MORPHOLOGICAL CHANGES OF UTERINE AND CERVICAL EPITHELIUM DURING EARLY PREGNANCY IN RABBITS

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Introduction

It is usually held that fertilized eggs lie freely in the uterine cavity for a few days before implantation, the cervix acting to hold them in the uterine lumen. Recently, contradicting evidence for this concept was provided by TSUTSUMI *et al.*[41], who reported that some intrauterine eggs, including blastocysts, could be expelled through the cervix into the vagina during the preimplantation stage in normal pregnant rabbits. They also found that a large number of eggs were discharged into the vagina of the superovulated rabbit at early times after mating. It has been recognized that administration of ovarian steroids or prostaglandin $F_{2\alpha}$ causes a rapid egg transport and results in the expulsion of fertilized eggs into the vagina[6~10,26,31~35]. These observations aroused our interest in the cervical contribution to the maintenance of pregnancy. Concerning the function of the cervix, considerable interest has been focused on sperm passage through the cervix[4,20] ; however, no attention has been payed to the functional role of the cervix in egg transport following the entrance of ova into the uterus. A previous study in this laboratory[31] demonstrated that the rabbit cervix displays a narrower lumen and contains more fibrous connective tissue in comparison with the uterine horn. These features of the cervix may be important in preventing discharge of intrauterine eggs into the vaginal lumen during the preimplantation stage.

In the present study, uterine and cervical epithelia of rabbits in estrus and during the postcoital period were examined with light and scanning electron microscopy, hopefully to elucidate the unclear phenomenon of egg expulsion into the vagina during the preimplantation stage. The histology of the endometrial epithelium might serve as a useful indicator of the pro-

gestational effects already described exhaustively for the rabbit in early stages of pregnancy and pseudopregnancy[1,11,12,16,25,29,38].

Materials and Methods

Twenty-four Japanese White female rabbits were mated twice to proven fertile males and then received an intravenous injection of 50 IU of human chorionic gonadotropin (Primogonyl, Schering) to ensure ovulation. Tissues were obtained on the 2nd, 4th, 5th, 6th, 7th and 9th days of pregnancy. Three other estrous, non-pregnant rabbits were used as control animals. The rabbits were sacrificed by an intravenous overdose of sodium pentobarbital (Somnopently, Pitman-Moore Inc.), and their reproductive tracts were immediately removed and trimmed of excessive fat.

For light microscopy (LM), the uterus-attached cervix was pinned on Sealant (Dow-Corning 780) and immediately fixed in Bouin's or Carnoy's solution. After one or two hours, samples were cut into smaller segments, followed by fixation for a longer period, and were embedded in paraffin. Paraffin sections of 5–7 μm thickness were stained with hematoxylin and eosin (H. E.) or azan, or by the periodic acid-Schiff (PAS) method.

For scanning electron microscopy (SEM), tissues were obtained from the mid-portions of uterus and cervix and their mucosal surfaces were very moderately washed with physiological saline solution. The tissues were then pinned on the Sealant and pre-fixed with 3% glutaraldehyde in 0.1 M phosphate buffer at 4°C. After 24-hour fixation, these samples were rinsed in 0.1 M phosphate buffer and post-fixed in 1% buffered osmium fixative for 1–1.5 hours at 4°C. The specimens were then washed in distilled water and dehydrated in a graded series of ethanol at room temperature. Following dehydration the specimens were transferred to liquid CO_2, using isoamyl acetate as the intermdeiate fluid for critical-point drying. Dried samples were mounted on aluminium studs and sputtered with approximately 200 Å of gold in an Ion Coater (EIKO Engineering). All specimens were examined using an Hitachi S-310 Scanning Electron Microscope operated at 5 kV accelerating voltage.

Results

I. Uterus

A. Estrous stage

SEM observation confirmed the results of our previous study[31]. The endometrial surface was relatively smooth and had many glandular openings

(Fig. 1). The endometrium was commonly lined by non-ciliated cells and occasional ciliated cells, exhibiting a cobblestone-like arrangement. The non-ciliated cells were characterized by crowded, short, nub-like microvilli on their free surfaces (Fig. 2), and some of the cells showed slight elevation of the cell surface and well-developed microvilli (Fig. 2). Occasionally, microvillous cells possessing a single cilium were located in groups (Figs. 2, 3). Such single cilia were apparently more slender than the cilia of ciliated cells. In rare cases, cells having short cilia were found (Fig. 4). The diameter of the short cilia seemed to be greater than that of the microvilli, but similar to that of the well-developed and usual cilia. Speculatively, this feature may be associated with generation of new cilia (ciliation).

B. Postcoital stage

2 days after mating: Folding of the mucosal surface had increased slightly and the epithelium had proliferated (Fig. 33). Numerous mitoses were found in both surface and glandular epithelium, especially in the glands. Uterine glands were increased in number, and showed elongated and expanded lumina. Abundant leucocytes were located within the epithelium and the subepithelial stroma. Pseudostratification of the epithelium was occasionally observed in histological sections, although most of the glandular epithelium remained simple cuboidal or columnar. SEM observation indicated that the boundary of each epithelial cell had become partially unclear, and ciliated cells were extremely increased in number as compared with the estrous stage (Fig. 5). Many droplets of various size, probably secretory granules, were attached on the surface of the microvillous cells and cilia (Figs. 5, 6). No microvillous cells possessing a single cilium were observed throughout the progestational stages. Clusters of very short cilia, such as observed in estrus, still appeared among the microvilli (Fig. 7).

4 days after mating: Branching of the mucosal folds was further advanced than on the 2nd day. In the parts of the mucosa which branched conspicuously, the folds, with narrow connective tissue cores, were more slender and were interconnected in complicated fashion, showing a labyrinthine structure. Uterine glands were increased in number and their lumina enlarged markedly. The uterine surface epithelium and the epithelium of the superficial part of the glands were almost pseudostratified (Fig. 34), but the epithelium of the deeper part of the glands was mostly a single layer. Mitoses were less numerous and leucocytes within the surface epithelium and subepithelial stroma had decreased in number. SEM revealed that the endometrial surface contained two types of non-ciliated cells (Fig. 8). One type exhibited a relatively flat, free surface and its boundaries appeared to

be delineated with crowded microvilli. The other cell-type showed a slightly convex and rounded apical surface. Cells of the two types were not only intermixed but also occurred as groups of single-type cells. Consequently, endometrial cells with flat, free surfaces seemed to form a smooth convex apex. Ciliated cells varied in number from animal to animal. They were more numerous than on the 2nd day in two of four animals at this stage, whereas only a few ciliated cells were evident in the other two.

5 days after mating: The mucosal folds were branched more delicately than before. The volume of the nuclei in the epithelial cells was increased, because of a reduction in the amount of cytoplasm, and densely crowded nuclei were common in the surface epithelium (Fig. 35). The free surface of the epithelium had become irregular, due to the formation of dome-like protrusions of apical cytoplasm (Figs. 9, 35). Microvilli on these protrusions were very sparse and smaller in size than those on the cells which remained unaltered (Fig. 9). Narrow and short string-like bridges frequently appeared between adjacent protrusions. Ciliated cells were rarely found among such non-ciliated cells, and their cilia, of various lengths, were sparsely arranged in most such cells. There was a tendency for cilia on the central part of a cell surface to be shorter than those on its marginal surface. It was noted by SEM that large cells rarely appeared in this stage (Fig. 10).

6 days after mating: The expanding blastocysts were easily identified, and reduction of mucosal folds was noted macroscopically at blastocyst sites when the uteri were cut open. The mesometrial folds in the conceptal areas, however, appeared broader throughout their length, and subepithelial stroma was more substantial. In the interconceptal areas, the connective tissue in the stroma was increased and the surface of the apical parts of the folds had become somewhat smooth. As the epithelial cell membranes had seemed to disappear and the crowding nuclei become arranged more compactly, it was difficult to identify each epithelial cell by LM (Fig. 36). Groups of closely packed nuclei had formed in the epithelium on the apical parts of the folds, showing cell fusion (multinucleated cells). However, the degree and distribution of cell fusion seemed to vary in different sites in the uterine mucosa. The process of cell fusion appeared advanced in the conceptal area rather than in the interconceptal area, and in the mesometrial folds rather than in the antimesometrial folds. Ciliated cells were rarely located in the peri- and antimesometrial folds, but a few were found in the mesometrial folds. In SEM, the endometrial surface was seen to be composed mainly of spheroid, non-ciliated cells having very sparse microvilli on their free surfaces (Figs. 11, 12). Large non-ciliated cells, probably due

to the formation of multinucleated cells, were frequently observed among normal-sized non-ciliated cells in both conceptal and interconceptal areas.

