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Morphological Observation on the Fibrous Materials in the Gilt's Vagina

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Summary

The fibrous materials in the gilt's vagina were observed by light microscope and SEM to investigate their relationship to leucocytes.

A large number of the neutrophil leucocytes appeared in the epithelium of the gilt's vagina in the stage of standing estrus. The microscopic observation indicated that the lobulation of the neuclei of leucocytes became more prominent. At the same time, they elongated into fiber-shape toward the cervical cannal and vulva of the genital organ in the gilt.

The fibrous materials were identified as the nuclei of the leucocytes.

It has been known that a large number of leucocytes appear in the epithelium of the vagina of gilts (1) and other mammals such as cows (1), mares (1), dogs (2), weasels (8), golden hamsters (4), rats (5, 6) and humans (7) following ovulation.

However, their role has not been elucidated (8, 9). Nishikawa *et al.* (1) reported that "the fibous materials" appeared in the smear of the gilt's vagina and that their number tended to shift in proportion to that of the leucocytes.

The purpose of this study was to investigate the morphology of leucocyte's neuclei in the gilt's vagina and trace their possible change to fibrous materials using light microscope and scanning electron microscope (SEM).

Materials and Methods

Materials used in this experiment were obtained from six Landrace gilts reared at the Animal Breeding and Reproduction Laboratory, Iwate University.

Biopsy: A punch-type forceps was inserted into the gilt's vaginal vestibule from the external genitalia. The forceps was then pressed toward the side to collect samples.

The biopsy was performed only once for each animal. Thus, materials in proestrus, standing estrus, metaestrus and diestrus were obtained from four different gilts.

A part of the tissue was fixed with Bovin fluid immediately after the collection. The fixed tissue was then embedded in paraffin and cut into 5–7 pieces, followed by staining with Hematoxylin-Eosin and Alcian blue. The remainder of the tissue was fixed with 2.5% glutalaldehyde (0.1 M phosphate buffer pH 7.4) followed by the dehydration in alcohol, the critical point drying in liquid CO_2 and the gold coating for the scanning microscopic observation using a FE type electron microscope (HITACHI S–700).

Collection of smears: For the purpose of collecting the smear sample effectively, two pieces of equipment were devised. The stamp type smear collector (STS collector) consisted of a bmaboo stick of 30 cm in length. One end of the stick was elevated and rectangular slide glass was attached to the raised portion by means of tape.

The smear collecting tube consisted of a vinyl tube, 2 cm in diameter and 11 cm long. One end of the tube was cut to the dimension of 3 cm in length and 1.5 cm in width. The tissue smear was collected by inserting the tube into the vagina followed by the insertion of the STS collector. The rectangular opening of the tube was adjusted to meet the sldie glass of the STS collector, which was pressed toward the side. The smear samples were obtained from the two gilts once in the diestrus and the proestrus, and three or four times during a period from the standing estrus to the postestrus. As soon as the tissue smears were collected, the slide glass was removed from the STS collector and they were transferred to the other slide glass, making sure the longitudinal direction of the sample.

After dried in air, the tissue smears were stained with the following four different dyeing fluid.

Fixation fluid	Staining
1. metylalcohol	H & E
2. Carnoy's fluid	Feulgen reaction
3. Carnoy's fluid	Methylgreen-Pyronin
4. Carnoy's fluid	Azan

Results

Light microscopic observation on the epithelium of the gilt's vagina.

During the estrous cycle the epithelium appeared to be of the stratified squamous type. At the proestrus, it consisted of about 5 cell layers and 6.3 μ thick on the average. Concurrent with reddening and swelling of the external genitalia of the gilt, the epithelium began to grow. The vessels in the lamina propria developed and the surface of the epithelium was smooth.

At the standing estrus, the epithelium was 110 μ thick and consisted of 13 layers on the average. This was the maximum thickness observed in this experiment.

The epithelium was deeply stained with Alcian blue. A large number of

leucocytes had corwded in the lamina propria. This phenomenon was oberved more intensively under the epithelium (Fig. 1–1).

At the metaestrus and epithelium appeared as thinner cell layers (5.3 cell layers and 50 μ thick on the average) and was characterized by a rough and uneven surface.

