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Scanning Electron Microscopical Observation on the Mucosal Epithelium of Hens' Oviduct with Special Reference to the Transport Mechanism of Spermatozoa through the Oviduct

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(Figs. 1-15)

The mucosal epithelium of the oviduct of fowls is constructed principally with two types of cells, ciliated and nonciliated cells, through the whole length. The epithelial cells play an important role in the formation and transport of eggs, as well as in the conveyance of spermatozoa through the oviduct. Besides the role of nonciliated cells which are secretory in nature, the ciliary movement of ciliated cells toward the cloaca supports the downward transport of ovulated eggs kinetically, but, in turn, it may hamper the upward progress of the spermatozoa toward the infundibular region, considered to be the site of fertilization. The simultaneous occurrecne of the transport of spermatozoa and that of eggs in opposite directions is a particularly unique mechanism in hens' oviduct, differing markedly from its counterpart in other animals. Taking this specific character of hens' oviduct into account, several workers have investigated the transport mechanism of spermatozoa through the oviduct; yet without satisfying results.

Recently, FERENCZY and RICHART *et al.* (1972[1], 1972[2]), JOHANNISSON and NILSSON (1972[3]), HAFEZ (1972[4], 1973[5]), and PATEK and NILSSON *et al.* (1972[6], 1972[7], 1973[8], 1973[9]) demonstrated the fine three-dimensional structure of the female reproductive tract of mammls, including man, by means of a scanning electron microscope. No such attempts, however, have been made yet to elucidate the structure of the oviduct of fowls, although it has always been a much-disputed problem.

The purpose of this present investigation, therefore, is to examine the delicate structure of the mucosal epithelium of hens' oviduct by scanning electron microscopy. In addition, the mechanism of spermatozoal transportion through the oviduct is studied from a standpoint of the structure of the mucosal epithelium.

MATERIALS AND METHODS

Oviducts of White Leghorn hens in laying were used in this study. Prior to dissection, the hens were injected intravaginally with large amounts of semen (0.3 m*l*). The semen had been collected from healthy cocks by means of abdominal massage. Twenty hours after insemination, the hens were bled to death. Uninseminated hens served as controls. The oviducts were taken out as a whole mass by laparotomy. Small tissue blocks were collected from the following organs: the infundibulum (middle and posterior parts), the magnum (middle), the isthmus (middle), the uterus (middle), the uterovaginal junction, and the vagina (middle). They were fixed in formalin gas for a short time to inhibit the movements of spermatozoa and kinocilia. Then they were refixed in 2.5 % gultaraldehyde buffered with phosphate (pH 7.4). Fixed sections were dried in the conventional manner by the critical point drying method using CO_2 (type HCP 1). After drying, they were coated with gold and examined by a scanning electron microscope (JSM type U) at an accelerating voltage of 10 KV.