7 days after mating: The attachment of the trophoblast to the uterine mucosa was initiated on this day on the antimesometrial fold. The antimesometrial folds in the area of conceptus were extremely reduced in height, and formation of the symplasma had taken place wherever surface epithelium was in contact with the trophoblast. The mucosal folds in the interconceptal area were diminished in height and width, resulting a broader uterine lumen. The formation of symplasma was not found in the surface epithelium of the interconceptal areas, where multinucleated cells were common.

As indicated by SEM, the surface epithelium was lined by non-ciliated cells of various size (Fig. 13), on which microvilli were sparse and small. These cells were possibly the multinucleated cells noted by LM (Fig. 37). Groups of small cells which exhibited a wrinkled surface devoid of microvilli were noted in places (Fig. 14). These cells had wide gaps among them, and were interconnected to each other by several thin, string-like bridges.

9 days after mating: In the interconceptal areas the glandular and cryptal lumina appeared to be extended because of reduction of the connective tissue cores. The size and number of the multinucleated cells had increased markedly and the mucosal surface appeared to be lined by irregular compartments which were constructed from the increasing number of nuclei. The process of cell fusion had advanced even to the deeper part of the glandular epithelium (Fig. 38).

II. Cervix

A. Estrous stage

In cross section, the cervical folds protruded centripetally into the lumen; and in longitudinal section they were arranged in parallel from the external os to the internal os, as reported previously[31]. The mucosal folds branched delicately, resulting in the formation of cervical crypts. The presence of the longitudinal folds and the crypts was quite evident in SEM (Fig. 15). The cervical mucosa, like the uterine mucosa, was covered with both ciliated and non-ciliated cells (Fig. 16). Ciliated cells, however, occupied more than half of the cervical epithelium, and non-ciliated cells occurred only sporadically in the "carpeting" of long cilia of ciliated cells (Fig. 17). The majority of cilia displayed slender and well-formed shafts with blunt tips, but cilia having thinner and bending tips appeared frequently (Fig. 18). In addition, areas of shorter cilia were found among the normal longer cilia (Fig. 19), and clusters of even shorter cilia (ciliary buds) were seen to emerge from

the microvillous surfaces of several cells, as observed in the endometrium. These types of cilia were found in all stages examined. Microvillous cells possessing a single cilium, as observed in the uterine mucosa of estrous does, were never found in the cervical epithelium ; but in general, there was no discernible difference between SEM characteristics of the ciliated cells in uterine and cervical epithelium.

In contrast, the surface morphology of non-ciliated cells in the cervix was clearly different from that of those in the uterus. The apical surfaces of the non-ciliated cells in the cervix formed dome-like protrusions on which the microvilli were greater in diameter and length as compared with those of endometrial cells, although the number of microvilli per cell was smaller (Fig. 18). The microvilli of most cells were regularly arranged, but in some cases they were sparse or absent in part (Fig. 19). Occasional secretory cells exhibiting an apocrine secretion were evident from the cytoplasmic projections on their apical surfaces (Fig. 20). The projections were raised to the level of the tips of the adjacent ciliated cells, and microvilli were scarce or absent in these cells. Some of these non-ciliated cells possessed one or more small hollows (depressions) on their free surfaces (Fig. 19). Small globular droplets were randomly attached on the surfaces of microvillous cells and on the cilia. These droplets were suspected to be secretory granules, because stained materials were seen at the apical ridges of cells and around the cilia in the PAS- or azan-stained sections (Fig. 39).

B. Postcoital stage

In contrast with the typical transformation of the endometrium, there was no clear macroscopic alteration of the cervical folds during the progestational stages examined. Based on the microscopic aspect, however, changes occurring in the cervix were obvious in the epithelium, especially in the non-ciliated secretory cells as compared to the ciliated cells. Postcoital changes in the ciliated cells seemed to be limited to their height and size; their internal structure did not appear to be altered histologically and their surface morphology at each stage was similar to that of estrous state.

2 days after mating: The cervical epithelium exhibited intense proliferation, and appeared to be pseudostratified in many places. Most of the secretory cells were filled with secretory materials in their supranuclear portions, but the density of the secretory materials was somewhat low as compared with that of the estrous state (Figs. 39 vs. 40). Peg cells were decreased in number. Abundant secretory granules were found in both the crypts and the cervical lumen in histological sections. In SEM, a great number of small droplets were obvious on the surfaces of microvillous cells

and cilia (Fig. 21). Remarkable changes were notable on the surfaces of non-ciliated cells. The microvilli of some cells exhibited various changes: swelling, elongation, or more dense distribution. Other cells had lost their microvilli partially or completely, exposing smooth apical surfaces, but some of them showed very irregular surfaces, due to the formation of several small hollows (Fig. 22). The low density of secretory granules within the cells and the appearance of an increased amount of secretion in the cervical crypts and lumen suggest active secretion from secretory cells in this stage.

4 days after mating: The thickness of the cervical epithelium was similar to that of the estrous state (Fig. 41). The secretion within the secretory cells was further diminished, and cells containing secretion near the apical ridges were a common finding. Cells containing numerous secretory granules in supranuclear portion were smaller in number than on the 2nd day of pregnancy. Peg cells had increased in number. In SEM, some droplets were seen attached to the surfaces of cilia, but few were found on the free surfaces of non-ciliated cells. Microvilli of most non-ciliated cells had the same slender shape observed in estrous does, but they remained more abundant. Some non-ciliated cells were covered by abundant, well-developed microvilli (Fig. 23) which resembled the microvillous promontories illustrated by FERENCZY and RICHART[15] in human endometrium. It was noted that long cilia were often located only at the peripheries of cells, with a bare, microvillous surface appearing in the center portion, with or without a few short cilia (Figs. 24, 25). This appearance of cells was also found in the cervical epithelium on days 5, 6, and 9 of pregnancy. An unique SEM image of a non-ciliated cell was obtained at this stage. A part of the plasma membrane of the cell had ruptured, and several droplets, probably secretory granules, were seen exposed in the cytoplasm (Fig. 26).

5 days after mating: The cervical epithelium exhibited intense proliferation again. Although the number of cells containing secretory materials in their apical portions was extremely diminished, some secretory cells contained PAS- or azan-stained materials under the nuclei, near the basement membrane (Fig. 42). In these cells, secretory material was no longer located in the supranuclear region, and their nuclei were pushed up toward the apical portion. Peg cells were rare in the epithelium. It was considered from these observations that secretory granules had begun to be produced again in the epithelial cells. In SEM, most of non-ciliated cells displayed short, thin microvilli on their free surfaces (Fig. 27). Some cells possessed occasional cytoplasmic projections or small hollows, on which microvilli had disappeared in part. It was of interest that a part of the free surface of the non-ciliated

cell was connected with secretory material by a strand of the same material (Fig. 28). This feature is regarded as part of the secretory process, whereby secretory material appears to be released through the small hollow on the cell surface.

6 days after mating: The cytoplasm of the non-ciliated cells again contained secretory materials which stained strongly or moderately (Fig. 43). Based on the observations on the 5th day after mating, it was estimated that the secretory output of such cells had increased further before implantation. The non-ciliated cells had lost microvilli on their free surfaces to varying degrees (Fig. 29), and some cells exhibited one or more small hollows on the apical surfaces, as seen in SEM (Figs. 29, 30).

7 days after mating: The epithelium had become thinner again, and increasing numbers of peg cells were noticed (Fig. 44). A little secretion was commonly seen at the apical ridges of the cells. In SEM, it was seen that microvilli on the surfaces of secretory cells had increased generally in density and hight, compared with the earlier stages (Fig. 31). In some areas, microvilli were further developed and elongated, and several contiguous cells displayed fields of long microvilli (Fig. 32). Occasionally, cytoplasmic projections were observed among the long microvilli.

9 days after mating: The epithelium remained thin and contained many peg cells. Most of the secretory cells exhibited only moderately staining secretion in the apical region, although cells containing mucin in their supranuclear regions were frequently found (Fig. 45). In the fields observed by SEM, microvilli on the cell surfaces were short and thick, and abundant secretory material adhered to the cilia.

Cytoplasmic cell inclusions: Cytoplasmic cell inclusions, initially noticed by LOEB and SMITH[26], were found in the cervical epithelium at all stages examined. These inclusions were spheriod bodies of various size (Fig. 46). In H. E. preparations, they were stained distinctly with eosin, and were easily distinguished from other components of the cytoplasm. In PAS and azan preparations, several granules were recognized within the inclusions. The granules stained red-violet with PAS and blue with azan stain, whereas the rest of the inclusions were red or pink with PAS and light blue with azan. Cytoplasmic cell inclusions were distributed randomly in the epithelium, and their incidence was quite variable. Their position within the epithelial cell was also variable. During estrus and up to 6 days after mating, the inclusions were generally found in the basal parts of the cells, varying in location from near the basement membrane to near the nucleus. On 7 and 9 days of pregnancy, however, many inclusions were noticed in the

supranuclear region, varying in location from just above the nucleus to the apical ridge of the cell.

