## Scanning Electron Microscopic Observation on Ciliated Cells of the Chicken Oviduct in Various Functional Stages

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The mucosal epithelium of the chicken oviduct contains two types of cells, ciliated and nonciliated secretory cells, throughout its entire length, although their distribution varies considerably with the segments of the oviduct<sup>1-3)</sup>. The ciliary motion of the chicken oviduct is considered to play a particular role in the transport of eggs and spermatozoa through the oviduct. Several investigators, including Palmiter and Wrenn (1971)<sup>4)</sup> and Anderson and Hein (1976)<sup>5)</sup>, examined the ciliogenesis of the oviductal epithelium. They made studies, however, mainly on the morphogenesis of cilia in a limited portion of the oviduct.

In the present study, observations were made on the general aspects of ciliated cells in functionally different oviducts by light and scanning electron microscopy. The birds observed were adult hens, pullets in the growing stage, and pullets treated experimentally with estrogen.

#### MATERIALS AND METHODS

All the hens used were of the White Leghorn breed. Observation 1 was carried out to examine the normal aspects of ciliated cells of the active oviduct and to compare these cells with those of the inactive one. For this purpose, three hens in laying and in long-term molting were used. Observation 2 was done to examine ciliation in the growing oviduct of pullets. For this purpose, three oviducts were collected from a group of the same hatching pullets 10, 15, 18, and 20 weeks after hatching. Observation 3 was done to examine the effect of estrogen on ciliation in the oviductal epithelium. For this purpose, 12-week-old pullets were injected subcutaneously with 1 mg/kg of 17  $\beta$ -estradiol in 1 ml of sesame oil every day for 5 or 10 days, and they were killed 12 hours after the last injection.

All the oviducts examined were removed from the infundibulum, magnum, isthmus, uterus, utero-vaginal junction, and vagina, as small blocks of tissue. These blocks were fixed in a 10% formalin and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin for histological examination. Tissues for scanning electron micros-

copy were fixed in a 2.5% glutaraldehyde (pH 7.4), dehydrated in increasing concentrations of alcohol, and dried by the critical-point drying method using  $CO_2$ . The dried specimens were coated with gold and examined by a Hitachi S-410 scanning electron microscope.

#### **FINDINGS**

#### Observation 1. Oviducts of laying and molting hens

The functionally active oviducts of laying hens developed into a maximum size and

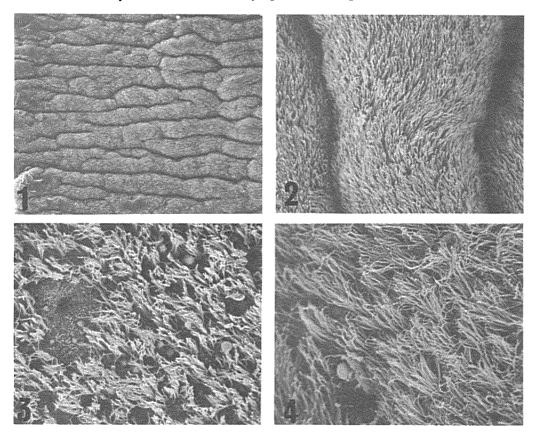
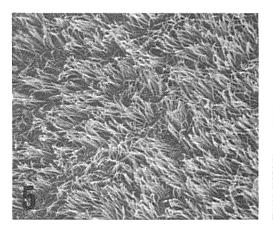


Fig. 1. The general appearance of the infundibular epithelium in a laying hen. The mucosa is thrown into longitudinal folds. x100.

- Fig. 2. High-power magnification of the epithelial surface of the fimbrial lips. It is covered exclusively with ciliated cells. x600.
- Fig. 3. The magnal epithelium in a laying hen. The epithelial surface is covered with about equal numbers of ciliated and nonciliated cells. The opening of the magnal glands is surrounded by nonciliated cells with abundant microvilli. x600.
- Fig. 4. The isthmic epithelium in a laying hen. Ciliated cells are prodominant on the epithelial surface. x600.