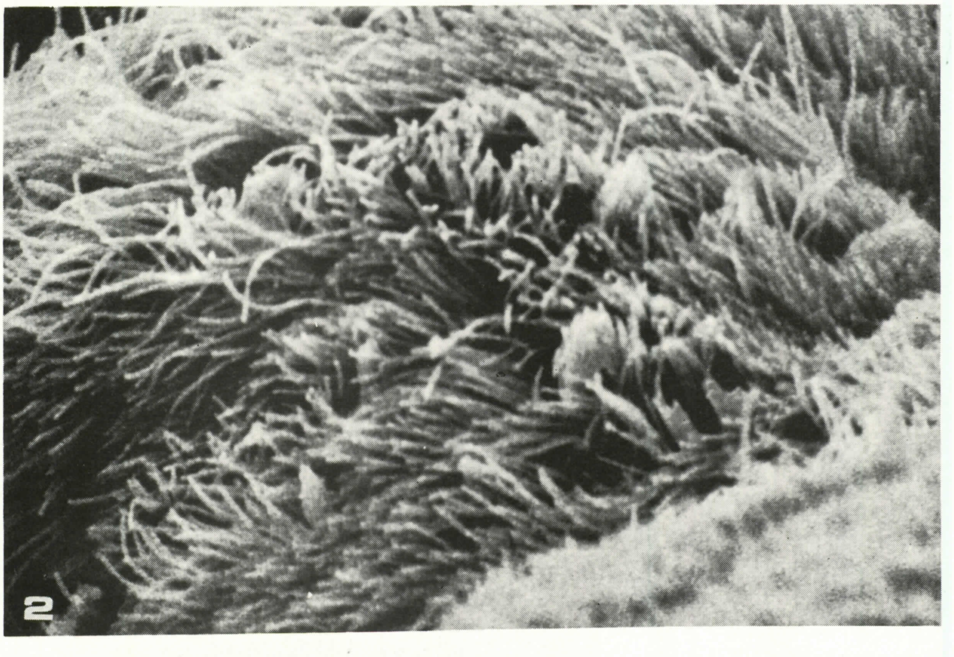
RESULTS

Infundibulum. Figs. 1 and 2 show the luminal surface of the middle part of the infundibulum. As is clear from them, the epithelium consisted almost exclusively of densely-ciliated cells. Nonciliated cells were hardly visible. Thus the epithelial surface looked like a carpet. The cilia of ciliated cells were slender in appearance and budded straight from the cell surface. They were about 2.5 ~ 3.0 μ tall. In a more caudal part of the infundibulum, varying numbers of spermatozoa were found on the ciliary layer (Figs. 3–5). This indicates that the spermatozoa which had been introduced into the vagina prior to the examination had reached and lodged themselves into the infundibular lumen. Spermatozoa were located at the tip of cilia. Some of them had their heads inserted among cilia, probably to get in touch with the surface of the cell. Occasionally, spermatozoa were lodged in groups in the crypts of the mucosal folds, their heads put inside the folds (Fig. 5). These crypts harboring spermatozoa were identical with the infundibular "sperm-nests" which had been found in histological sections of the oviduct by VAN DRIMMELEN (1946[10], 1949[11]) and FUJII and TAMURA (1963)[12]. With the transition to the magnum, the nonciliated cells increased gradually in number (Fig. 4).

Magnum. Fig. 6 shows the luminal surface of the middle part of the magnum. The magnal epithelium was found to be composed of approximately an equal number of ciliated and nonciliated cells, both distributed alternately. Each ciliated cell had tuft-like appearance. The cilia of ciliated cells were slender, but taller and more waving than those in the infundibular region. Nonciliated cells located among ciliated cells were rich in microvilli, protruding high above the free surface of the cells (Fig. 7).

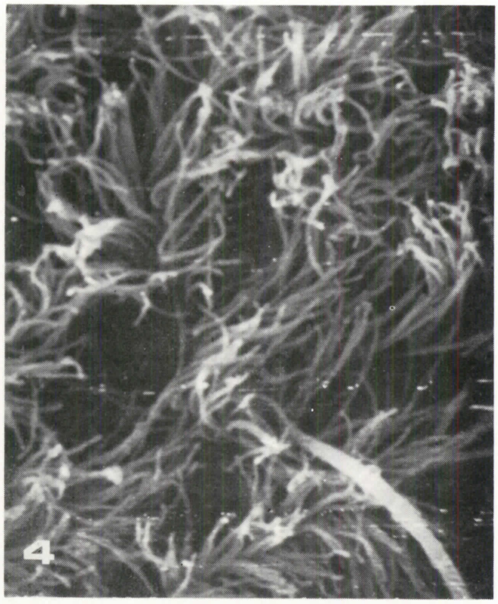
Fig. 1. The mucosal epithelium of the middle part of the infundibulum of the hen's oviduct. Scanning electron micrograph. ×1,000.

Fig. 2. A high-power magnification of the mucosal epithelium shown in Fig. 1. The epithelium is covered by ciliated cells with numerous cilia. ×3,300.



Sometimes, microvilli developed so well that they looked like cilia (Fig. 8). The openings of albumen-secreting glands were found dispersed on the lateral side of longitudinal mucous folds (Fig. 7). There were no ciliated cells around these openings. No spermatozoa were present in the magnum.

Isthmus. Fig. 9 shows the luminal surface of the isthmus. The isthmic epithelium was found to be composed of ciliated and nonciliated cells similar to those of the magnal epithelium. Due to the relatively dense arrangement of cilia, however, the ciliated cells seemed to be more numerous than the nonciliated cells. The cilia were slightly wavy and a little shorter in the isthmus than those in the magnum. No spermatozoa were present in the isthmus.