Discussion

The present results on the surface morphology of the uterine and cervical mucosa of estrous rabbits have extended previous macro- and microscopic observations[31], and have confirmed SEM findings by HAFEZ[18,19], KANAGAWA *et al.*[23] and KANAGAWA and HAFEZ[24]. They are also in harmony with the SEM images at 12 hours after HCG injection reported by RICHES *et al.*[30]. The present observation on the progestational endometrium has reaffirmed the histological findings of previous investigators[1,16,25,29] and confirmed earlier SEM findings by BARBERINI *et al.*[3]. Our results show that mucosal folding increased markedly at 4 days of pregnancy and that the process of cell fusion began at 5 days of pregnancy and later extended progressively. The formation of multinucleated cells was evident in the uterine epithelium, with or without blastocysts, but symplasmic changes were observed only in the areas of the epithelium in contact with blastocysts. LARSEN[25] has reported that the formation of multinucleated cells is induced by hormones, while that of the symplasma requires a local effect produced by the blastocyst. DAVIES and HOFFMAN[11] have considered, based on the report by HILLIARD and EATON[22], that the morphological events in the rabbit endometrium up to 3 days following HCG injection are not dependent on progesterone, but that morphological changes after the fourth day are progesterone-dependent. HILLIARD and EATON[22] have shown that ovarian production of steroid hormones during the 3-day period of tubal transport is extremely low, but that estradiol and progestin outputs rise between 4 and 6 days after mating. It is reasonable to suppose, therefore, that the cervical epithelium, as well as the uterine epithelium without blastocysts, is regulated only by the ovarian hormones during early pregnancy and is beyond the limits of any local stimulus provided by the blastocysts. Nevertheless, morphological alterations of mucosal folds in the cervix were not clearly demonstrable during the postcoital periods which we examined. Moreover, the proportion of ciliated cells did not appear to be altered by hormonal events after coitus. Since the various SEM images of cilia and ciliated cells of the cervical epithelium were obtained during different reproductive stages, changes which were observed might be due to cytological changes in the maintenance of cervical epithelium rather than progestational effects.

Stubby, short cilia were considered to be immature or developing cilia,

because of their resemblance to the SEM images reported by other investigators[2,13,15,20]. When ciliary generation occurs almost simultaneously in a group of several cells, fields of uniformly shorter cilia, as observed in the cervix, may appear during the process of maturation. Such developing cilia appeared as blunt cylinders, while normal, long cilia were of two types: those with blunt tips and those having tapered, bending tips. It seems, therefore, that cilia are able to bend as their length increases. Although most cells were fully ciliated, some cells had cilia only at their peripheries with shorter cilia occasionally emerging at the center of the cell. This phenomenon probably represents the process of ciliation as observed in the mouse oviduct by DIRKSEN[13], who mentioned that cilia first grow at the periphery of the cell and then fill in the central area. Our observations suggest that two types of ciliation may exist in the cervical epithelium of rabbits.

More distinct changes occurred in the production and secretion of mucus, in the shape and density of microvilli, in the free surfaces of cells, and in the movement of cytoplasmic cell inclusions in non-ciliated cells. Changes in the number of different secretory cells of the cervix have been reported by HAFEZ *et al.*[17], who examined rabbit and bovine cervices histochemically at estrus and at 1, 3, and 7 days after mating. They demonstrated that rabbit cervical epithelium contained many columnar cells which were filled completely or moderately with secretory granules at estrus and 1 day after mating, and that cells containing secretory granules located just beneath the luminal surface, and peg cells, were very common in the epithelium at 3 and 7 days after mating. Although the intervals of time after coitus examined in this study were different from those in the report just mentioned, it was clear in our study that the production of secretory granules, which decreased gradually following coitus, increased shortly before implanatation (5 to 6 days after mating). The height of the epithelial cells was also altered along with changes in their secretory activity. Those epithelial cells which contained abundant secretory granules in their cytoplasm were tall-columnar, and the epithelium appeared to be hyperplastic, while the epithelial cells which contained few secretory granules were cuboidal rather than columnar in shape. On the other hand, there was a negative relationship between secretory activity and the incidence of peg cells in the cervical epithelium. Peg cells were most numerous when the secretory cells contained few secretory granules. Peg cells are believed to be the remnants of emptied secretory cells and to be lost ultimately from the epithelium[5].

Variation in the height of the cervical epithelium is known to be as-

sociated with the secretory activity during the estrous cycle in most animals[14]. The height and the secretory activity of the epithelium reach their peaks during estrus and then undergo various degrees of reduction, indicating that estrogens cause an increase in the size and secretory activity of cervical epithelium, while progestins decrease them. In the rabbit, ODOR[28] and RICHES *et al.*[30] have demonstrated that secretory granules disappear and cilia undergo a patchy loss following castration, and that rapid restoration can be caused by estrogen administration. These observations suggest that the production of secretory granules in the cervical epithelial cells is regulated by hormonal events, especially by estrogen output by the ovaries. From 4 to 6 days after mating, estradiol production by the ovaries (as well as progestin production) rises, reaching its peak on the 6th day of pregnancy ("Preimplantation peak"); and then it falls by the 7th day[22]. We think it likely, therefore, that the recovery we observed in output of secretory granules in the cervical epithelial cells at preimplantational stages (5 to 6 days following coitus) is dependent on the increase in estrogen production by the ovaries.

The variability in surface characteristics of non-ciliated secretory cells, as revealed by SEM, may depend on their secretory activity. When the non-ciliated secretory cells contained abundant secretory granules, they frequently had fewer microvilli, cytoplasmic projections, and small hollows on their surfaces. The small hollows on the surfaces of secretory cells seemed to resemble the "surface indentation" in the epithelium of rat extrapulmonary respiratory tract described by ANDREWS[2], or the "small pit or hole" in the epithelium of ovine cervix illustrated by WERGIN[42]. Such structures on cell surfaces have been thought to result from mucin secretion. WERGIN[42] reported that a single small pit or hole on the cell surface gradually enlarged by growing toward the cellular margins, and that consequently the cytoplasmic contents of the cells gained access to the lumen. In the present study, degeneration of epithelial cell membranes was seldom observed in the specimens obtained at 2 and 4 days after mating (Figs. 22, 26). In the rabbit cervix, therefore, it is possible that the cytoplasmic contents of secretory cells pass through a single small hollow as observed at 5 days after mating (Fig. 28).

Cytoplasmic cell inclusions are common in the cervical epithelium.[17,21,26,37] According to LOEB and SMITH[26], such inclusions occur in normal estrous, pseudopregnant (15 days following pseudofertilization), pregnant (18 and 24 days after mating) and hysterotomized (a few days following copulation) animals. The present study, as well as the report by HAFEZ *et al.*[17], in-

dicates that cytoplasmic cell inclusions occur likewise in the cervical epithelium during early pregnancy. It is believed that the inclusions move upwards toward the cervical lumen and that their content is discharged into the lumen.[17,26] HAFEZ *et al.*[17] reported that the inclusions were confined to the basal part of the cells during estrus and at 3 days after mating, but that many were apically located at 7 days after mating. The present study confirms the possibility of such movement within the epithelial cell with the advance of pregnancy. The physiological function(s) of such inclusions and the mechanism of their generation remain to be clarified.

The present results suggest that the non-ciliated cells in the cervix are more responsive to progestational effects than are the ciliated cells. It was also demonstrated that the cervix, an organ adjacent to the uterus, does not undergo gross morphological changes during early pregnancy. Morphological constancy may play an important role in the retention of preimplantation embryos in the uterine lumen, since the cervix is a rigid organ containing a great amount of connective tissue and having a smaller lumen.

Summary

Changes occurring in the uterine and cervical epithelium of the rabbit during estrus and early postcoital periods were examined by light and scanning electron microscopy. Materials for observation were taken from the central parts of both organs. In estrous animals, the uterine and cervical epithelium consisted of both ciliated and non-ciliated cells. Non-ciliated cells in the uterus and the cervix, respectively, could be distinguished by their surface characteristics, but ciliated cells were similar in both organs. At increasing time intervals following mating, progressive changes occurred in the endometrium. The surface and glandular epithelia showed extensive proliferation and the branching of mucosal folds gradually advanced, with continuing development of uterine glands and crypts. Ciliated cells progressively disappeared from the uterine mucosa up to the time of implantation. The process of cell fusion was initiated 5 days after mating, and from then on epithelial cells were converted gradually into multinucleated cells.

