975)¹⁹⁾ observed the mucosa of the infundibular and the ampullary parts of the oviduct of farm animals including mares by scanning electron microscopy. HAMMOND and WODZICKI (1941)¹¹⁾ briefly described the anatomical and the histological changes in the reproductive organs of the mare during the estrous cycle. Recent studies on the egg transportation in the mare, however, have presented some peculiar phenomena that unfertilized ova are retained around in the ampullary-isthmic junction (AIJ) of the oviduct for several months^{1-5,14,16,17,20,22,23,25)}, and that gelatinous masses are frequently found in the distal part of ampulla near AIJ of the oviduct^{14,16,17)}. Since more eggs tend to be retained in the oviduct containing the gelatinous masses, the masses might cause the retention of the tubal eggs directly or indirectly¹⁶⁾. VAN NIEKERK and GERNEKE (1966)²³⁾ considered that the gelatinous masses were originated from the ovarian follicular fluid. While, ONUMA, MATSUOKA, NAKAMURA, NAKANISHI and FUJISHIRO (1972)¹⁷⁾ and ONUMA and OHNAMI (1975)¹⁶⁾ claimed that the masses may be derived from the desquamated cells of the tubal mucosa because the mass consists of collagen fibers. They found various numbers of the degenerated cells in the mass, probably from the tubal mucosa, also. However, an interesting appearance suggesting the mucosal origin of the mass was noted by one member (Y. T.) of our team in a study of the transport of fertilized equine eggs through the oviduct¹⁵⁾; some gelatinous masses were indeed connected with the oviductal mucosa by a thin string.

The objects of this investigation are to obtain the general informations about the equine oviductal mucosa, to confirm the connection between the

mass and the mucosa, and to answer a question for the origin of the mass.

Materials and Methods

A total of 22 oviducts of 11 mares (seven Hokkaido native ponies, two hybrid horses and two race horses ranging from 2 to 22 years in age) were obtained after killing at a slaughterhouse. A majority of the oviducts was trimmed and straightened in moist condition after transportation to our laboratory. The status of the ovaries was checked. The oviducts of a mare (race horse) which were obtained previously and stored in 10% formalin solution were used additionally; one oviduct was used for histological investigation and the other was used for macroscopic observation on the surface of the mucosa. Five oviducts were dissected into the different segments and fixed for scanning electron microscopy (SEM) or light microscopy (LM) immediately after killing at the slaughterhouse.

For SEM, the specimens were prefixed overnight in 3% glutaraldehyde in 0.1 M phosphate buffer at 4°C. The tissues were rinsed in 0.1 M phosphate buffer and postfixed in 1% osmic acid-phosphate buffer solution. After fixation for 1 hour, the tissues were rinsed and dehydrated in a graded series of ethanol, and then dried using CO₂ and isoamyl acetate as intermediate fluid in a critical point drying apparatus. The dried specimens were spattered with approximately 200 Å of gold and observed in Hitachi S-310 Scanning Electron Microscope operating at 5 kV accelerating voltage. For histology, the specimens were fixed in Bouin's fixative and embedded in paraffin. Sections in 7 to 10 μm thickness were stained with hematoxylin-eosin (H. E.), Azan, Gomori's trichrome, aldehyde-fuchsin, or by the periodic acid-Schiff method (PAS). Tissues from oviductal mucosa were embedded in epoxy resin after glutaraldehyde-osmium fixation, also. Semithin sections in 1 μm thickness were taken for LM and stained with 0.5% toluidine blue.

For examination of the gelatinous masses, 18 oviducts were immersed in a physiological saline solution. The masses were located by the aid of a dissecting microscope, cutting open the oviducts longitudinally along the oviductal lumen. The length of the ampullary and isthmic portions of each oviduct was measured to determine the location of the masses. Size and shape of the masses were recorded. The masses isolated free in the oviductal lumen and the masses connected with the oviductal mucosa, together with the part of mucosa, were placed in buffered glutaraldehyde solution. The fixed specimens were subjected to the procedures for SEM or LM. Some of the masses were embedded in epoxy resin for transmission electron microscopy after dehydration in ethanol. Ultrathin sections, cut on a ultra-

microtome (Porter-Blum MT-2 B, Sorvall), were stained with uranyl acetate and lead citrate, and examined with Hitachi 11 B electron microscope. Semi-thin sections of 1 μm thickness were made and stained with 0.5% toluidine blue for LM, also.

Results

A. Morphology of the oviduct of the mare

1. Macroscopic observations on the oviduct

The infundibulum, in which an abdominal ostium opened in the basal part, had two large longitudinal folds and was covered with numerous longitudinal small folds of mucosa, radiated from the abdominal ostium in inner surface (Fig. 1). Free margin of the infundibulum was the fimbriae covered by mucosa and the outer surface of the infundibulum was composed of the serosa. The ampulla and isthmus of the oviduct were easily identified by their outer diameters. The mean length of 19 oviducts was 20.9 ± 1.2 cm (S. E.). There was no significant difference between the lengths of the ampulla and the isthmus. The diameter of the ampulla increased from the abdominal ostium to the slightly upper part of the central portion of the ampulla (4-6.5 mm in diameter), then gradually decreased toward the isthmus. Distal part of the ampulla became narrow and connected with the isthmus. The transitional area (AIJ) was within 1 cm in length. The narrowest diameter of the isthmus (about 1 mm in diameter) was attained in the part passing AIJ, and the diameter increased slightly approaching to the uterus. The part of the tube traversing the uterine wall (intramural part) was about 1 cm in length.