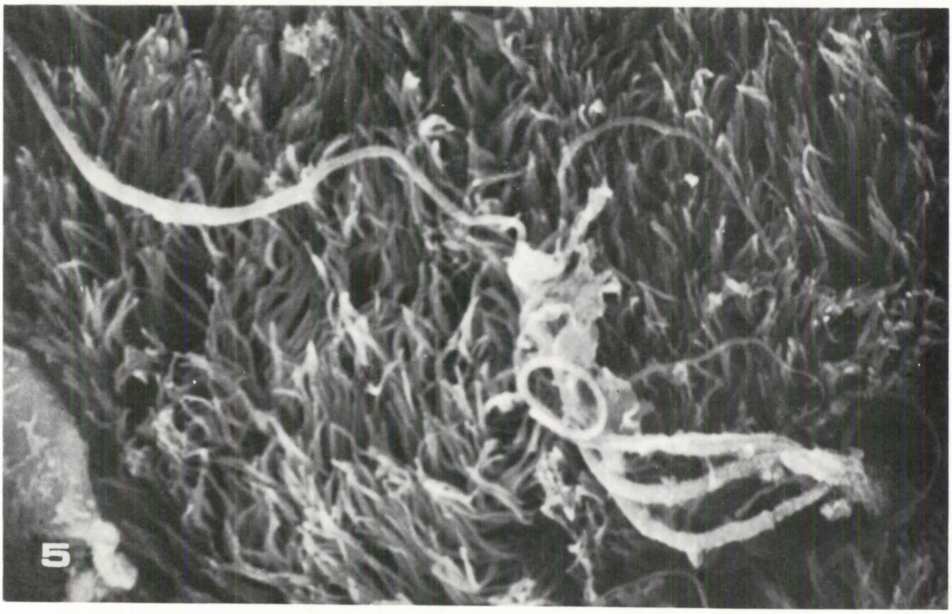
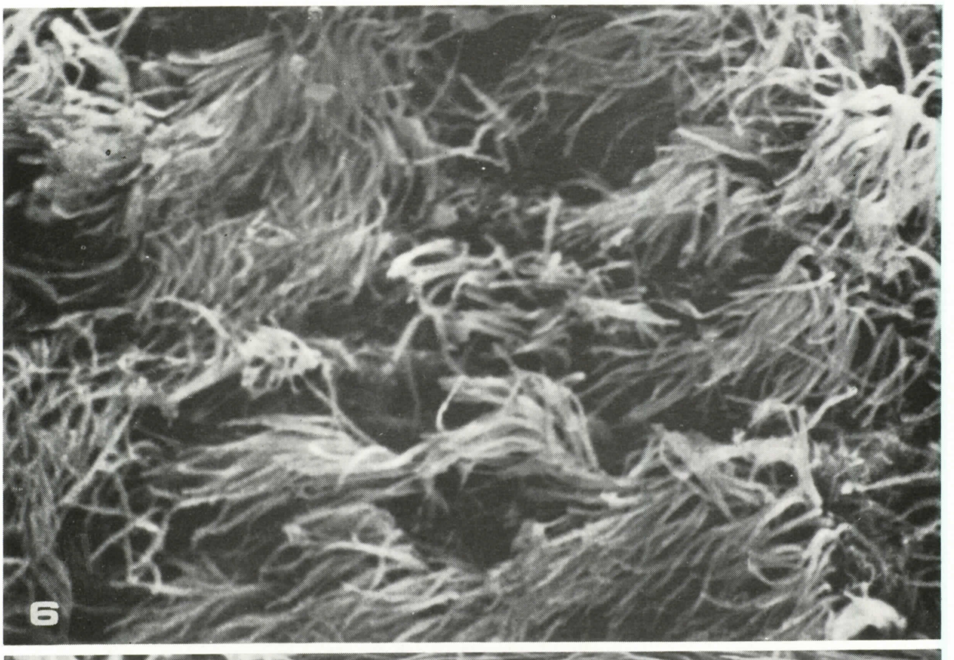


Fig. 3. A spermatozoon located on the mucosal surface of the middle part of the infundibulum, with its head inserted among cilia. × 2,200.

Fig. 4. The mucosal epithelium of the junctional region between the infundibulum and the magnum. It is covered with approximately equal numbers of ciliated and nonciliated cells. × 3,300.

Fig. 5. Some spermatozoa located on the mucosal surface of the posterior infundibulum. Note a group of spermatozoa with their heads inserted in the crypt of the mucosa. × 3,300.

Uterus. With the transition to the uterus, the oviduct abruptly became a dilated pouch covered with numerous leaf-like papillae. Fig. 10 shows the luminal surface of the uterus. The uterine epithelium consisted of an approximately equal number of ciliated and nonciliated cells. Both cells showed almost the same proportions as those in the magnum. Accordingly, the appearance of the uterine epithelium closely resembled that of the magnal epithelium. The cilia of the ciliated cells were tall and wavy like those in the magnum. The nonciliated cells had few microvilli. No spermatozoa were found in this region.