A large number of leucocytes crowded in the epithelium and penetrated into the epithelial cell layers (10, 11). The nuclei of leucocytes were slightly stretched (Fig. 1–2). The epithelium was stained slightly darker than the lamina propria with Alcian blue.

At the diestrus, the epithelium appeared very thin (3 cell layers and 33 μ thick on the average) with a smooth surface (Fig. 1–3). The epithelium was stained weaker than the lumina propria with Alcian blue.

Light microscopic observation on the smears:

At the proestrus, the dead epithelial cells appeared and their number increased with the progress of the estrous stage. On the first day of the standing estrus, the amount of the cells reached their maximum number. On the second day, leucocytes appeared in the intercellular space (1, 2, 3, 4, 5, 7). These leucocytes were identified as neutropil leucocytes (11). Following the appearance the leucocytes which had 3 or 4 nuclear lobes were commonly present (4, 7) (Fig. II–1). The nuclei then elongated and stretched to string shape. They were further extended in the longitudinal direction of the gilt's genital organ (Fig. II–2), forming fibrous materials. These observation clearly indicated that the fibrous materials were the deformed nuclei of leucocytes. At the metaestrus, the fibrous materials adhered to the agglomeration of the epithelial cells as well as to the outer edge of the dead epithelial cell layer (the space between the dead epithelial layer and vital remaining epithelium), which covered the posterior region of the dead epithelial layer (Fig. II–3).

At the diestrus, a small amount of the fibrous materials adhered to the tiny clusters of dead epithelial cells. The fibrous materials showed positive reactions to Hematoxylin, Feulgen, Methylgreen and Carmine. This observation showed conclusively that the fibrous materials originated from the nuclei of the leucocytes and that they were chemically DNA'S.

Observation on the fibrous materials by SEM.

On the surface of the epithelium, a spherical body was observed (Fig. III–1). The projection appeared in the first phase (Fig. III–2). By further extension, the size of the remaining body elongated further (Fig. III–4, IV–3).

As a result, the remaining body disappeared into the fibrous strand in the third phase (Fig. III–5, IV–2, 6).

In the intercellular gap, the fibrous material was observed (Fig. IV–1). The fibrous materials tended to agglomerate with their neighbor (Fig. IV–4, 5).

Discussion

The phenomenon of the growth and the thinning out of the epithelium of the gilt's vagina due to peeling has been reported by Bal *et al.* (12), Maruyama *et al.* (13, 14). Mortor *et al.* (15) and Walker *et al.* (16). Our results support their observations. The results of the Alcian blue staining test indicated that the active secrection of the mucoid fluid occurred in the early stage of the standing estrus in gilts.

The results of our investigation on the time of appearance of the leucocytes in the vagina, the staining with Hematoxylin, Feulgen and Methylgreen, and the origin of the fibrous materials, also supported the observation reported by Nishikawa et al. (1). Our observation made it clear that "the fibrous materials" observed by Nishikawa *et al.* (1) were the nuclei of the leucocytes.

Our observation by SEM and the light microscope demonstrated that the nuclei of the leucocytes elongated as the pseudopodiums extended (8, 10) and that the nuclei became fibrous shape as the leucocyte's body deformed to fibrous shape.

By the STS method, it was demonstrated that the fibrous materials extended only in the longitudinal direction, that is, toward the cervical cannal and vulva of genital organ. The fibrous materials agglomerated and exclusively covered a large extent of the posterior portion of the degenerated epithelial cells, that is, the space between the normal epithelial cells and the degenerated epithelial cells. Observation by SEM confirmed the sequence of morphological changes observed by light microscope.