In the internal surface of the uppermost area of the ampulla, there was a distinct folding continuity of the mucosa from the infundibulum (Figs. 1, 2). The arrangement of the mucosal folds at the middle part of the ampulla (most expanded segment) became irregular due to large and complicated branching of the folds (Fig. 3). Approaching the AIJ, the folds decreased in size and in number (Fig. 4). The transition of the mucosal folds from ampulla to isthmus occurred rather suddenly (Figs. 4, 5). Very poorly developed folds running longitudinally through the whole isthmus were intersected at places (Fig. 6).

2. Scanning electron microscopic observations

Two mares used for LM or SEM were estimated to have ovulated several hours prior killing. The oviductal mucosa was covered with numerous clusters of ciliated cells and of non-ciliated dome-shaped secretory

cells through the whole oviduct (Figs. 9, 17, 21). Non-ciliated cells were easily found among the crowded cilia and possessed many microvilli on their surface. The ciliated and non-ciliated cells were located in equal numbers through the whole oviducts.

1) Infundibulum

The longitudinal mucosal folds in the fimbriae (Fig. 7) changed to irregular and wavy folds near the abdominal ostium (Fig. 8). In about half of the non-ciliated cells in the fimbriae of one oviduct, plasma membranes at apical portion of the cells were ruptured and secretory granules were seen exposed in the cytoplasm (Fig. 10). This phenomenon was limitedly recognized in this area through the oviduct.

2) Ampulla

The folds of the ampulla were largest in size in comparison with the folds of the other segments of the oviduct, and formed a series of independent large protrusions located closely packed together (Fig. 11). The formation of secondary folds of the protrusions was remarkable (Fig. 12). Although the majority of non-ciliated secretory cells were densely covered with well-developed microvilli, the microvilli in some cells were short, or irregularly distributed due to the protruding buds of various size (Figs. 13, 14). The surface of these cells transformed was sometimes elevated beyond the level of other non-ciliated cell surface, and the apical ridges of them reached to the same level of the tips of the cilia (Fig. 14).

3) Ampullary-isthmus junction

At the distal portion of ampulla, the shape of the folds was similar to that of the ampullary portion, except that the mucosal folds decreased in size and the shape of mucosal protrusion became irregular (Fig. 15). The transition of the fold from the ampulla to the isthmus seemed to occur at the lower part rather than the position judged by the macroscopic observation. Approaching the isthmus, the mucosal folds diminished remarkably in size and the mucosal projections were not so dominant (Fig. 16). In some non-ciliated cells, irregular surface was noted as found in ampullary portion.

4) Isthmus

Small mucosal folds running longitudinally through the whole isthmus portion occasionally intersected with each other, showing a net-like fold pattern especially in the upper part of the isthmus (Fig. 18). The formation of secondary folds was scantily observed on the longitudinal folds (Fig. 19). There was no clear transition of the folds to the intramural part, except for the many crypts located on the mucosal surface of the intramural part (Fig. 20).

3. Histological observations

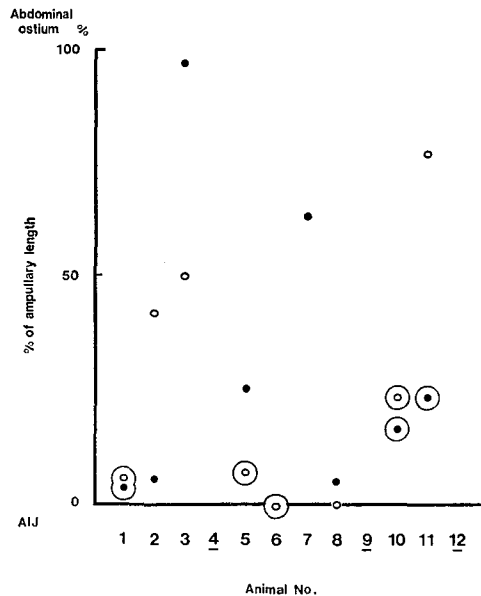
The outermost of the oviduct was lined by a tunic of serosa. The muscular layer consisted of thin outer longitudinal and thick inner circular layers. The muscular coat in the isthmus was extremely thick in relative proportion to that of ampulla. The muscular bundles of the intramural part of the oviduct diminished slightly in number compared with isthmus portion, and a sphincter-like structure was not noted.

The mucous membrane was extremely well developed in the ampulla through the oviduct (Fig. 22). Many large protrusions constructed of the primary folds and the secondary folds of the mucosa were marked in the ampulla. The lumen, therefore, showed a labyrinthine system of narrow spaces. While, the mucosal folds in the isthmus had low ridges and were scanty branched (Fig. 23). No clear transition from the ampulla to the isthmus was found in histological composition, except for the shape of the mucosal folds. The epithelium was highest in the ampulla and slightly diminished in height toward the isthmus. In the infundibulum, the folds were less highly branched in contrast with those in the ampulla. The axial core of mucosal folds was supported principally by connective tissue and blood vessels.

The oviductal epithelium was lined with a layer of columnar cells, ciliated and non-ciliated, but appeared frequently pseudostratified mainly in the isthmus (Fig. 24). Peg cells were observed occasionally in the ampulla (Fig. 25). Cytoplasm of the ciliated cells was relatively lucent and few cytoplasmic granules were situated at the perinuclear zone. While, the secretory cells had densely stained cytoplasm and protruded at the apical end. The nucleus of the ciliated cells was spherical and located in the central region of their cytoplasm. The secretory cells possessed oblong nuclei located in the basal region. PAS reaction indicated that secretory activity was much higher in the isthmus than in the ampulla (Fig. 26 vs. Fig. 27).