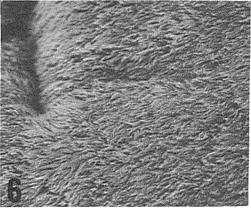
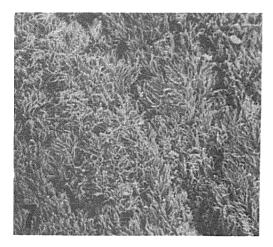


Fig. 5. The uterine epithelium in a laying hen. Ciliated and nonciliated cells are arranged alternately on the epithelial surface. Nonciliated cells located among ciliated cells are rich in microvilli. x600.

Fig. 6. The epithelium of the utero-vaginal junction in a laying hen. The epithelial surface is covered exclusively with ciliated cells. x600.

length with marked tortusity. The mucosa of the funnel region of the infundibulum was thrown into low folds running somewhat longitudinally (Fig. 1). The mucosal epithelium extended over the fimbrial lips towards the outer peritoneal side for a width of about 1.5-2 mm. Both inner and outer fimbrial epithelia were composed of ciliated cells alone, and no nonciliated cells were present (Fig. 2). The cilia of ciliated cells appeared to be long and erect. Converging to the neck region, ciliated cells gradually disappeared and, in turn, nonciliated cells showed up. At the basis of the infundibulum, the mucosal epithelium was composed of approximately equal numbers of ciliated and nonciliated cells, except the one at the bottom of the grooves between folds.



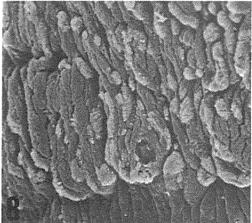
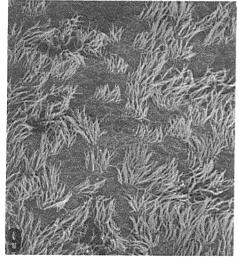
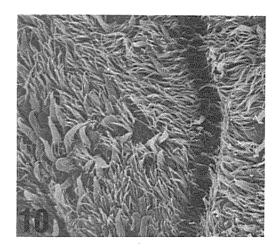


Fig. 7. The vaginal epithelium in a laying hen. Ciliated cells are predominant on the epithelial surface. x600.

Fig. 8. The external mucosa of the infundibulum in a sexually inactive molting hen. The epithelium shows a large denuded surface by losing cilia. x100.





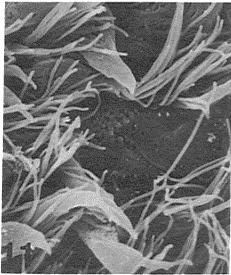


Fig. 9. The uterine epithelium in a molting hen. There are markedly deciliated cells and hexagonal cells remaining with few protrusions. x600.

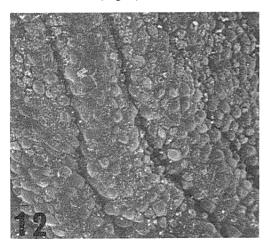
Fig. 10. Degenerative ciliated cells in the isthmic region of the oviduct of a molting hen. Their cilia have been pasted together to take the shape of a painting brush. x600.

Fig. 11. High-power magnification of the area shown in Fig. 10. A deciliated cell remains to have pectinated protrusions. x4,000.

The magnal mucosa was made of tall and wide longitudinal folds. The epithelial surface was lined alternately by ciliated and nonciliated cells similar to those of the caudal infundibulum. The areas surrounding the openings of the magnal glands were devoid of ciliated cells. The nonciliated cells among the ciliated cells were rich in microvilli (Fig. 3).

The isthmic mucosa was thrown into longitudinal folds narrower than those of the magnum. The epithelial surface was covered with ciliated and nonciliated cells, the ciliated cells appearing to be predominant in number (Fig. 4). The uterine mucosa was composed of numerous leaf-shaped folds. The epithelial surface was lined with ciliated and nonciliated cells of almost the same proportion. The cilia appeared slender and wavy. Nonciliated cells had abundant microvilli on the apical surface (Fig. 5). The

epithelial surface of the utero-vaginal juncture was occupied exclusively by ciliated cells (Fig. 6). The vaginal mucosa was composed of tall and narrow longitudinal folds, forming secondary and tertiary folds. The epithelial surface appeared to be covered with more ciliated cells than nonciliated cells, although the grooves between folds were devoid of ciliated cells (Fig. 7).



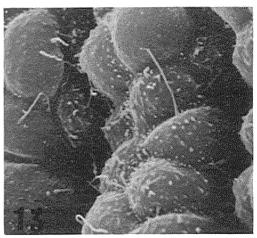


Fig. 12. The infundibular surface in a 10-week-old pullet. The surface is covered with epithelial cells with only a few protrusions and looks like a dome. x600.

