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Scanning Electron Microscopic Studies of Uterine Epithelial Cytoplasmic Protrusions during the Estrous Cycle, Pregnancy, Pseueopregnancy and Early Stages of Decidualization in Rat

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Summary

Scanning electron microscopic studies on the change in number, size and shapes of epithelial cytoplasmic protrusions were undertaken in rat uterus during the estrous cycle, pregnancy, pseudopregnancy and early stages of decidualization. The cytoplasmic protrusions were not observed on the surface of uterine lumen in any stages of the estrous cycle nor in the early stages of progestation. However, many morel-like shaped protrusions abruptly appeared on Day 5 of pregnancy and pseudopregnancy. The cytoplasmic protrusions in the oviduct-ligated pregnant and pseudopregnant rats were fewer than those in the normal pregnant rats uterus on Day 5. However, when the uterus was mechanically stimulated on Day 5 of pseudopregnancy, the number of these protrusions dramatically increased by 6, and 12hr after the stimulation to become about the same as those on Day 5 of pregnancy. Accordingly, it is conceivable that the formation of cytoplasmic protrusions on the luminal surface of rat uterus is influenced not only by ovarian hormones, but also by the mechanical stimulus to the uterus, i.e. existance of the blastocyst in the lumen of the uterus.

The apcial surface of the uterine luminal epithelial cells not only constitutes the point of initial physical association with the trophoblast at implantation, but in addition is the interface between the uterine lumen and the rest of the maternal organism (1). Large cytoplasmic protrusion from the apical surface were demonstrated by Nillson (2) in the mouse and Warren and Enders (3) in the rat. In the rat uterus, more extensive illustrations of these protrusions were presented by Nillson (4～6), Psychoyos and Mandon (7) and Ljungkvist (8). Since these cytoplasmic protrusions appear only in the antimesometrial region of rat and mouse uterus at the progestational stage, their roles are considered to be closely concerned with ovo-implantation. Although it is reported that these protrusions are influenced by ovarian hormones (7, 9), little is yet known about the

physiological role of these protrusions and the corelation between these protrusions and the ovarian function. Especially, the relationship between the number, size or shapes of protrusions and the presence of blastocyst in the uterine lumen or mechanical stimulus to the uterine epithelium to induce decidual cell formation is not known at all. Accordingly, scanning electron microscopic studies on the change of number, size and shapes of epithelial cytoplasmic protrusions in the rat uterus during the estrous cycle, pregnancy, pseudopregnancy and early stages of decidualization have been undertaken.

Materials and Methods

Mature virgin female rats of the Wistar strain, weighing 230±24 gram, were housed in an air-conditioned room (22±1°C). The light was automatically turned on at 7:00. and off at 7:00 p.m. A vaginal smear of all animals was recorded prior to and throughout the course of all experiments. Only animals showing more than four successive regular estrous cycles were used in this experiment. The experimental design is shown in Fig. 1.

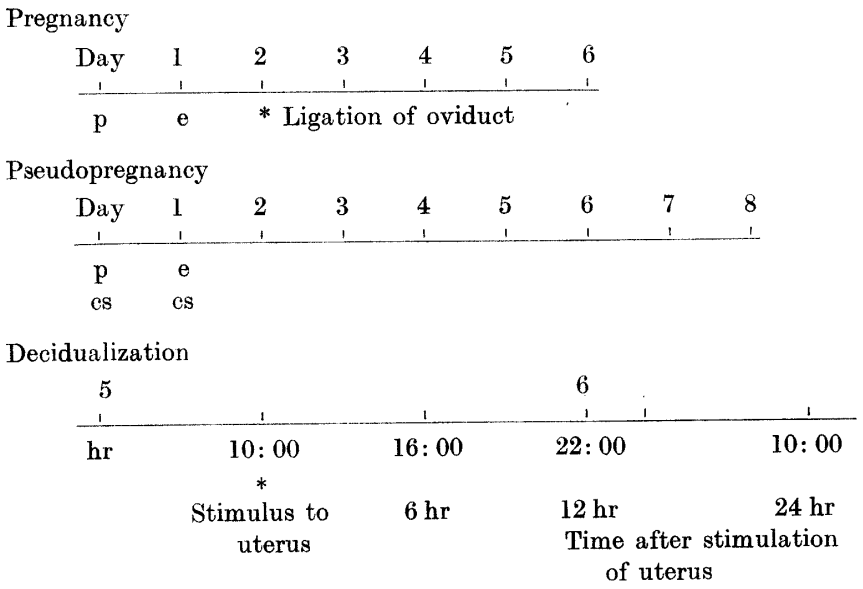

FIG. 1. Experimental design

Preparation of animals was made as follows.