B. Observations on the masses

The masses were easily recognizable as a brightly white mass in oblique light under the dissecting microscope (Fig. 28). The masses were obtained from 16 of 24 oviducts (67%). They varied in shape and size: thread-like masses (3.5-7.5 mm in length, and 1-1.2 mm in width) or the globular masses (1-1.5 mm in diameter). A few masses were seen variously branching and showed cluster-like shape. All masses obtained were located in the ampullary portion of the oviducts and 78% of them were found at the distal half of the ampulla (Text-fig. 1). In 7 out of 16 oviducts having the masses



Text-fig. 1. Locations of the gelatinous masses in the ampullary portion of the oviduct in each mare.
 ● ; Mass in the right oviduct.
 ○ ; Mass in the left oviduct.
 ◌ ; Mass connected to the oviductal mucosa.
 — ; Mare having no gelatinous masses.

(44%), the masses were observed to be connected with the oviductal mucosa. Especially, at least five of the masses were clearly connected with the mucosa at one point, and two of them were connected by a narrow string (Fig. 28). Such masses connecting with the mucosa were located limitedly in the area near AIJ (Text-fig. 1).

The masses were consisted of many lobules in various size, which were constructed of swirling bundles of fine fibers (Fig. 29). The bundles were stained weakly with eosin, blue with Azan stain, green with Gomori's trichrome stain, and light red with PAS. The elastic fibers were not demonstrated in the masses by the aldehyde-fuchsin stain. These results strongly suggested that the masses consisted of collagen fibers. Transmission electron microscopy of the mass presented a distinct banding, with an average of every 640 Å, in the longitudinal view of the collagen fibrils (Fig. 30). Scanning electron microscopic studies of the masses demonstrated the lobulous structure (Fig. 31) which consisted of numerous fine fibers (Fig. 32).

A thin string of the masses which were connected with the mucous

membrane, was twisted and appeared to penetrate into the mucosa (Figs. 33, 34). Histological sections distinctly demonstrated that a mass was connected with the mucosa through the epithelium (Fig. 35). The same fibrous structure as the masses, was observed in the lamina propria in the SEM. Therefore, the fibrous connective tissue of the lamina propria appeared to be transformed into the masses, although the epithelium was lacking on the surface of the masses and some degenerating cells were engulfed in the masses. There were no special features noted histologically in the mucosa surrounding the penetrating massive material.

Discussion

In general view of animals, the mucosal folds of the oviduct are arranged longitudinally from the isthmus to the ampulla, and an arrangement of the folds in the fimbriae is irregular.¹⁰ KANAGAWA, HAFEZ, PITCHFORD, BAECHLER and BARNHART (1972)¹² reported that the tree-branch-like folds were arranged longitudinally from the isthmus to the ampulla in the rabbit. The ampullary folds in the rabbit were less branching villi-like folds in the cross section and long and slender folds in the longitudinal section.²¹

Although the mucosal folds of the equine oviduct were mainly longitudinal, they showed irregular arrangement of large mucosal protrusions which were constructed with the primary and secondary folds in the main portion of the ampulla. Such branching of the ampullary folds in the mare seems to be specific in comparison with that in the other animals, such as rabbits, sows, cows and humans.^{4,7,9,21}

Two main types of epithelial cells of the equine oviduct were observed similar to those in other animals: the ciliated cell and the non-ciliated cell. The peg cells were rarely found in the ampullary portion. HAMMOND and WODZICKI (1941)¹¹ mentioned that the mucosa consists of a ciliated epithelium and a thin connective tissue with small blood and lymphatic vessels in the mare. They added also that some authors^{13,24} found non-ciliated epithelial cells in narrow size, having a longitudinal nucleus. SISSON and GROSSMAN (1958)¹⁸ described that the equine tubal epithelium is a single layer of columnar ciliated cells. The main types of epithelial cells, however, were non-ciliated secretory and ciliated in the SEM.¹⁹ Our observation confirmed that non-ciliated cells as well as ciliated cells distributed in the whole epithelium through the oviduct light and scanning electron microscopically.

Distribution rate of ciliated cells varied in different parts of the oviduct and in different segments of the same tissue. The ciliated cells in the oviducts of subprimates and rabbits were unevenly distributed. They were

abundant in the infundibulum, and gradually decreased from the ampulla to the isthmus.^{8,12)} In the oviduct of human, on the other hand, no significant cyclical or geographical morphologic variations in ciliated cells were reported during the menstrual cycle.^{4,6)} According to STALHAIM *et al.* (1975)¹⁹⁾ ciliated and non-ciliated cells in the equine oviduct were evenly distributed as clusters in SEM. In the present study, no significant differences in proportion of ciliated cells were observed through the whole oviduct, confirming the description by STALHAIM *et al.* (1975).¹⁹⁾

The masses in oviduct were found in 87% of 135 oviductal flushings by OGURI and TSUTSUMI (1972)¹⁴⁾ and in 76.2% of 424 flushings by ONUMA and OHNAMI (1975)¹⁶⁾. The detectability of the masses in the present study (67%) was somewhat low compared with the above two reports, and this may be due to the limited number of the oviduct. The masses in 44% of the oviducts holding the masses were connected with the oviductal mucosa, and this percentage seems to be rather high. This indicates that such phenomenon may be common and widespread in the oviduct of the mare.

Two opinions have been presented as to the origin of the masses; namely, the follicular origin²³⁾ and the oviductal mucosal origin^{16,17)}. There are some contradictions in the follicular origin of the masses, nevertheless the newly ovulated eggs were enveloped by a viscous and elastic material of follicular origin. The contradictory facts are that some masses have been recovered from the oviducts on the ipsilateral side of the ovaries which had never ovulated¹⁴⁾, and that old unfertilized eggs have never been found in the masses. The results in the present study clearly show that the masses originated from the oviductal mucosa and did not originate from the viscoelastic material of follicle. As the masses consists of the bundles of fibers, probably from the lamina propria, the so-called gelatinous mass may be referred to as a fibrous mass.