Fig. 13. Epithelial cells with a single cilium seen in the magnal portion in a 10-week-old pullet. x4,000.

The inactive oviducts of molting hens were markedly reduced in size and length, but retained their basic structure. The atrophic oviducts had undergone extensive alterations in the ciliated epithelium. The ciliated cells that covered the epithelial surface were remarkably reduced in number to varying degrees throughout the whole length of the oviduct, except the vaginal portion. The reduction in number of ciliated cells, as a whole, was found in about one-half or more of the fully differentiated epithelium of the active oviduct. A very conspicuous deciliation occurred in the infundibular region, particularly on its external epithelial surface. In this region, the completely ciliated epithelium had lost its cilia in group and its denuded surface only remained (Fig. 8). Degenerative ciliated cells had more sparse and wavy cilia than the normal ones (Fig. 9). The cilia in process of degeneration often changed in shape and looked like a painting brush as a whole (Fig. 10). Deciliated cells occasionally remained in pectinated protrusions showing the pinching off of cilia on the free surface (Fig. 11). The nonciliated cells among the ciliated ones had a smooth surface with a few short microvilli. Unlike the above-mentioned segments, the utero-vaginal juncture had an epithelium appearing only slightly deciliated. No deciliation was seen in the vaginal epithelium.

### Observation 2. Oviducts in growing pullets

The oviducts of 10-week-old pullets were so slender and straight, measuring about 7—10 cm in length, that the different segments of the oviduct were hardly distinguishable. The mucosal epithelium at this stage was generally covered with smooth-surfaced cells showing a dome-like appearance (Fig. 12). Frequently, a single stubby cilium was located at the center of the apical surface (Fig. 13). In some instances, a few ciliated cells were present sparsely in the regions which were to develop into the magnum and uterus (Fig. 14). These newly ciliated cells had short and tangled cilia. While the vaginal epithelium at the caudal end of the oviduct was already ciliated to a considerable extent (Fig. 15). In this region, ciliation was seen in about 50% of the whole surface of the fully differentiated vagina.

In 15-week-old pullets, the oviducts were almost the same in size as in the 10-week-old ones, but the mucosal epithelium became more ciliated in the magnum, isthmus, and uterus than in those pullets. The developing oviductal epithelia had growing cilia of various stages. Nonciliated cells among the ciliated cells presented a bulbous appearance (Fig. 16). The ciliated cells at this stage spread over about 50% of the epithelial surface of the magnum, isthmus, and uterus and to a lower degree in the cranial infundibulum than in that of the active oviduct, but were as dense in the vagina as in the active oviduct.

In 18-week-old pullets, the oviducts were developed considerably in size, measuring about 30 cm in length. Each segment of the oviduct became clearly distinguishable. Ciliation progressed rapidly in the epithelium (Fig. 17). It extended over about 80% of the surface of the magnum, isthmus, and uterus and over  $20 \sim 50\%$  of the surface of the infundibulum of the active oviduct. The external epithelium of the fimbria began to be ciliated. By 20 weeks of age, ciliation became so dense in all the oviductal epithelia that it was comparable to that of the active oviduct.

Observation 3. Oviducts injected with estrogen

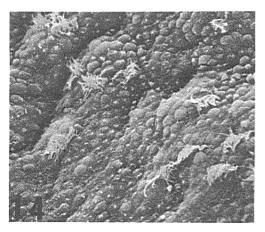
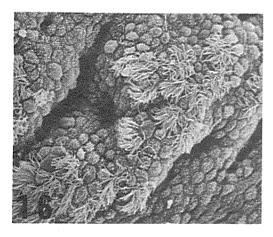




Fig. 14. The uterine epithelium of a 10-week-old pullet. Cells with growing cilia are scattered. The cilia are short and tangled. x600.

Fig. 15. The vaginal epithelium of a 10-week-old pullet. Ciliation has occurred in a considerable extent of the epithel. x100.



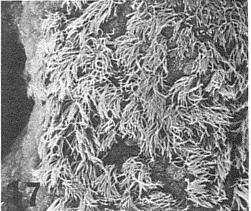


Fig. 16. The magnal epithelium of a 15-week-old pullet. The epithelial cells begin to be ciliated actively. x600.