In the cervix of the postcoital rabbit, no marked transformation of the mucosal folds was noticed, and the proportion and morphology of ciliated cells in the cervical epithelium were similar to those in estrous animals. Changes following coitus were limited to the epithelial cells, especially the non-ciliated secretory cells. Secretory material in the secretory cells decreased gradually up to 4 days after mating. However, the amount of secretory material had increased again on the 5th and 6th days after mating and then

had subsequently diminished, indicating that secretory granules are produced anew before implantation. Signs of ciliation and possible secretory activity were also noted and discussed.

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PLATE 1
EXPLANATION OF FIGURES

Scanning electron micrographs of the uterine epithelium

Fig. 1. Many glandular orifices are located in the surface of the endometrium, displaying a cobblestone arrangement of the epithelial cells, in the estrous rabbit. Ciliated cells are sparse.

Fig. 2. Ciliated and non-ciliated cells lining the endometrium in estrus. Non-ciliated cells are common and characterized by crowded, short microvilli. The microvilli of some cells are further developed and the free surface of the cells is elevated (arrow). Note the microvillous cells each possessing a single cilium.

Fig. 3. Higher magnification of microvillous cells displaying a single cilium.

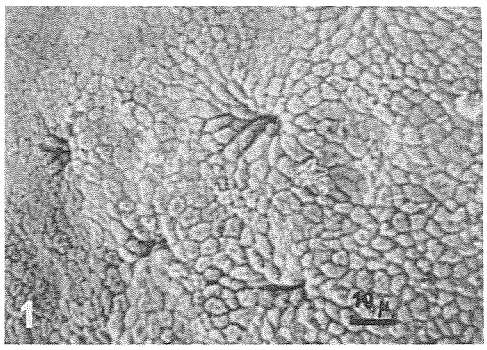
Fig. 4. Cilia of various lengths in an endometrial cell during estrus, may indicate the process of ciliation in a cell which is covered with microvilli. The diameters of immature cilia are approximately equal to those of mature cilia, and greater than those of microvilli.

Fig. 5. Endometrium on 2nd day of pregnancy showing expanded glandular openings (arrows) and increased number of ciliated cells. The cell boundaries have become indistinct in part. A number of blebs can be seen on the cell surfaces.

Fig. 6. Higher magnification of the endometrium on 2nd day of pregnancy. Many droplets of various size are attached on the surfaces of cells and cilia.

Fig. 7. Short cilia emerge in clusters among numerous microvilli on 2nd day of pregnancy. These cilia can be distinguished by their greater diameter. Secretory granules also adhere to some cells and immature cilia.

Fig. 8. Endometrium on 4th day of pregnancy, illustrating two types of non-ciliated cells. Cells of one type from relatively smooth areas of endometrial epithelium. These cells are covered with microvilli, and their boundaries are delineated by more densely crowded microvilli (A). Cells of the other type exhibit slightly convex and round-shaped apical surfaces and are intermixed with cells of the first type, either solitarily or in small groups (B).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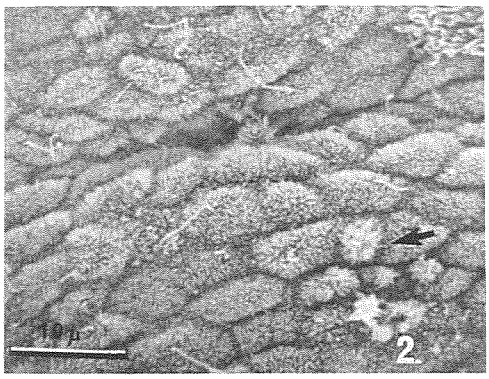
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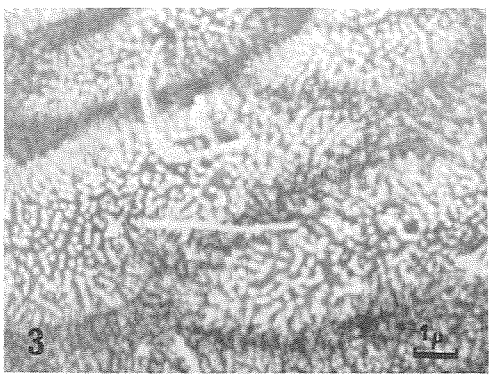
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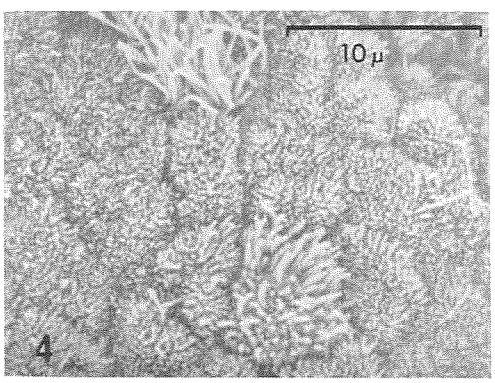
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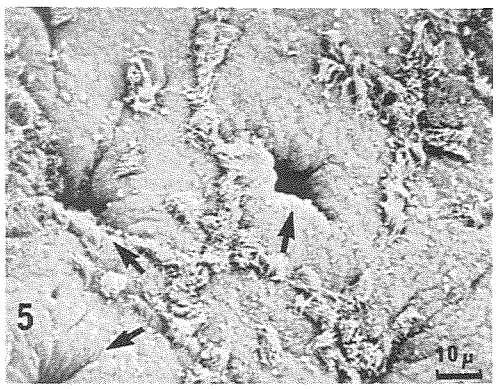
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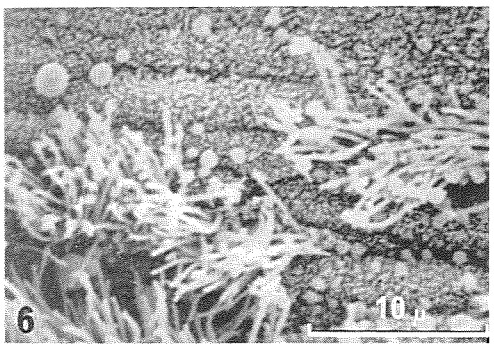
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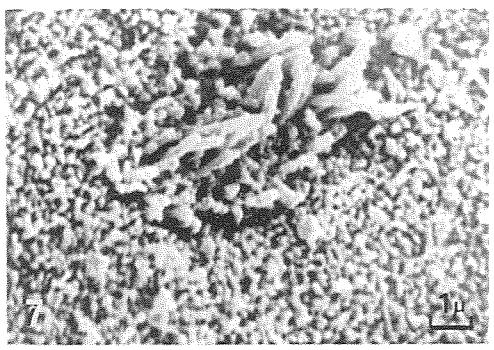
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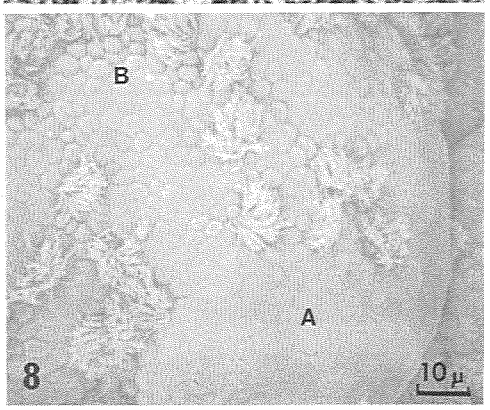
B
A
8
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PLATE 2
EXPLANATION OF FIGURES

Scanning electron micrographs of the uterine epithelium

Fig. 9. Endometrial surface on 5th day of pregnancy showing dome-like protrusions of the apical parts of cells. These protrusions show fewer microvilli than do non-protruded cells. Note the ciliated cell exhibiting long peripheral cilia. The central surface of this cell is densely covered with short microvilli.

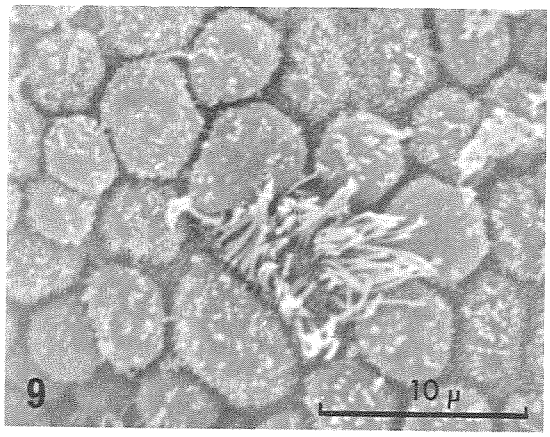
Fig. 10. Large cells, probably resulting from cell fusion, are first observed on 5th day of pregnancy.

Fig. 11. Endometrial surface on 6th day of pregnancy. Note the increasing number of large cells.

Fig. 12. Higher magnification of an area in Fig. 12. Microvilli on the cell surfaces have decreased in number.

Fig. 13. Endometrial surface on 7th day of pregnancy showing advanced cell fusion. Note the large cells of variable size.