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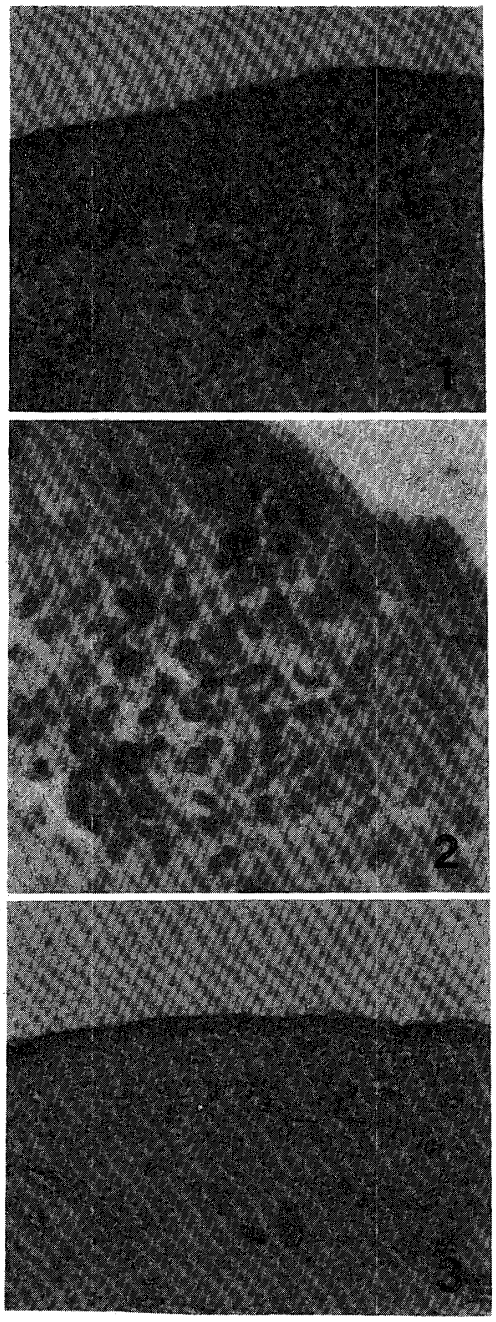

FIG. 1. Morphological change of vaginal epithelium.
1–1. At standing estrus.
Alcian blue-Hematoxylin stained
× 490
1–2. At postestrus
H.E. stained ×910
1–3. At diestrus
H.E. stained ×210

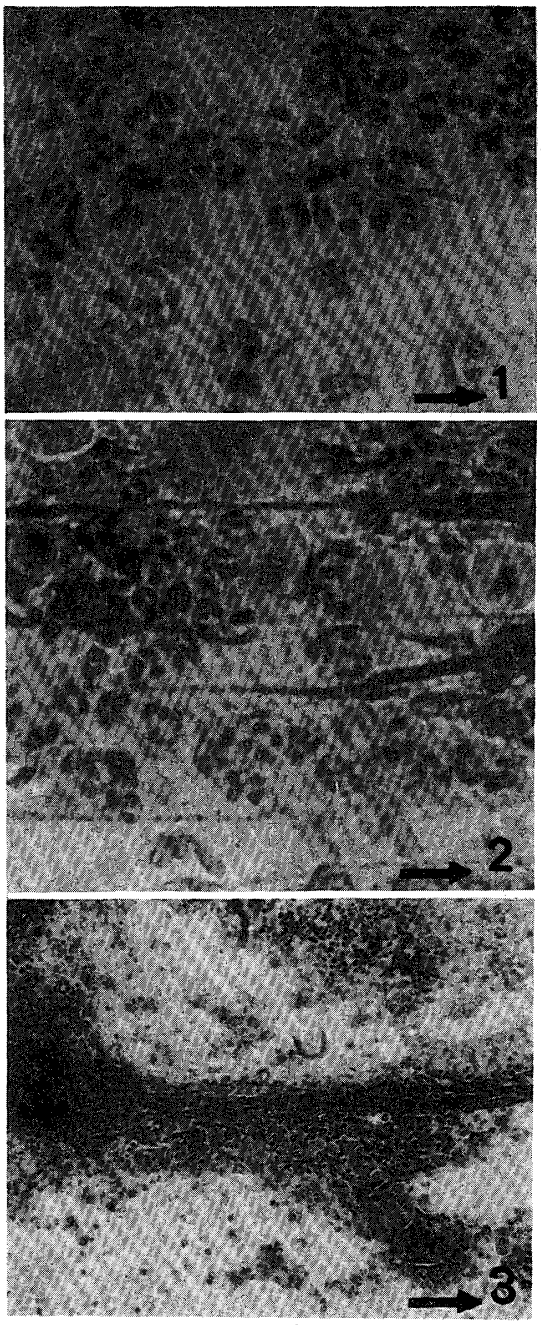

FIG. II. Morphological change of leucocyte's nuclei in stamp smear
(→: longitudinal direction of genital organ)
II–1. At postestrus × 980
H.E. stained
II–2. At postestrus × 980
H.E. stained
II–3. At postestrus × 210
H.E. stained