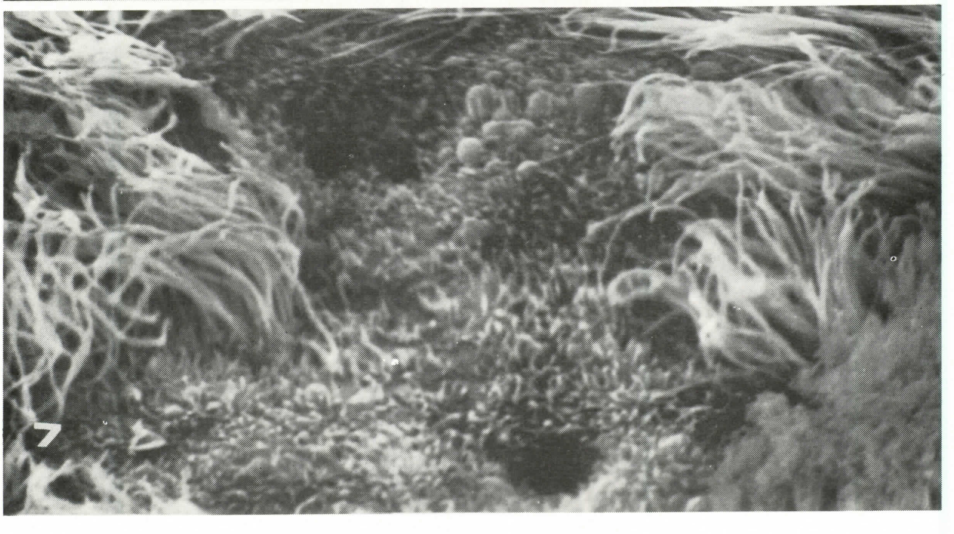


Fig. 6. The mucosal epithelium of the middle part of the magnum. Note ciliated cells with relatively tall, wavy cilia. × 3,300.

Fig. 7. The openings of the albumen-secreting gland of the magnum. Note the lack of ciliated cells and the presence of nonciliated cells riched in microvilli around the openings. × 3,300.

Fig. 8. Nonciliated cells with tall microvilli in the magnum. × 6,000.

Fig. 9. The mucosal epithelium of the middle part of the isthmus. It is covered with predominant ciliated cells. × 6,000.

Uterovaginal junction. The uterus becomes a narrow tube, connected with the uterovaginal junction. This junctional region between the uterus and the vagina is about 1 cm long. It may be distinguished externally by its S-shaped tube. The papillae of the uterine mucosa change gradually into low longitudinal folds.

Figs. 11 and 12 show the luminal surface of the uterovaginal junction. As it clearly appears from these figures, the epithelium is composed of ciliated cells alone, and its appearance is essentially the same as that of the infundibular epithelium. Each ciliated cell projects numerous straight cilia. The epithelial surface looks like a carpet. It can be observed that the uterovaginal lumen as well as the infundibular lumen contains a large number of spermatozoa (Figs. 13 and 14). The spermatozoa are lodged on the surface of the ciliary layer, and most of them have their heads inserted among cilia. Strangely, they are deformed into a neck-bending shape, although it was impossible to distinguish immotile dead spermatozoa from motile living ones. Fujii and Tamura (1963[12], 1964[14]) and Bobr and Lorenz *et al.* (1964)[13] found that the mucosa of the uterovaginal junction had specific tubular glands (called vaginal glands by Fujii and sperm-nests glands by Bobr), which harbored large numbers of spermatozoa for a long time. Although no whole glands were discovered in any section examined, their openings presented themselves in Fig. 12, as hollow crypts among the cilia.