Fig. 14. A portion of endometrium on 7th day of pregnancy showing wide gaps among the epithelial cells and intercellular connections of thin "strings". Each cell exhibits a wrinkled free surface devoid of microvilli.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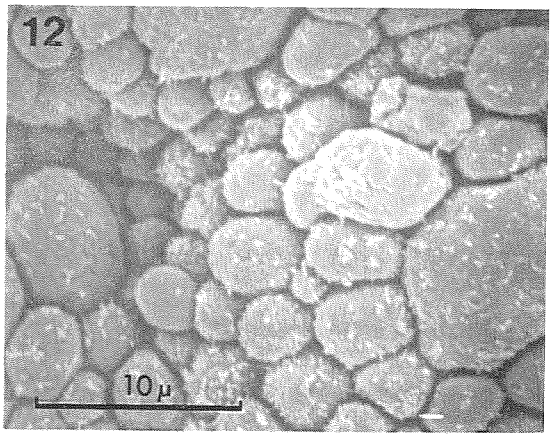
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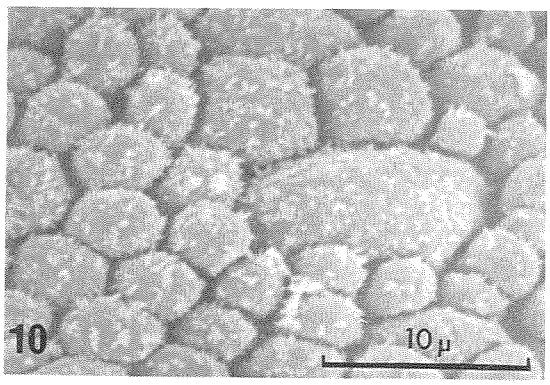
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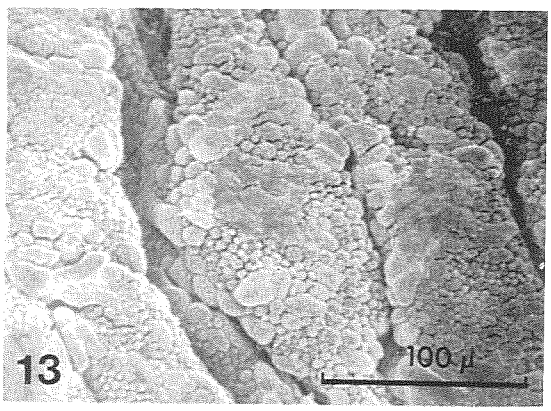
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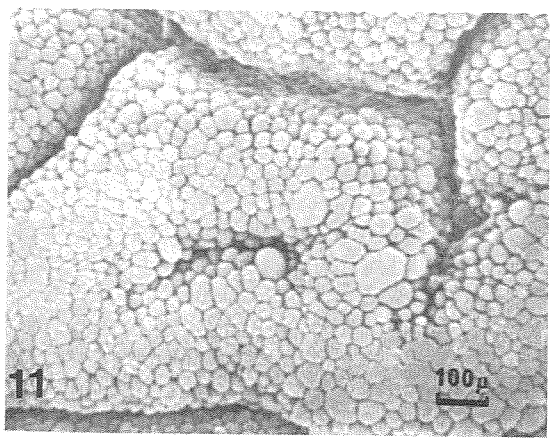
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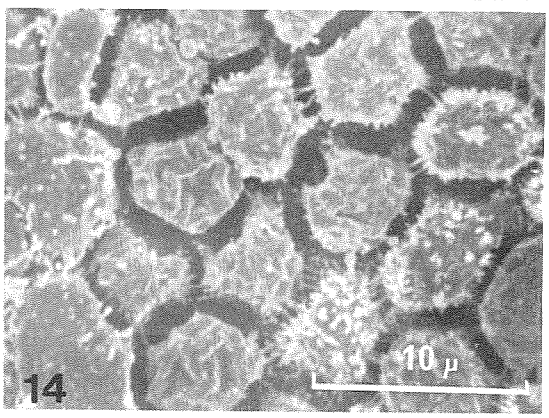
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PLATE 3
EXPLANATION OF FIGURES

Scanning electron micrographs of the cervical epithelium in estrous rabbits.

Fig. 15. Longitudinal folds of cervical mucosa. Note the cervical crypts.

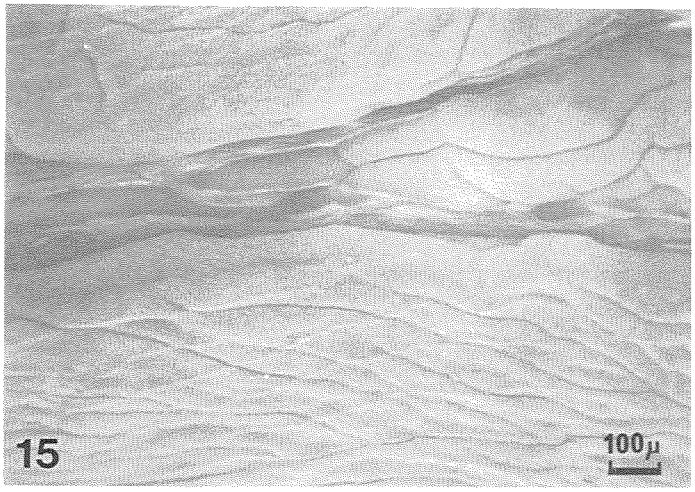
Fig. 16. Cervical epithelium composed of both ciliated and non-ciliated cells. The surface epithelium is densely ciliated. Well-preserved erythrocyte can be seen near the center of this figure.

Fig. 17. Non-ciliated cells display dome-like free surface with regularly arranged microvilli.

Fig. 18. Higher magnification of the cervical epithelium. Note the cilia displaying thinner and bending tips. The microvilli exhibit greater diameter and length than those of endometrium.

Fig. 19. A field of shorter cilia, easily distinguished from more typical longer cilia, is evident. Non-ciliated cells display a few small hollows on their free surfaces (arrows). Note sparsely arranged microvilli.

Fig. 20. Cytoplasmic projections from secretory cells, suggesting an apocrine secretion. No microvilli can be seen on the surfaces of cytoplasmic projections.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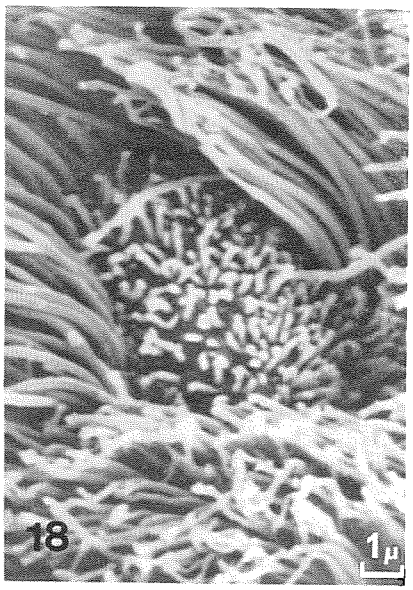
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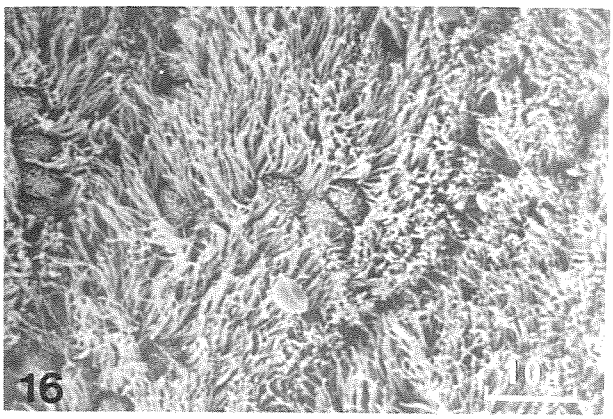
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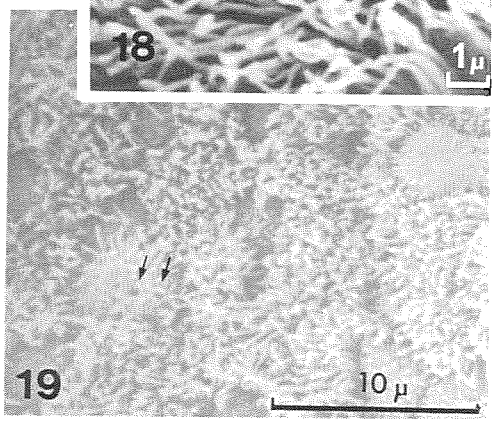
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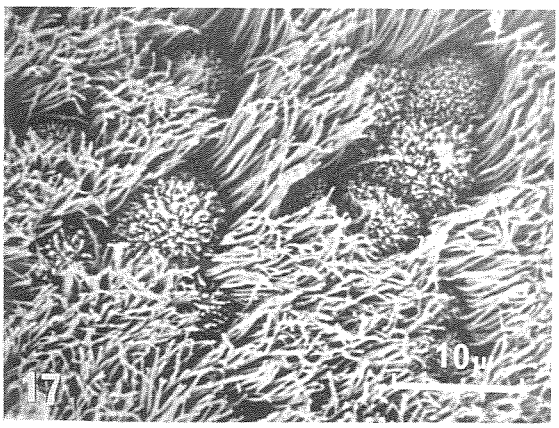
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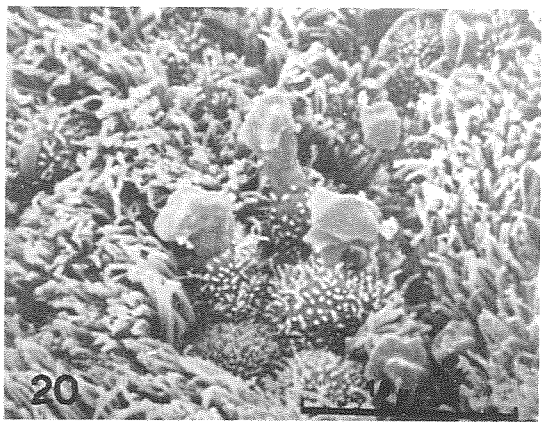
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PLATE 4
EXPLANATION OF FIGURES