Highly frequency of the occurrence of the masses and the connection with the mucosa might suggest some changes in the oviductal mucosa, although the histological changes in the tube of the mare were reported to be relatively slight during the estrous cycle.¹¹⁾ It might be reasonable that highly developed folds in the ampulla or near AIJ, as shown in this study, break away from the mucosa into the oviductal lumen and then the epithelium is degenerated. It is considered, as a result, that an abundant fibrous tissue originated from the lamina propria is exposed into the lumen and condensed to form a mass. Distribution of the masses, in that all masses connected to the mucosa are located near AIJ and the separated masses are seen scattered throughout the ampulla, indicates the possibility of the

transportation of mass through the ampulla.

Further studies are needed to know the process of formation and the functional role, if any, of the fibrous masses.

Summary

The general structure of the equine oviduct and the gelatinous masses, which were reported originally by VAN NIEKERK and GERNEKE (1966)²³, were studied macro- and microscopically. The mucosal folds in the infundibulum were longitudinal and they became irregular in fold pattern near the abdominal ostium. Extremely branching and large protrusions of the folds are seen irregularly crowded in the main part of the ampulla. Such mucosal fold formation in the ampulla seemed to be specific in the oviduct of the mare. The projected folds decreased in size and number toward the isthmus. The poorly developed folds ran longitudinally through the isthmus.

Two main types of epithelial cells were recognized; ciliated cells and non-ciliated secretory cells. No significant differences in the distribution patterns of both cells were recognizable in whole oviductal mucosa of the mare.

The massive materials were found in 67% of 24 oviducts. Some of them were seen connected with the mucosa of the ampulla near the ampullary-isthmic junction. The masses consisted of collagen fibers which were estimated by light microscopy and electron microscopy, and were considered to be originated from the fibrous connective tissue of the lamina propria in the oviductal mucosa.

Acknowledgments

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Plate 1

Explanation of figures

- Fig. 1. Infundibulum and beginning of ampulla. Oviduct was cut opened longitudinally, and secured to a board before fixation in 10% formalin solution. Mid portion of the infundibulum was tied to the board. Arrows show the large mucosal folds located inner surface of the infundibulum.
- Fig. 2. Mucosal folds in upper portion of ampulla.
- Fig. 3. Mucosal folds in middle portion of ampulla, showing irregular arrangement.
- Fig. 4. Mucosal folds in distal portion of ampulla near AIJ.
- Fig. 5. Mucosal folds in isthmic portion near AIJ.
- Fig. 6. Mucosal folds in central portion of isthmus.
- Fig. 7. Longitudinal mucosal folds in fimbriae in SEM. Gauge indicates 100 μm in length.
- Fig. 8. Mucosal folds near the abdominal ostium showing wavy arrangement in SEM. Gauge indicates 100 μm in length.

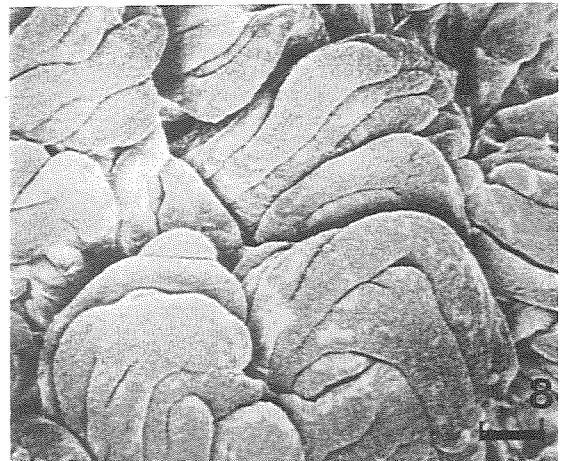
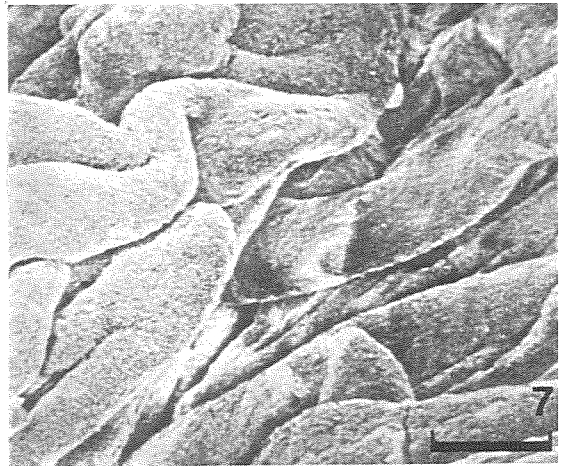
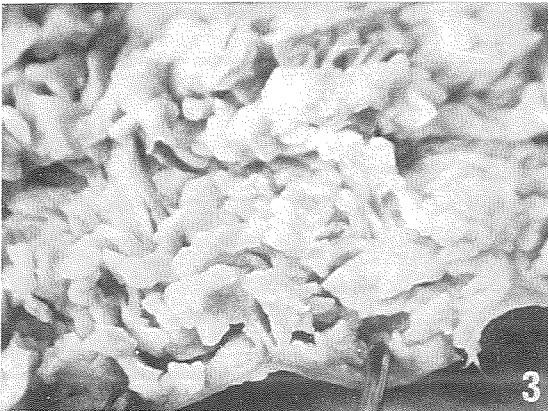
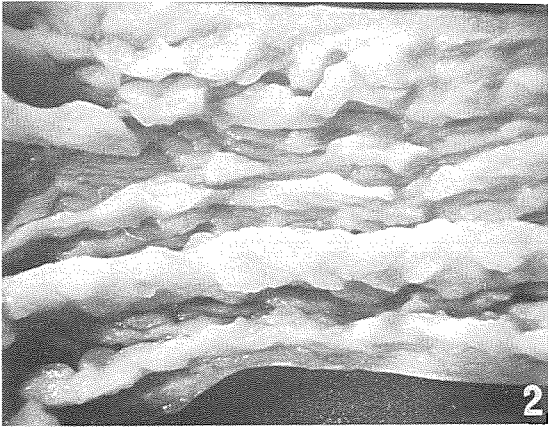
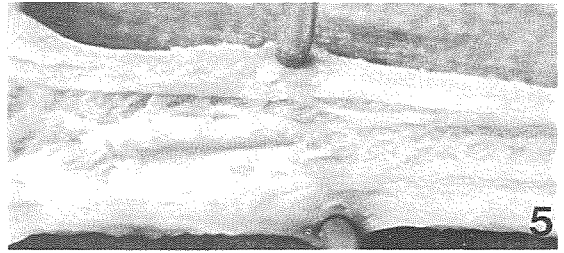
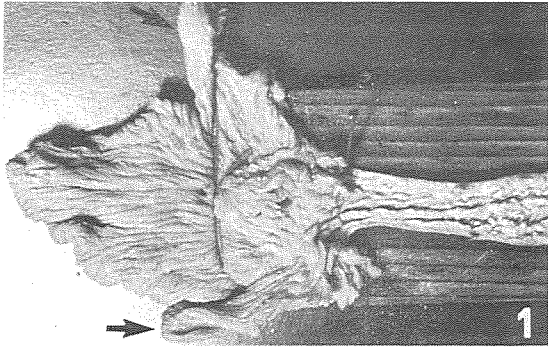