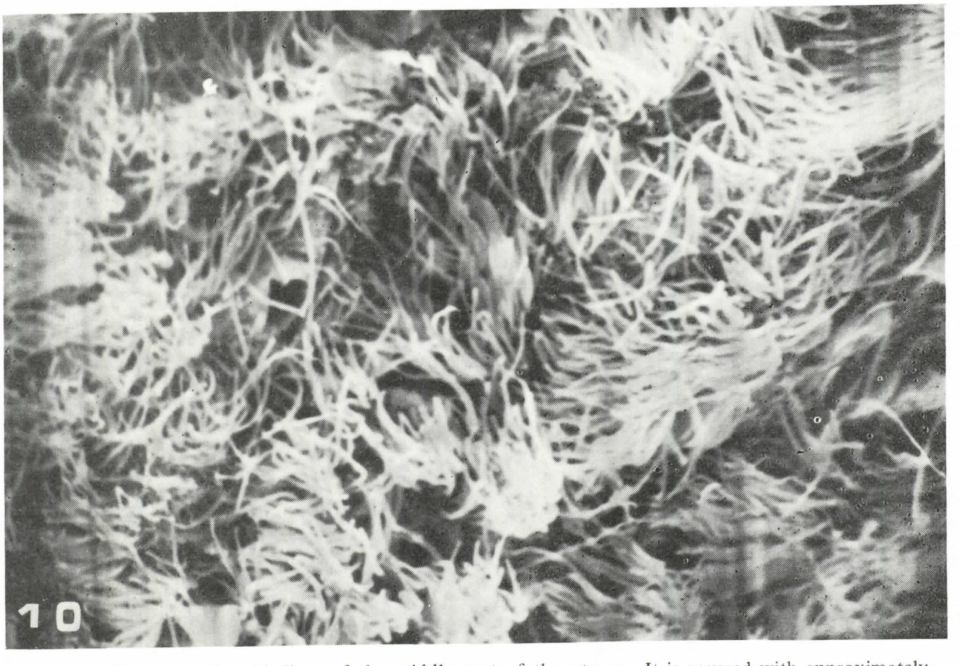


Fig. 10. The mucosal epithelium of the middle part of the uterus. It is covered with approximately equal numbers of ciliated and nonciliated cells. Ciliated cells have numerous, wavy cilia. × 3,300.

Vagina. Fig. 15 shows the luminal surface of the vagina. The vaginal epithelium was composed of ciliated and nonciliated cells. From the disposition of cilia, ciliated cells seemed to be larger in number than nonciliated cells, just as in the isthmic region. The cilia of ciliated cells were straight and relatively low. No spermatozoa were found in any vaginal section observed.

DISCUSSION

The present scanning electron microscopy of hens' oviduct revealed clearly the delicate three-dimensional structure of the mucosal epithelium. The epithelium of the

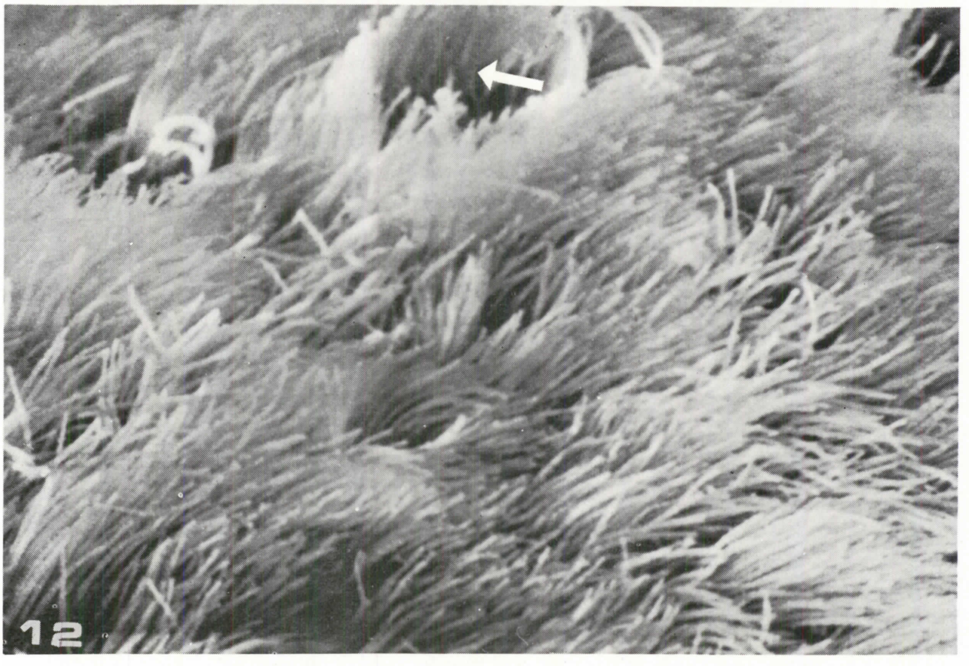
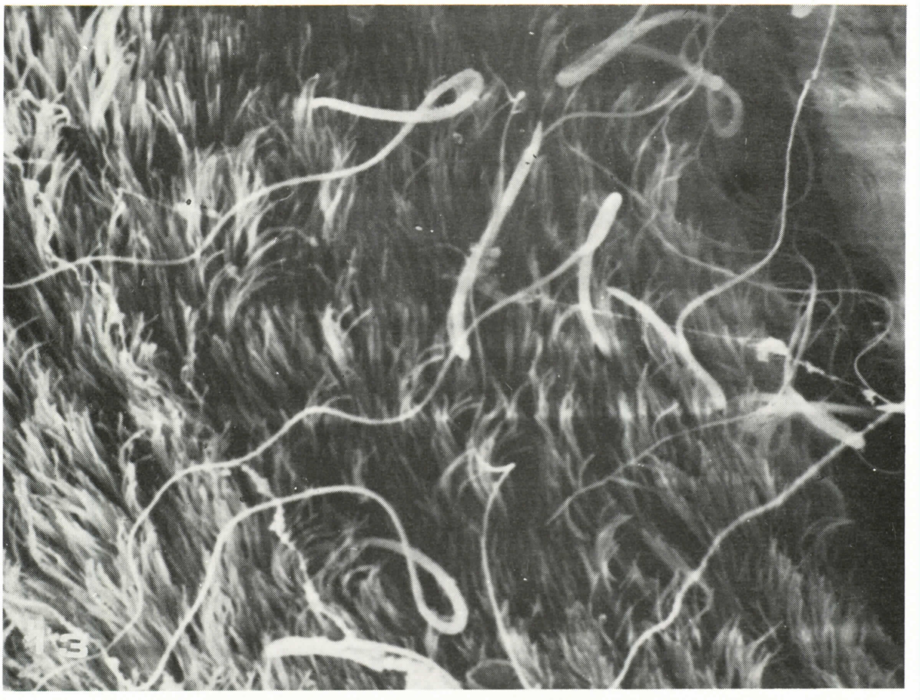