Fig. 17. The uterine epithelium of a 18-week-old pullet. Ciliation in the epithel progresses remarkably. x600.

When injected daily with 17  $\beta$ -estradiol for five days, the oviducts of 12-week-old pullets underwent dramatic alterations in size and structure of the mucosal epithelium. They were so enlarged to about 20 cm in length that they were two times as large as those of the controls and the same in size as those of about 16-week-old pullets. The mucosa became complex to show remarkable proliferative figures in histological examination. Many mitotic figures of cells were observed in the oviduct. The epithelial surface was ciliated markedly throughout the oviduct, although the density of ciliated cells differed from one part to another. The ciliated epithelium consisted of various types of cells possessing fully formed or growing cilia. Nonciliated cells became bulbous

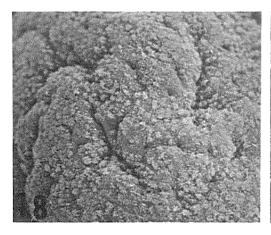




Fig. 18. The magnal epithelium of a 12-week-old pullet treated with 17  $\beta$ -estradiol for five days. Ciliation in the epithel progresses remarkably. x100.

Fig. 19. High-power magnification of the area shown in Fig. 18. The epithelial surface is intermingled with ciliated cells of growing cilia of various types and nonciliated cells showing a bulbous appearance. x600.

with abundant microvilli (Figs. 18 and 19). The density of ciliated cells on the epithelial surface was about 20–50% in the active oviduct, and only about 20% in the infundibulum. When given estradiol for ten days, the oviducts became hypertorphic markedly to be 40 cm in length, or the same size as those of about 20-week-old-pullets. In all the segments of the oviduct, the density of ciliation reached a maximum by this treatment.

#### DISCUSSION

The present findings on the aspects of ciliated cells in the functionally different oviducts are summarized in Table 1.

Portion Oviduct	Infundibulum	Magnum	Isthmus	Uterus	Utero- vaginal junction	Vagina
10 weeks old	<del>-</del>	-~ ±	_	-~±	+	++
15 weeks old	+	++	++	++	1-1-1	++++
18 weeks old	+~++	+++ ~ ++++	+++ ~ ++++	+++~+++	++++	++++
Inactive oviduct of molting hens	+	+	++	++	+++	+++~++++
5th day of estro- gen treatment	+~++	<del>.1 1</del>	++	+++	+++	+++
10th day of estogen treatment	+++~+++	+++~+++	+++~+++	, <del>1111</del>	++++	1111

Table 1. General aspects of ciliation of the oviductal epithelium in various functional phases.

Key to signs.  $-\sim \pm$ , no or slight: +, 20%: +++, 50%: ++++, 80%: +++++, 100% of fully ciliated epithelium of active oviducts.

It appears from the table that ciliation in the chicken oviduct, on the whole, may begin at 10 weeks of age and be completed at 20 weeks of age before the onset of laying. According to KAR (1947)<sup>6</sup>), the growth of the oviduct after hatching is slow and progressive up to 20 weeks of age. Thereafter, it elongates very rapidly. It is interesting to note that ciliation in the oviductal epithelium is accomplished by the end of this slow stage of development.

In the present study, it was found that there were characteristic differences in the pattern of ciliation of the mucosal epithelium among the segments of the oviduct. The first ciliation of the epithelium occurred in the magnum and uterus at about 10 weeks of age, the second one in the isthmus, and the last one in the infundibulum at about 15 weeks of age. As an exception, ciliation seemed to have started in the vaginal epithelium before 10 weeks of age, since it was already noticed at that time. From these results, it is certain that ciliation in the mucosal epithelium tends to progress upwardly from the caudal vagina to the cranial infundibulum. These differences in the mode of ciliation make it possible to assume that the stimulatory effects of ovarian hormones on the oviduct may vary with the segments of this organ.