Plate 2

Explanation of figures

- Fig. 9. General view of the surface of the epithelium in infundibulum in SEM. Epithelium consisted of ciliated and non-ciliated secretory cells.
- Fig. 10. *Ruptured plasma membrane (arrow) at an apical portion of the non-ciliated cell in fimbriae. Secretory granules are seen exposed in the cytoplasm.*
- Fig. 11. Large mucosal protrusion arranged in abundance in the ampulla in SEM.
- Fig. 12. Marked formation of secondary folds in the mucosal protrusion in ampulla. Gauge indicates 100 μm in length.
- Fig. 13. Non-ciliated cells with irregular apical surface due to protruding buds of various size.

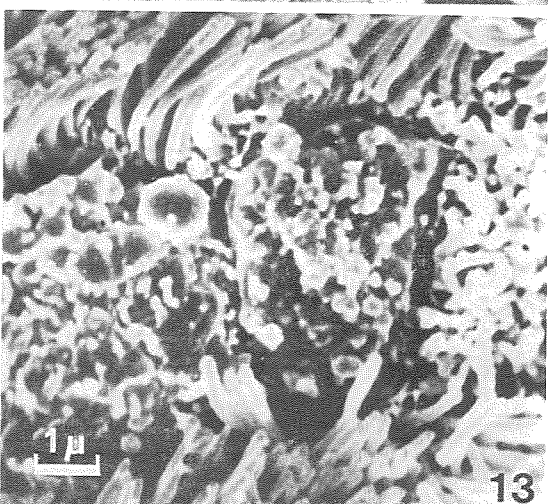
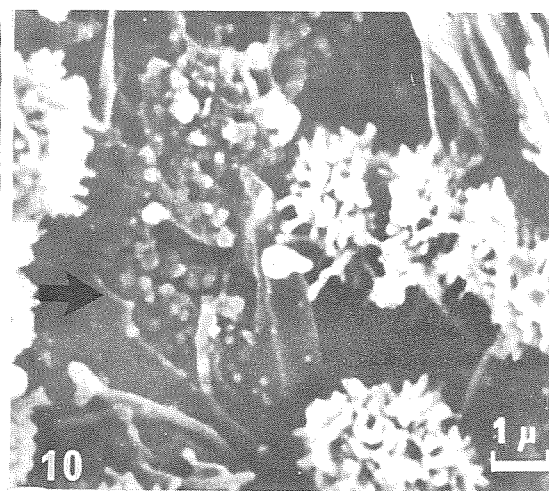


Plate 3

Explanation of figures

- Fig. 14. The surface of non-ciliated cells was slightly more elevated than the usual level (arrow).
- Figs. 15 & 16. Mucosal folds of AIJ in SEM. Mucosal protrusions become small in size and irregular in shape.
- Fig. 17. General view of the surface of the epithelium in AIJ in SEM.
- Fig. 18. Net-like fold pattern of the mucosa in the upper portion of the isthmus, due to intersecting of longitudinal small mucosal folds, in low magnification in SEM.
- Fig. 19. Scanty secondary folds on the surface of the longitudinal folds in isthmus. Gauge indicates 100 μm in length.