Fig. 11. The mucosal epithelium of the uterovaginal junction. × 1,000.

Fig. 12. A high-power magnification of the mucosal epithelium shown in Fig. 11. It is covered only with ciliated cells with numerous, straight, relatively low cilia. An arrow indicates the opening of the vaginal gland. × 3,300.

oviduct was constructed uniformly by ciliated and nonciliated cells over the whole length, although the distributional proportion and structural appearance of both cells varied considerably from one region of the oviduct to the other. The mucosal epithelium of the oviduct can be tentatively classified into three types on the basis of the structural pattern of the epithelial surface. The first type is characterized by such distribution of only ciliated cells which have numerous, straight, low cilia as is seen in the infundibulum and in the uterovaginal junction. The second type is characterized by such distribution of approximately equal numbers of nonciliated and ciliated cells that have tall and wavy cilia as is seen in the magnum and uterus. The third type is characterized by such predominant



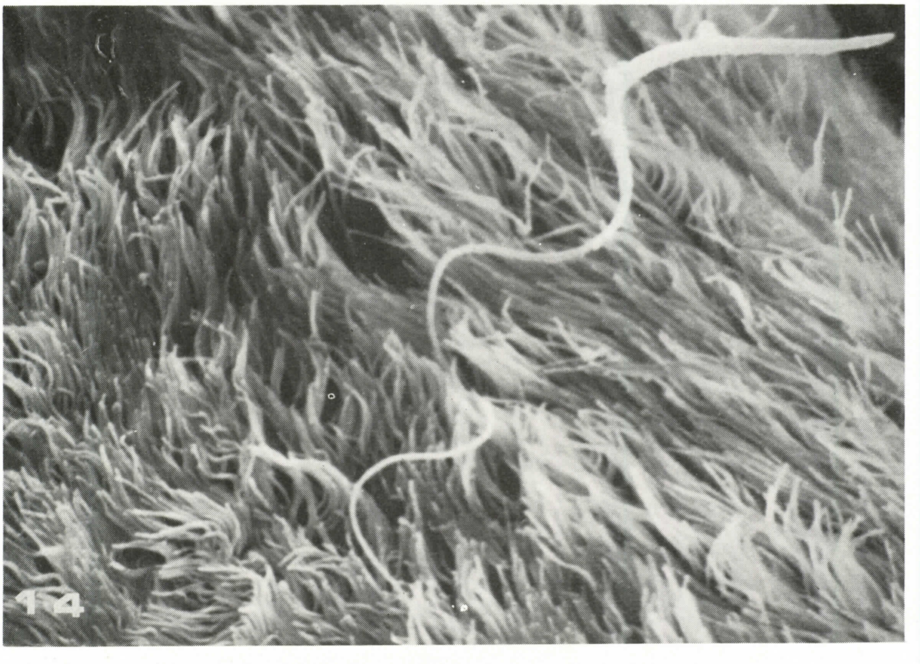


Fig. 13. Spermatozoa located on the mucosal surface of the uterovaginal junction. Most of them are deformed into a neck-bending shape. Each spermatozoon inserts its head among cilia. × 2,200.