Scanning electron micrographs of the cervical epithelium

Fig. 21. Cervical epithelium on 2nd day of pregnancy. A great number of secretory granules are attached on the surfaces of non-ciliated cells and cilia. Microvilli on the non-ciliated cell are densely distributed.

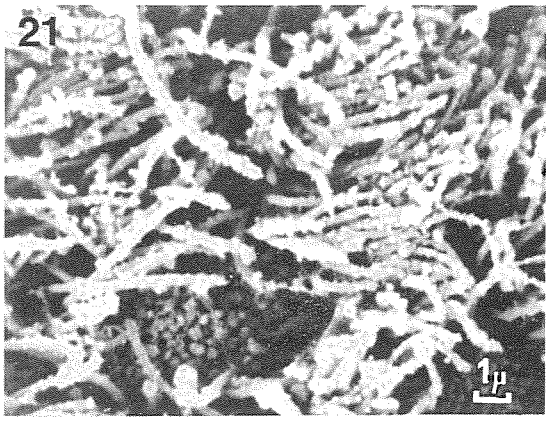
Fig. 22. Cervical epithelium on 2nd day of pregnancy. Some non-ciliated cells exhibit irregular surfaces due to the presence of several small hollows.

Fig. 23. Cervical epithelium on 4th day of pregnancy. The non-ciliated cells have numerous well-developed microvilli with occasional microvillous promontories (arrows).

Fig. 24. Cervical epithelium on 4th day of pregnancy. Presumptive ciliated cells are frequently found. The normal long cilia are located only at the peripheries of cells, while microvillous surfaces are apparent at the centers.

Fig. 25. Higher magnification of an area in Fig. 24, showing the emergence of immature cilia at the center. Many short microvilli are also present.

Fig. 26. A non-ciliated cell on 4th day of pregnancy. Part of the plasma membrane is ruptured and several droplets are seen in the cytoplasm.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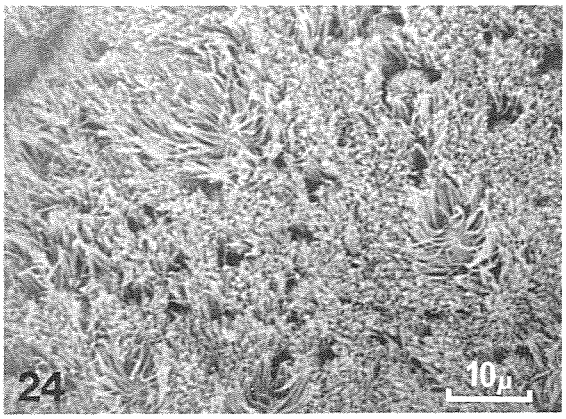
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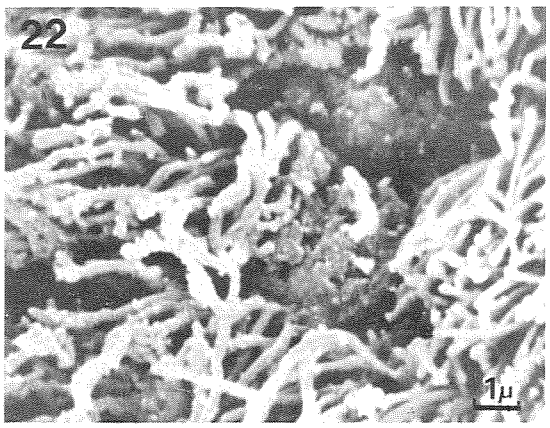
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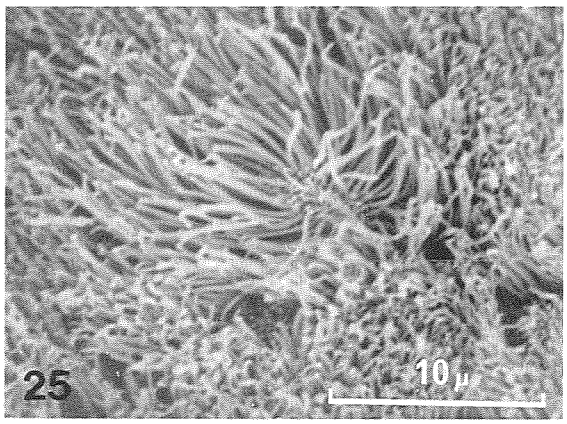
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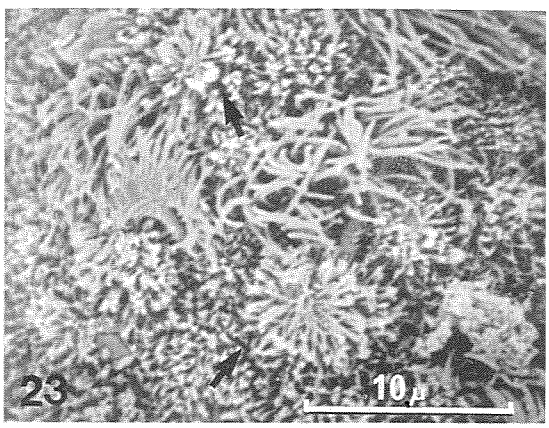
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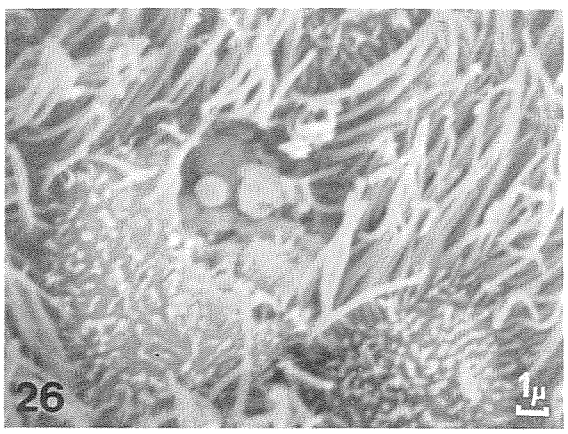
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PLATE 5
EXPLANATION OF FIGURES

Scanning electron micrographs of the cervical epithelium

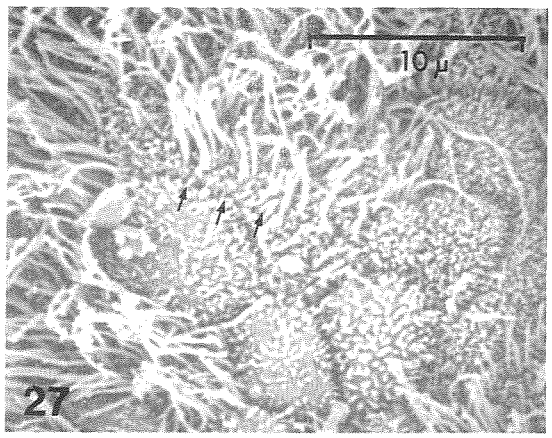
Fig. 27. Non-ciliated cells having short, thin microvilli on their free surfaces on 5th day of pregnancy. Some cells are generating new ciliary shafts (arrows).

Fig. 28. Cervical epithelium on 5th day of pregnancy. Secretory material is connected to the surfaces of the non-cilliated cells. This feature may reflect one of the processes of secretion.

Figs. 29 & 30. Cervical epithelium on 6th day of pregnancy. Note the non-ciliated cells showing one or more small hollows on their surfaces, which are partially devoid of microvilli.

Fig. 31. Microvilli on the surfaces of non-ciliated cells are distributed densely and have increased in height on 7th day of pregnancy.