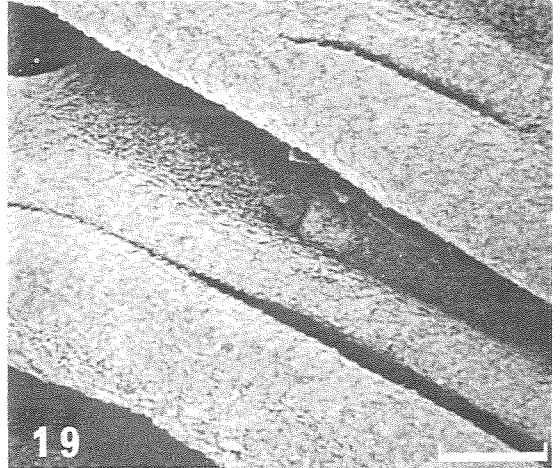
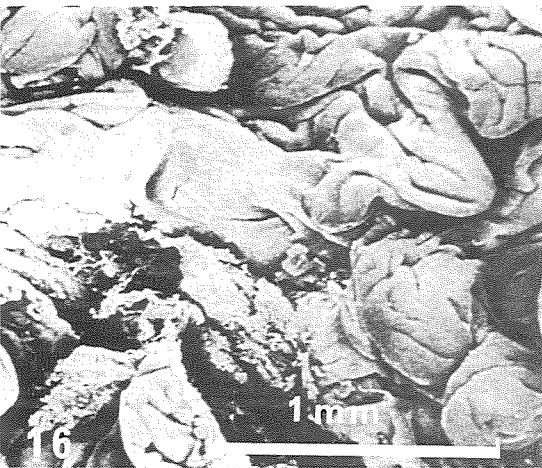
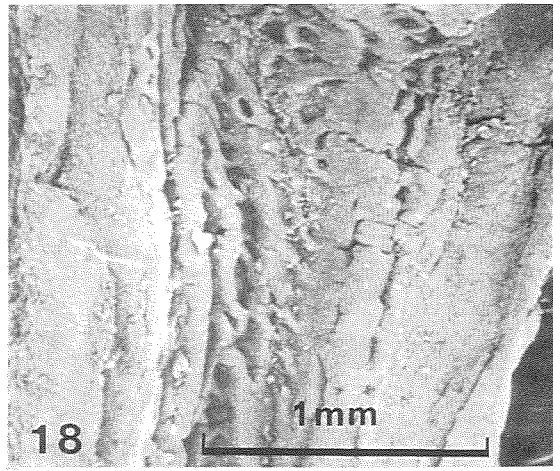
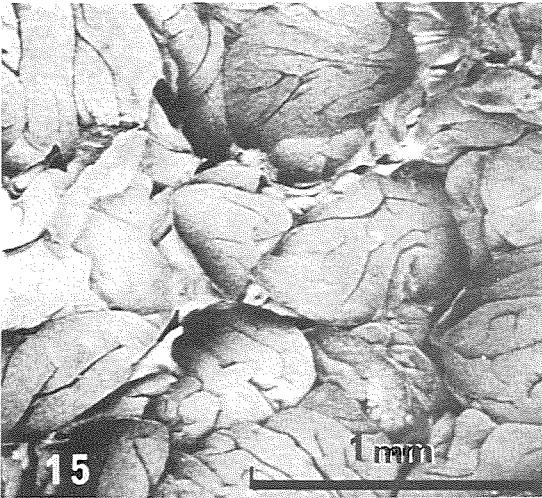
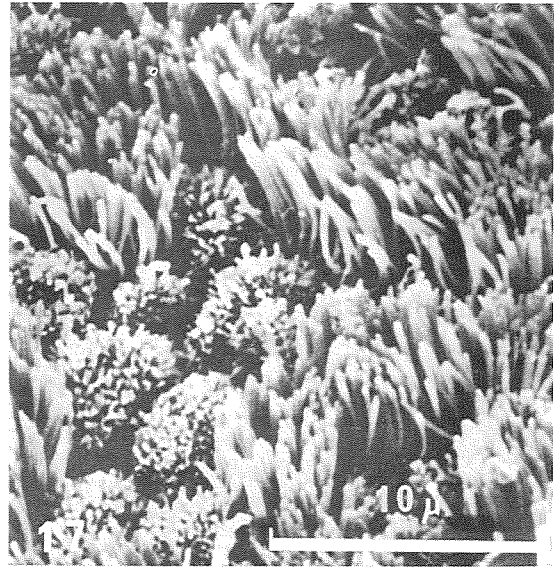
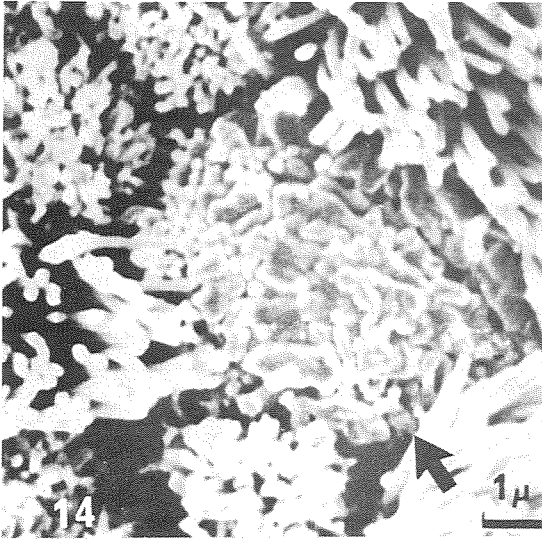


Plate 4
Explanation of figures

- Fig. 20. Mucosal surface of intramural part of oviduct in SEM. Many crypts were noted on the mucosal surface. Gauge indicates 100 μ m in length.
- Fig. 21. General view on the surface of the epithelium in isthmus in SEM.
- Fig. 22. Mucosal folds showing labyrinthine system in cross section of the ampulla in LM. (Bouin fixation, embedded in paraffin, H. E. stain, $\times 32$).
- Fig. 23. Poorly developed mucosal folds and thick muscular coat in cross section of the isthmus in LM. (Bouin fixation, embedded in paraffin, H. E. stain, $\times 32$).
- Fig. 24. Epithelial cells in the isthmus in LM. (Glutaraldehyde-osmium fixation, embedded in epoxy resin, toluidin blue stain, $\times 640$).
- Fig. 25. Epithelial cells in the ampulla in LM. (Glutaraldehyde-osmium fixation, embedded in epoxy resin, toluidin blue stain, $\times 650$).