Fig. 14. A spermatozoa located on the mucosal surface of the uterovaginal junction. × 3,300.

distribution of ciliated cells which have somewhat waving low cilia, as is seen in the isthmus and vagina. In the present investigation, it is of interest to note that the mucosa of the infundibulum closely resembled to that of the uterovaginal junction in the epithelial structure, and that both organs contained similarly varying numbers of spermatozoa.

Fig. 15. The mucosal epithelium of the middle part of the vagina. It is covered with ciliated and non-ciliated cells, the former being predominant. Cilia appear low and straight. ×3,300.

The mechanism for the travel of spermatozoa through the oviduct in hens has been studied in detail by MIMURA (1939[15], 1941[16]). The same author (1957[17], 1958[18]) also examined the distribution of spermatozoa within the oviduct by preparing smear specimens at different intervals after insemination. Similar findings were obtained by TAKEDA (1964)[19]. ALLEN and GRIGG (1957)[20] used spermatozoa with ^{32}P to examine the transport mechanism through the oviduct and reported that the juncture of the vagina and the uterus seemed to constitute a barrier against spermatozoal progress.

TAKEDA (1974)[21] carried out an experiment on the spermatozoal transport through the oviduct, using living and dead fowl spermatozoa, living heterogeneous spermatozoa, and methylene blue dye. He concluded that the uterovaginal junction proved to have a "spermatozoa-selecting function", since only living fowl spermatozoa was allowed to pass into the uterus. All these findings suggest that both regions, the infundibulum and the uterovaginal junction, may have some retention mechanism, or barrier system, to prevent spermatozoa from moving further through the oviduct.

As mentioned above, the presence of a "sperm nest" in the infundibulum and the uterovaginal junction has been demonstrated by histological observation of the oviduct. The present scanning electron microscopy also revealed that varying numbers of spermatozoa were harbored in both regions of the oviduct. Yet the problem of how spermatozoa are located selectively in these regions remains unsolved.

As is well known, the cilia of ciliated cells of the oviduct beat the cloaca from the infundibular region and make the luminal fluid flow. PARKER (1930)[22] found two types of ciliary movement, upward and downward, in pigeons' oviduct. MIMURA (1937)[23] also observed similar movements in the magnal epithelium of hens' oviduct. HAFEZ and KANAGAWA (1972)[24] found in mammals' oviduct that the ciliary movement was accelerated by administration of sexual hormones, and stated that cilia seemed to vary in sensitivity to changes in the blood level of estrogen-progesterone. According to BORELL and NILSON *et al.* (1957)[25], the frequency of the ciliary movement was about 1,500 per minute in rabbits' fallopian tube.

From these findings, we may conclude that for sexually matured hens, the ciliary movement is more intense in the infundibulum and the uterovaginal junction than in any other region of the oviduct. Accordingly, a barrier system against the spermatozoal transport, probably present in the infundibulum and the uterovaginal junction, may be mainly responsible for the ciliary movement of the luminal surface, although such factors as the contractile activity of the wall of the oviduct, the biochemical properties of mucin, and the innate directional motility of spermatozoa may be related to spermatozoal conveying. The strong downward ciliary movement of the uterovaginal junction prevents foreign bodies and dead spermatozoa from invading the uterus. At the same time, however, it retards the upward progress of the living spermatozoa. As a result, the ciliary movement expels foreign bodies out to the cloaca.

On the other hand, some living motile spermatozoa which have failed to enter the uterus immigrate passively into the vaginal glands, where they are retained. The same ciliary motion may probably occur in the infundibulum which shows essentially the same structural pattern as the uterovaginal junction. In the infundibulum, the vigorous ciliary movement supports the downward passage of an ovulated egg, but, in turn, it retards the upward progress of spermatozoa. As a result, spermatozoa are prevented from being driven from the infundibular lumen into the abdominal cavity and retained in the crypts of the epithelial folds, which have been called "sperm nests".

In conclusion, a barrier mechanism against the spermatozoal transport through the hen's oviduct, which has been suggested to exist in the infundibulum and the utero-vaginal junction, may be responsible for the vigorous ciliary movement of the luminal surface. Thus the uterovaginal junction of the hen's oviduct may act as barrier, selector, and reservoir of spermatozoa within the oviduct.

SUMMARY

The mucosal epithelium of the oviduct in laying hens was studied by scanning electron microscopy. It was constructed principally by ciliated and nonciliated cells over its whole length. The distribution and appearance of these two types of epithelial cells varied considerably from one region of the oviduct to another. The infundibulum and the uterovaginal junction were characterized by the distribution of only ciliated cells having abundant, somewhat low cilia, different from any other region of the oviduct.