In some mammals, several investigators<sup>7-10</sup>) have already reported that the epithelial cells of the oviduct, particularly ciliated cells, are under the control of ovarian hormones, and that they undergo marked cyclic changes connected with the sexual cycle. In birds, it is also known that the growth of the oviduct is affected with ovarian hormones<sup>11-14</sup>). On the ciliation in the oviductal epithelium, Palmiter and Wrenn (1971)<sup>4</sup>) reported that estrogens stimulated ciliogensis in the magnal portion of the immature oviduct. Anderson and Hein (1976)<sup>5</sup>) obtained a similar result. They stated that ciliogenesis started in the oviductal epithelium of pullets on the third day of estrogen treatment, and that more cells became ciliated on the tenth day, when ciliation occurred to 55% of the epithelial cells after treatment with 17  $\beta$ -estradiol and to 75% after treatment with diethylstilbestrol.

In the present study, the oviducts of 12-week-old pullets given 17  $\beta$ -estradiol exhibited approximately 50% ciliation on the 5th day and 100% ciliation on the 10th day, as compared with the fully differentiated active oviduct. This result indicates obviously that estrogens play an important role in ciliation and maintenance of ciliated cells in the oviductal epithelium. On the other hand, it was found that the sexually inactive oviducts in molting hens had undergone a marked deciliation in the epithelium of the whole oviduct, except the vaginal portion. Moreover, a most drastic deciliation occurred in the infundibulum, but no deciliation was visible in the vagina. These findings suggest that the response to ovarian hormones may be much lower or none in the vagina than in any other part of the oviduct.

#### **SUMMARY**

The behavior of ciliated cells in the mucosal epithelium of the chicken oviduct was studied by light and scanning electron microscopy. Oviducts were collected from functionally different hens, growing pullets, and estrogen-treated pullets. The findings obtained are as follows.

- 1. In functionally active laying hens, the oviductal epithelium was composed of ciliated and nonciliated cells. Both types of cells appeared to be almost equal in number in the magnum, uterus, and caudal infundibulum. In the isthmus and vagina, ciliated cells were predominant. The cranial infundibulum and utero-vaginal juncture were covered exclusively with ciliated cells.
- 2. In functionally inactive molting hens, the epithelium was deciliated markedly throughout the oviduct, except the vagina. Ciliated cells decreased in density to about one-half, as compared with those of the fully differentiated active oviduct. The vaginal epithelium was atrophic, but not deciliated.
- 3. Ciliation of the epithelium began in the oviduct, except the vagina, at about 10 weeks of age after hatching and was completed at about 20 weeks of age before laying. Ciliation in the vaginal epithelium was already noticed at 10 weeks of age and accomplished by about 15 weeks of age.

4. Ciliation in the oviductal epithelium was stimulated markedly by treatment with estrogen. In 12-week-old pullets given 17  $\beta$ -estradiol, the ciliation of the oviduct was approximately 50% on the 5th day after treatment and 100% of that in the fully differentiated active oviduct on the 10th day.

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# 種々の機能状態における鶏の卵管繊毛細胞の様態についての走査電顕的観察

#### 藤井俊策

鶏の卵管粘膜上皮は、卵管の全長を通して、繊毛細胞と非繊毛細胞(分泌細胞)の2種の細胞から成っている。とくに繊毛細胞は卵管内の卵の輸送、精子の移送に特別な役割を果たしている。この研究は種々の機能状態における繊毛細胞の様態を、主として走査電顕的に観察した。

- 1. 活動期 (産卵期)の卵管の織毛細胞は、遠位漏斗部、膨大部、子宮部では非繊毛細胞とほぼ同数に分布 していた。峡部と腟部では繊毛細胞が優勢であった。近位漏斗部と子宮・腟移行部は繊毛細胞のみから 成っていた。
- 2. 非活動期(換羽期)の退縮した卵管では、繊毛細胞は卵管の一部を除き退行変化し、その密度は活動期の卵管のほぼ半数に減少していた。とくに近位漏斗部の繊毛細胞の消失が顕著であった。 腔部繊毛細胞はほとんど変化していなかった。
- 3. 卵管の繊毛形成はふ化10週令頃から始まり、産卵開始前の20週令にほぼ完成した。腟部の繊毛形成は 例外的に早く、10週令時には既にかなりの程度に進行していた。漏斗部の繊毛形成は最も遅れた。
- 4. 卵管の繊毛形成はエストロゼンによって著しく促進された。 12 週令のヒナに 17  $\beta$ -estradiol を毎日 連続注射したところ、注射 5 日と10日後には、それぞれ活動期の卵管の50%と 100 %に相当する繊毛形成 が認められた。