Fig. 32. Microvilli are further developed, resulting in fields of long microvilli, on 7th day of pregnancy. Note cytoplasmic projection (large arrow) and immature cilia (small arrow).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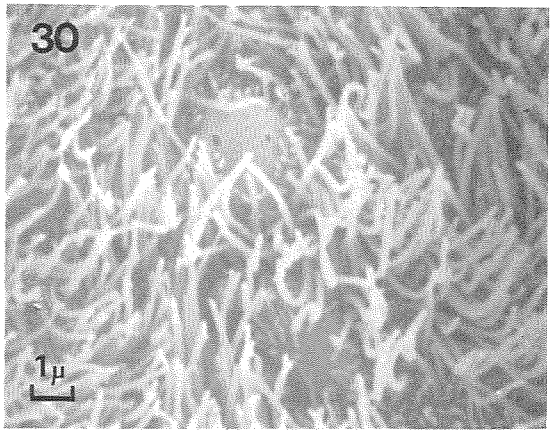
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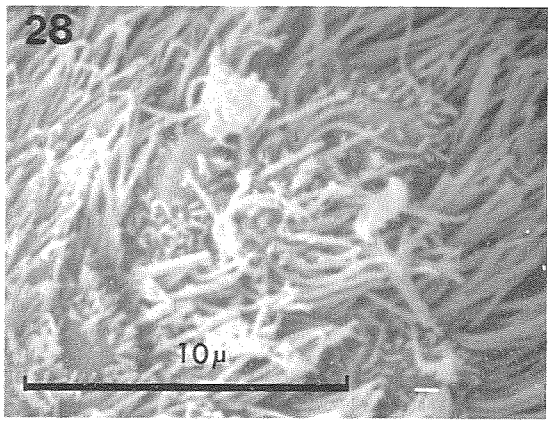
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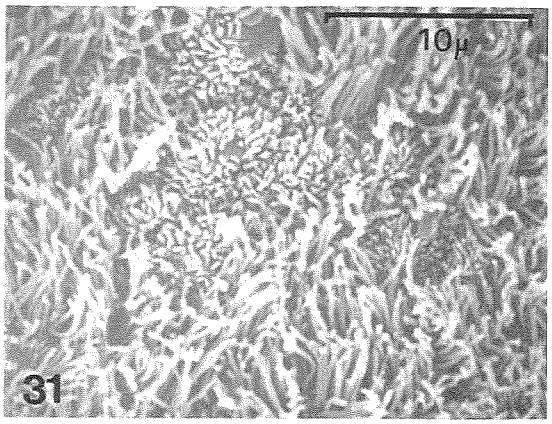
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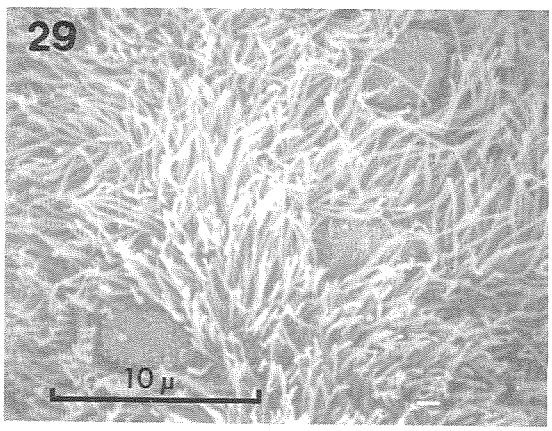
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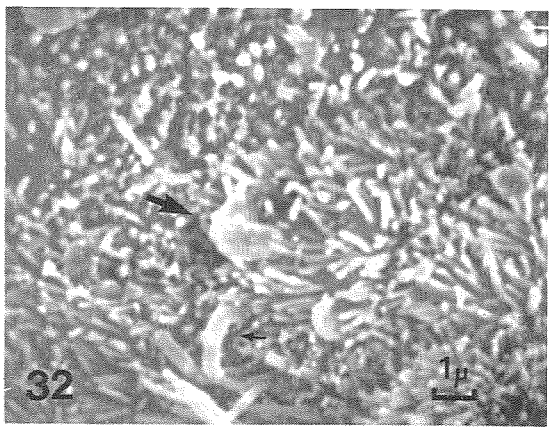
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PLATE 6
EXPLANATION OF FIGURES

Light micrographs of the uterine epithelium

Fig. 33. Endometrium on 2nd day of pregnancy showing pseudostratification and thickening of the epithelial layer. Glandular epithelium is lined with simple columnar cells. H. E. ×228.

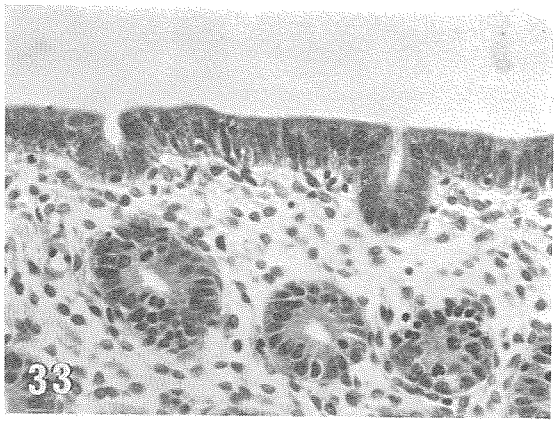
Fig. 34. Endometrium on 4th day of pregnancy. The mucosal folds are more branched. Surface and cryptal epithelia are extensively pseudostratified. H. E. ×228.

Fig. 35. Endometrium on 5th day of pregnancy, showing the complex pattern of mucosal folds. The epithelium is reduced to a single layer of closely packed cells possessing characteristic apical protrusions. The nuclei are crowded. H. E. ×228.

Fig. 36. Endometrium on 6th day of pregnancy. Cell membranes have disappeared and the epithelial cells are transformed into cells with multiple nuclei. H. E. ×228.

Fig. 37. Mesometrial surface of endometrium on 7th day of pregnancy, interconceptal area. Surface epithelium is now composed mainly of multinucleated cells. A few single cells (arrows) are located among the multinucleated cells. H. E. ×228.

Fig. 38. Mesometrial surface of endometrium on 9th day of pregnancy, interconceptal area. All surface epithelial cells have been converted into multinucleated cells. The epithelial cells in the deeper parts of the crypts also show cell fusion. H. E. ×228.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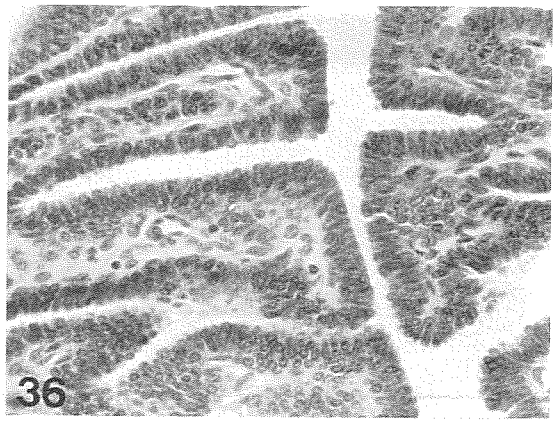
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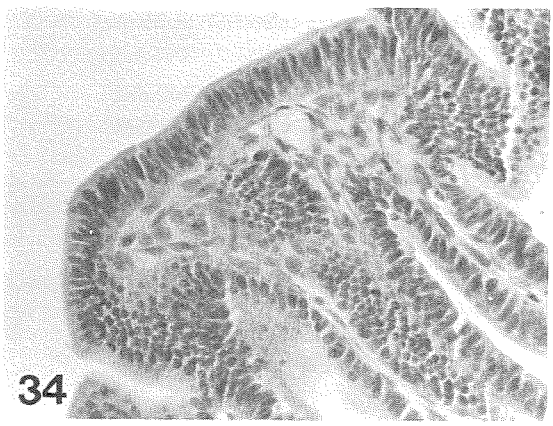
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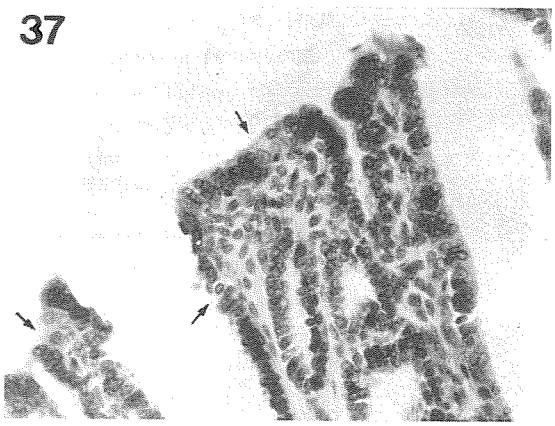
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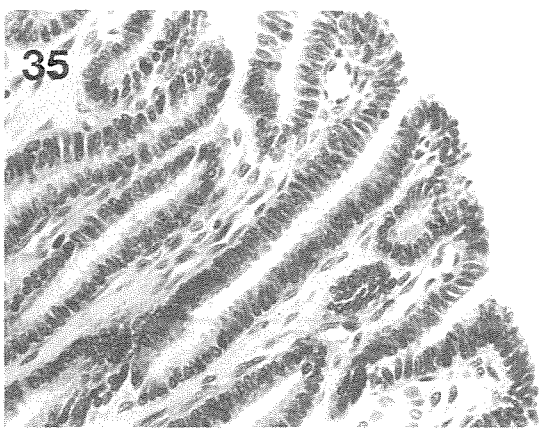
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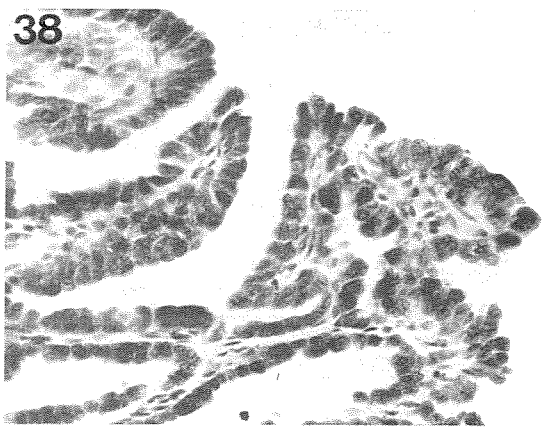
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PLATE 7
EXPLANATION OF FIGURES