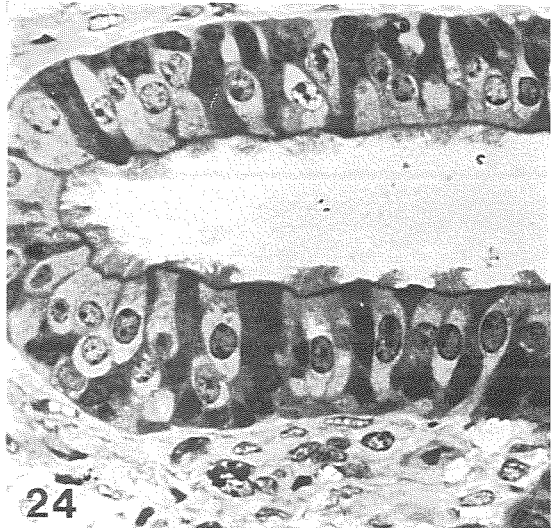
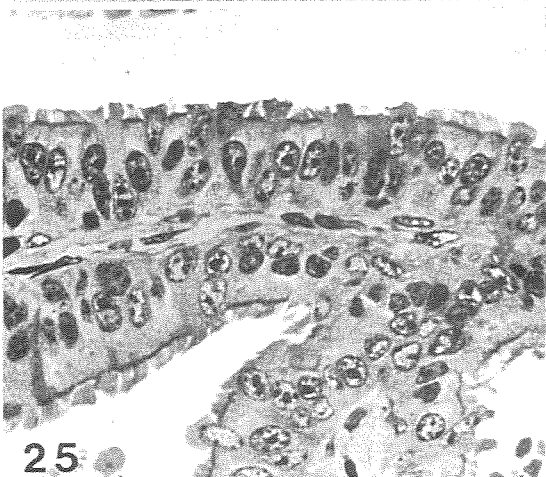
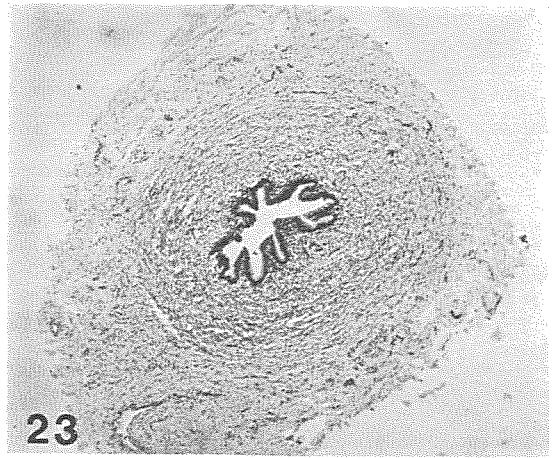
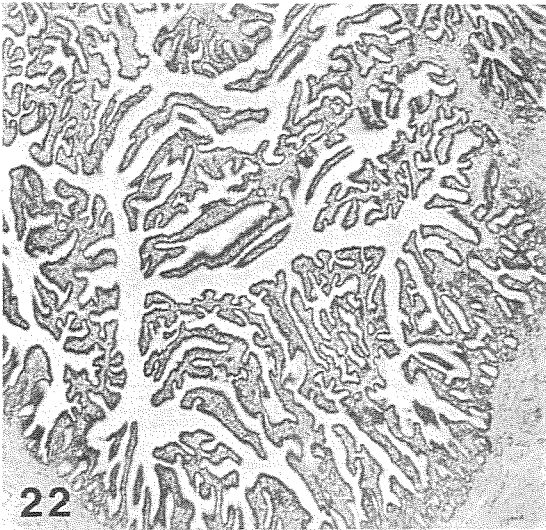
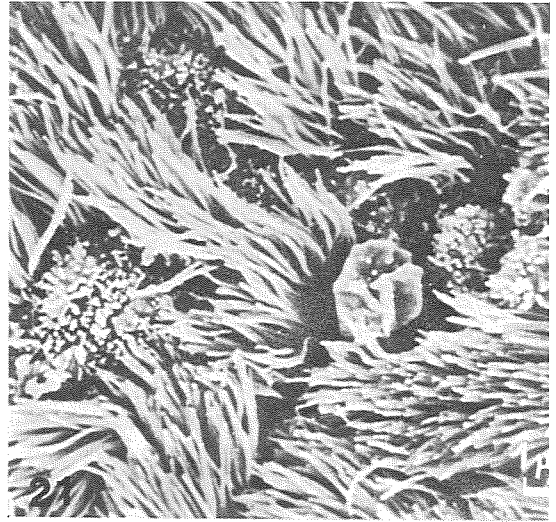
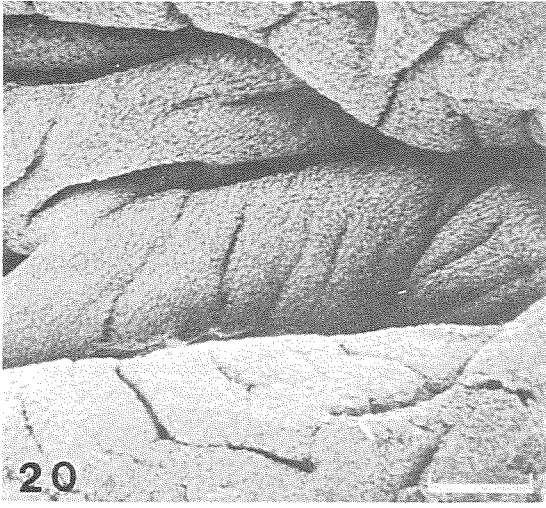