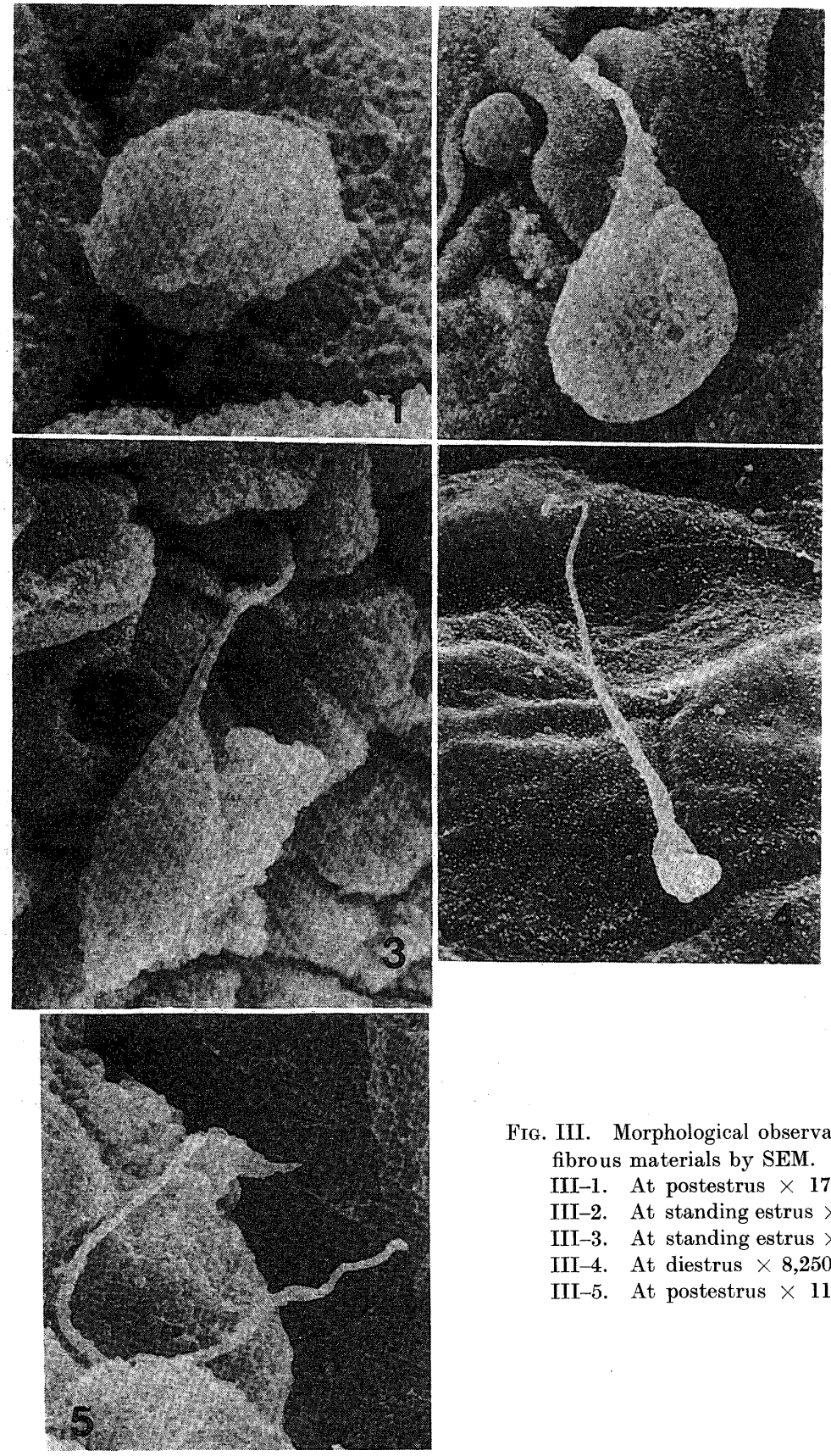

FIG. III. Morphological observation of fibrous materials by SEM.
III–1. At postestrus × 17,500
III–2. At standing estrus × 9,750
III–3. At standing estrus × 9,750
III–4. At diestrus × 8,250
III–5. At postestrus × 11,250