The spermatozoa introduced experimentally into the vagina were retarded and retained selectively in the lumina of these two regions of the oviduct. They were located among the cilia of ciliated cells. A barrier mechanism in the two regions against the spermatozoal transport through the oviduct in hens, which has been suggested by early workers, appeared to be a structural characteristic of the mucosal epithelium. The strong ciliary movement of the two regions may act as a simple mechanical barrier against the upward progress of spermatozoa and serve to make a "sperm nest" of these regions.

REFERENCES

1) FERENCZY, A., RICHART, R. M., AGATE, F. J., PURKERSON, M. L., and DEMSEY, E. W.: *Science*, **175**, 783–784 (1972).
2) ——— : *Fertility and Sterility*, **23**, 515–521 (1972).
3) JOHANNISSON, E. and NILSSON, L.: *Ibid.*, **23**, 613–625 (1972).
4) HAFEZ, E. S. E.: *J. Reprod. Fertil.*, **30**, 613–625 (1972).
5) ——— : *Am. J. Obstet. Gynecol.*, **15**, 703–717 (1973).
6) PATEK, E., NILSSON, L., and JOHANNISSON, E.: *Fertility and Sterility*, **23**, 459–465 (1972).
7) ——— : *Ibid.*, **23**, 719–733 (1972).
8) PATEK, E., NILSSON, L., JOHANNISSON, L., HELLEMA, M., and BOUT, J.: *Ibid.*, **24**, 31–43 (1973).
9) PATEK, E. and NILSSON, L.: *Ibid.*, **24**, 819–831 (1973).
10) VAN DRIMMELEN, G. C.: *J. S. A. Vet. Med. Assoc.*, **17**, 42–52 (1946).
11) ——— : *Nature*, **163**, 950–951 (1949).
12) FUJII, S. and TAMURA, T.: *J. Fac. Fish. Anim. Husb. Hiroshima Univ.*, **5**, 145–163 (1963).

13) BOBR, L. W., LORENZ, F. W., and OGASAWARA, F. X.: *J. Reprod. Fertil.*, **8**, 39–47 (1964).
14) FUJII, S.: *Arch. histol jap.*, **23**, 447–459 (1963).
15) MIMURA, H.: *Okajimas Folia Anat. Japon.*, **17**, 459–476 (1939).
16) ——— : *J. Dept. Agric. Kyushu Imp. Univ.*, **6**, 167–259 (1941).
17) ——— : *Jap. J. Zootech. Sci.*, **28**, Suppl., 22–23 (1957). (In Japanese.)
18) ——— : *Ibid.*, **29**, Suppl., 7 (1958). (In Japanese.)
19) TAKEDA, A.: *Japan Poultry Sci.*, **1**, 19–31 (1964). (In Japanese with English summary.)
20) ALLEN, T. E. and GRIGG, G. W.: *Aust. J. Agric. Sci.*, **8**, 787–799 (1957).
21) TAKEDA, A.: *Japan Poultry Sci.*, **11**, 45–54 (1974). (In Japanese with English summary.)
22) PARKER, G. H.: *Proc. Soc. Exp. Biol. Med.*, **27**, 704–706 (1930).
23) MIMURA, H.: *Okajima Folia Anat. Japon.*, **15**, 287–295 (1937).
24) HAFEZ, E. S. E. and KANAGAWA, H.: *J. Reprod. Fertil.*, **28**, 91–94 (1972).
25) BORELL, U., NILSSON, O., and WESTMAN, A.: *Acta. obstet. gynec. Scand.*, **36**, 22–28 (1957).

鶏の卵管粘膜上皮の走査電子顕微鏡による観察，とくに卵管内の精子移動の関連において

藤　井　俊　策

鶏の卵管粘膜上皮の構造と，これに関連して卵管内の精子移動の機構を，走査電子顕微鏡を用いて形態的に観察した。

卵管の粘膜上皮は，卵管の全長にわたって線毛細胞と非線毛細胞の２種の細胞から構成されていた。しかしこの両細胞の細胞形態，分布状態は，卵管の各部位によってかなり異なっていた。とくに漏斗部と子宮・腟移行部は類似の上皮構造をもっており，ここでは非線毛細胞を欠き，線毛細胞のみから成っているのが特徴であった。

一方，実験的に卵管腟部に注入された精子は，漏斗部と子宮・腟移行部に選択的に貯溜する傾向がみられた。この両部への精子の貯溜は，該部の線毛細胞の強力な線毛運動によって精子の前進が阻止されるためと推察された。