Plate 5

Explanation of figures

- Fig. 26. PAS-stained section of the ampulla, showing secretory activity of the epithelium in LM. ($\times 320$).
- Fig. 27. PAS-stained section of the isthmus, showing high secretory activity of the epithelium in LM. ($\times 320$).
- Fig. 28. Massive material on the oviductal mucosa, connected with the mucosa by a narrow string. After glutaraldehyde-osmium fixation, the mucosa was black and the mass was white in color. ($\times 13$).
- Fig. 29. The mass constructed of swirling bundles of fine fibers in histological cross section. (Glutaraldehyde-osmium fixation, embedded in epoxy resin, toluidin blue stain, $\times 320$).
- Fig. 30. A collagen fibril found in the mass by transmission electron microscopy.
- Fig. 31. A mass formed by many lobules in various size in SEM.

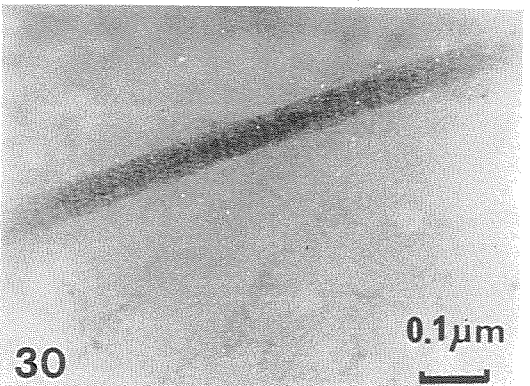
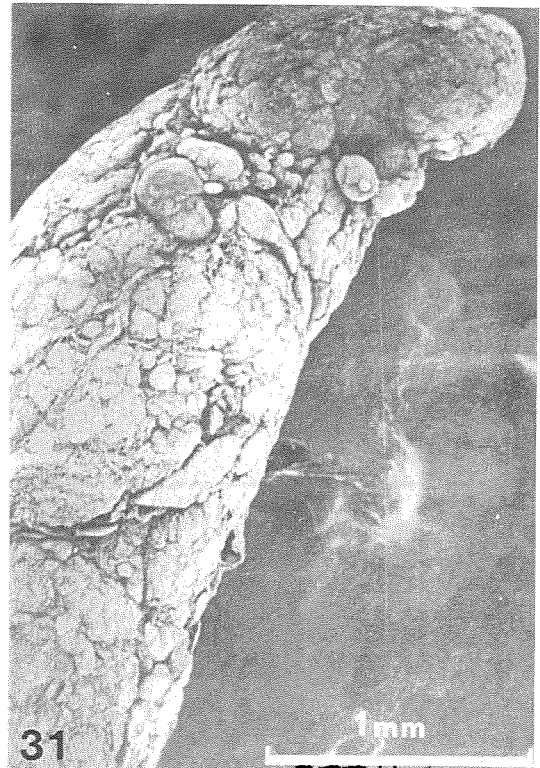
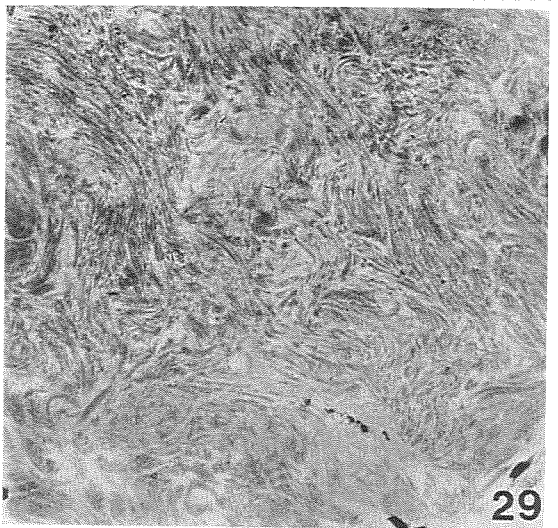
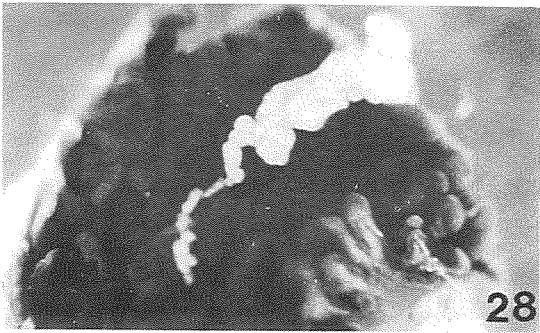
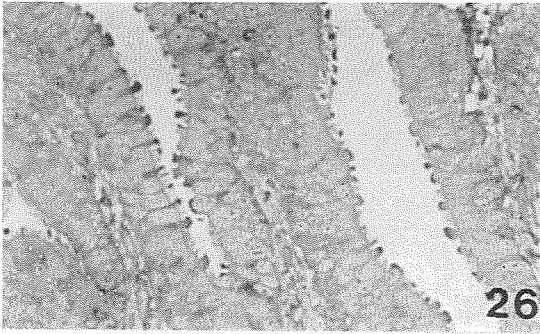


Plate 6
Explanation of figures

- Fig. 32. Surface view of the mass in higher magnification, showing no epithelium and fine fiberous constitution in SEM.
- Fig. 33. Twisted string connecting between the mass and the oviductal mucosa in SEM. Gauge indicates 100 μ m in length.
- Fig. 34. String of the masses penetrating into the mucosa in SEM.
- Fig. 35. Bundle of fibers of a mass, entwining and penetrating into the lamina propria of the oviduct in LM. (Glutaraldehyde fixation, embedded in paraffin, H. E. stain, $\times 160$).