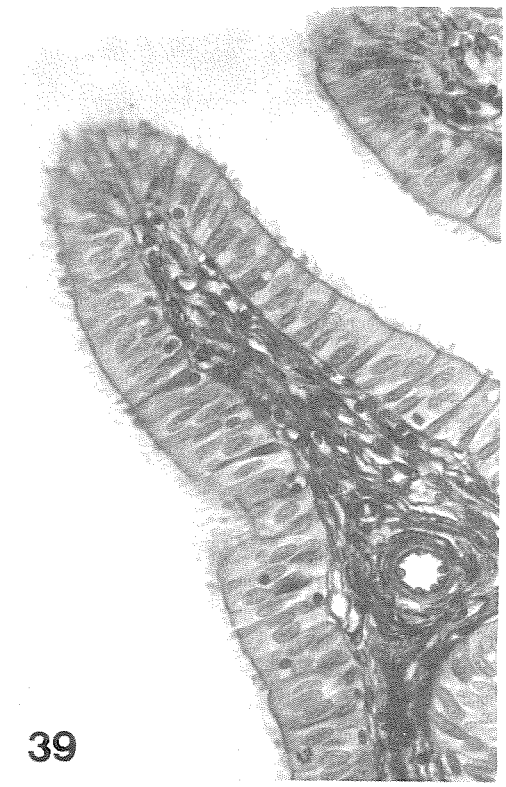
Light micrographs of the cervical epithelium

Fig. 39. Cervical epithelium of the non-pregnant estrous rabbit. The epithelium is a simple layer composed of tall-columnar ciliated cells and non-ciliated cells. Most non-ciliated cells are filled with secretory material in their supranuclear portions. Azan. ×283.

Fig. 40. Cervical epithelium on 2nd day of pregnancy showing the hemispheric apices of non-ciliated cells (arrows). Secretory material has decreased slightly in the cytoplasm of non-ciliated cells. Azan. ×283.

Fig. 41. Cervical epithelium on 4th day of pregnancy, showing reduction in height. Cells containing many secretory granules are fewer. Peg cells have increased in number. Azan. ×283.

Fig. 42. Cervical epithelium on 5th day of pregnancy, showing the beginning of mucin production and thickening of the epithelial layer. Non-ciliated cells contain mucin in their basal portions and their nuclei are pushed up toward the cervical lumen. Azan. ×283.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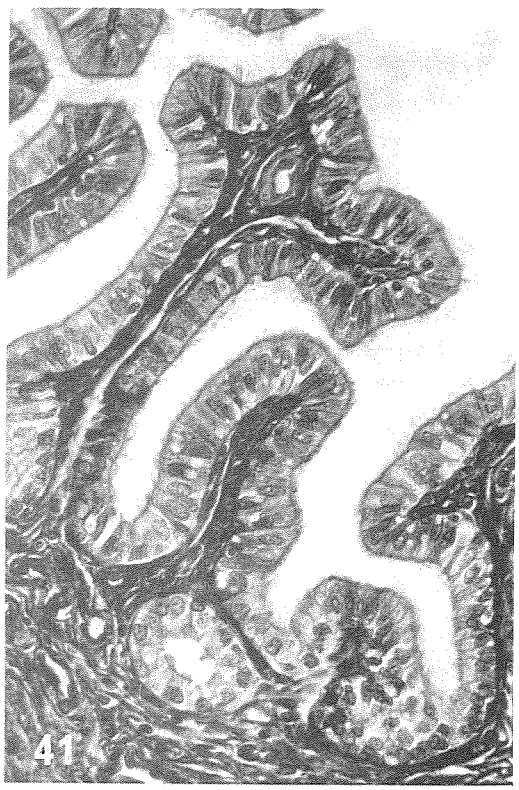
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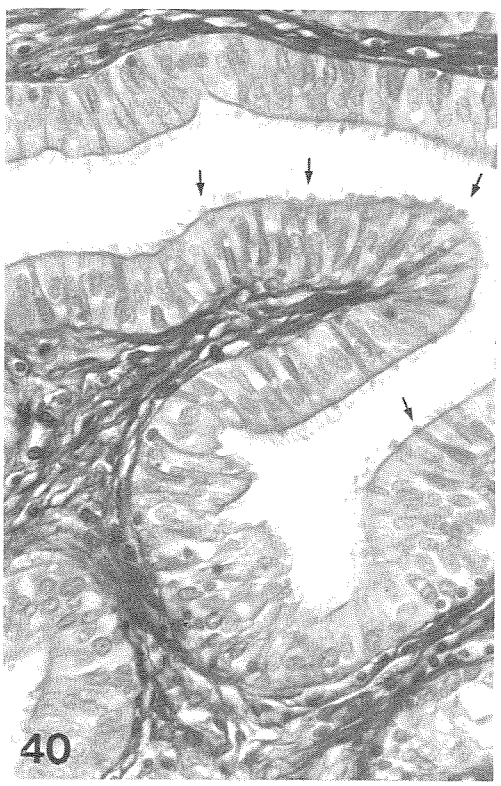
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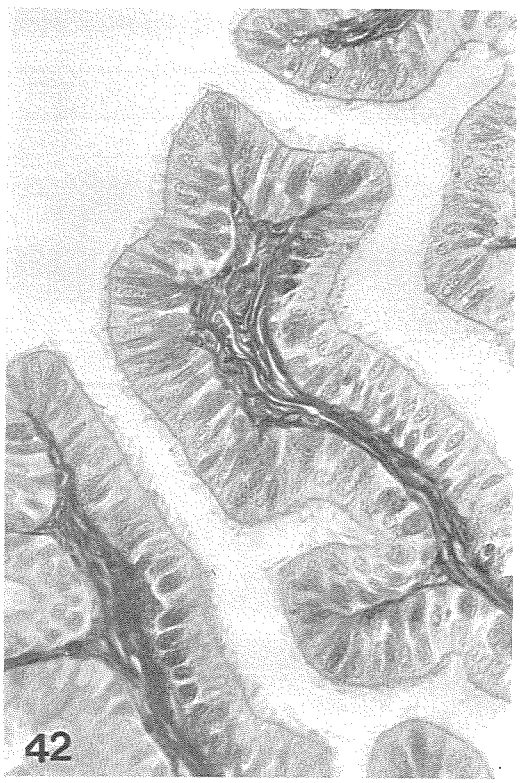
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PLATE 8
EXPLANATION OF FIGURES

Light micrographs of the cervical epithelium

Fig. 43. Cervical epithelium on 6th day of pregnancy, showing intense production of mucin. The secretory cells again display dome-like apical projections. Azan. ×283.

Fig. 44. Cervical mucosa on 7th day of pregnancy. The epithelium has become thinner. Secretory materail is located near the apical ridges of the non-ciliated cells. Peg cells are frequently observed. Azan. ×283.

Fig. 45. The epithelium remains thin and most secretory cells contain little mucin on 9th day of pregnancy. The features of this stage are much like those of 7th day of pregnancy. Azan. ×283.

Fig. 46. Cytoplasmic cell inclusions in the epithelium on 9th day of pregnancy. The inclusions are located in both the basal parts (large arrows) and the apical parts (small arrows) of the cells. PAS. ×283.