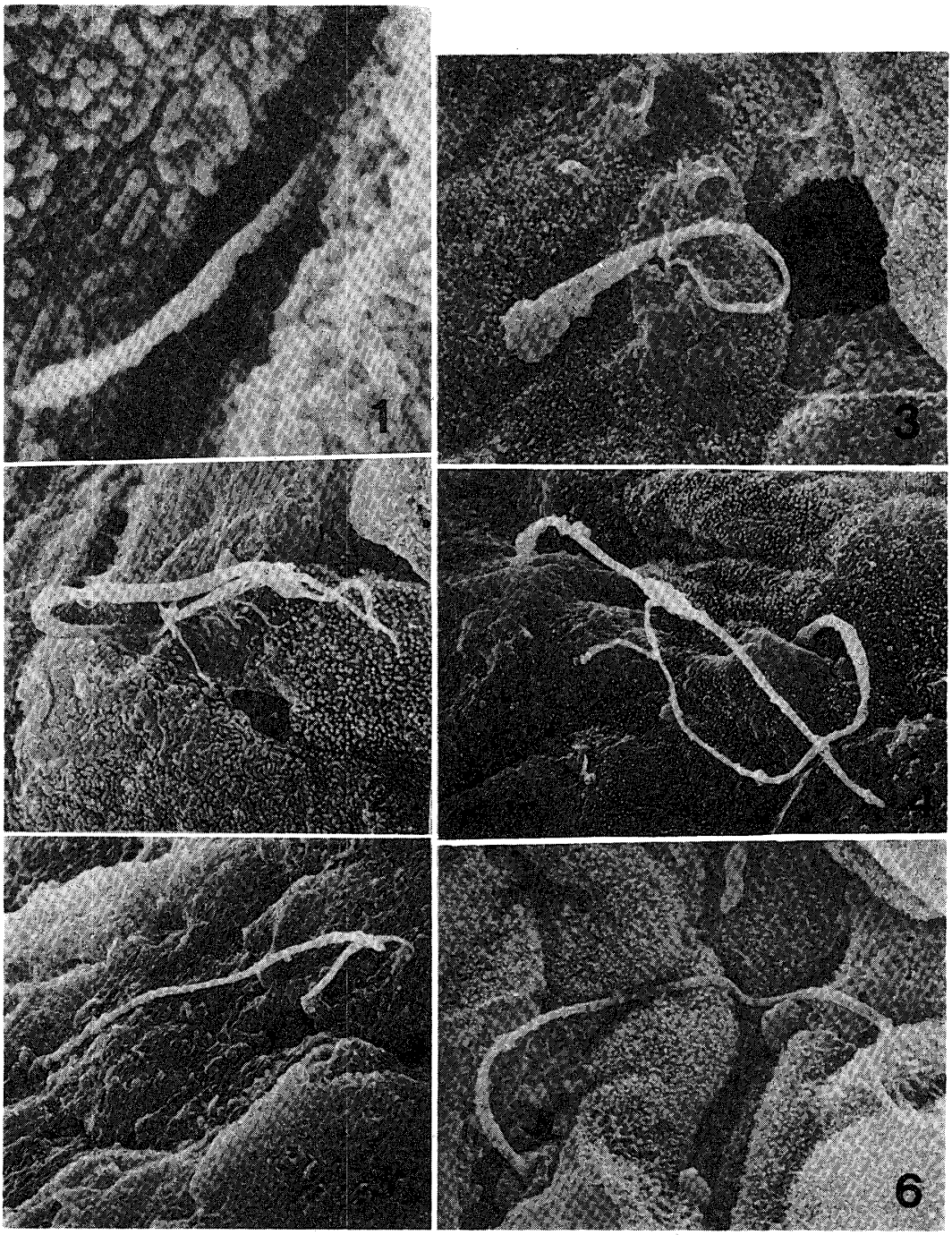

FIG. IV. Morphological observation of fibrous materials by SEM.
IV–1. At diestrus × 52,800
IV–2. At postestrus × 4,000
IV–3. At postestrus × 10,400
IV–4. At postestrus × 7,200
IV–5. At postestrus × 5,600
IV–6. At postestrus × 6,400