a) Normal estrous cycle; Animals were prepared for each stage of the estrous cycle, that is, proestrous, estrous, metestrou sand diestrous. They were examined at 9:00 and were killed at 10:00 on the same day.

b) Pregnancy; The females in proestrous were caged overnight with fertile

males and the day of copulation was determined by the presence of spermatozoa in vaginal smears taken between 8:00 and 10:00 daily. The copulation day was designated as Day 1 of pregnancy. They were killed at 10:00 on Days 2, 3, 4, and 6 of pregnancy respectively and at 10:00 and 16:00 on Day 5 of pregnancy.

c) Ligation of oviduct; In order to investigate the effect of blastocysts in the uterine lumen on the appearance of cytoplasmic protrusions, the utero-tubal junctions were ligated by a fine thread and the oviduct was cut off from the uterus on Day 2 of pregnancy. They were sacrificed on Days 4 and 5 of pregnancy.

d) Pseudopregnancy; Pseudopregnancy was induced by faradic stimulation of cervix uteri at proestrous and estrous (10). The day of estrous was noted as Day 1 of pseudopregnancy. Animals were provided for each day from Day 2 to Day 8 of pseudopregnancy.

e) Decidualization; Laparotomy was performed at 10:00 on Day 5 of pseudopregnancy and the uterine horns were lightly held between tips of forceps at five points of each horn at regular intervals. The animals were killed at 0, 6, 12 and 24 hr after the stimulation of the uterus.

Procedure for observation of the uterine luminal epithelial surface.

The animals were killed by decapitation and the uterine horns were rapidly removed. Each uterine horn was dissected at the mesometrial region and mounted on a hard square paper by pinning the four corners of the specimen. For scanning microscopical observation, specimens were fixed in a cold 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2). After fixation, tissues were rinsed in 0.2 M phosphate buffer (pH 7.2), dehydrated by graded ethanol, followed by replacing in iso-amyl-acetate. The iso-amyl-acetate was removed by the CO_2 critical point drying method (Hitachi, HCP–1 type) and the dried specimens were mounted and shadowed with gold ion (Eico Engineering Co., 1B–3 type Ion Coater). The specimens were viewed in a Hitachi MINI-SEM scanning electron microscope. The Anti-mesometrial luminal surface of the uterus obtained from each animal was photographed at random, ten times at one thousand magnification followed by counting the number of cytoplasmic protrusions per 2,500 μm^2 of uterine surface. Several blocks obtained from normal pregnant rats were subsequently postfixed in 1% osmium tetroxide in phosphate buffer, dehydrated, and embedded in epoxy resin, thin sectioned and viewed by JEM 100C Type transmission electron microscope.

The statistical significance of difference among the mean numbers of cytoplasmic protrusions per constant area of uterine luminal surface was determined with the Student's t-test.

Results

The uterine surface was covered by penta or hexagonal epithelial cells with many microvilli. The cytoplasmic protrusions were not observed on the surface of

TABLE 1. *The numbers of uterine epithelial cytoplasmic protrusion*

Day Hr	2	3	4	5 10:00 (Ohr)	16:00 (6hr)	22:00 (12hr)	6 10:00 (24hr)	7	8
Pregnancy	0.4 ±0.6	1.2 ±1.6	2.5 ±1.2	24.9 ±4.5	36.7 ±4.8	—	0.6 ±0.8	—	—
Ligation	—	—	0	17.7 ±3.5	16.9 ±2.2	—	—	—	—
Pseudopregnancy	0	0	3.7 ±3.4	7.3 ±3.8	10.6 ±3.4	—	4.4 ±2.8	0	0
Decidualization	—	—	—	7.3 ±3.8	29.7 ±4.2	30.0 ±3.9	1.7 ±1.3	—	—

the uterine lumen in any stages of the estrous cycle (Fig. 2), although the length of microvilli showed changes in each stage of the estrous cycle. Although the cytoplasmic protrusions were hardly observed on Days 2, 3 and 4 of pregnancy (Fig. 3), they significantly increased in number on Day 5 of pregnancy (Fig. 4), followed by flattening of the luminal surface and the appearence of distorted, rough microvilli on Day 6 of pregnancy (Fig. 5) as shown in Table 1. On the other hand, in the oviduct-ligated rats (Fig. 6), they were fewer than that of normal pregnant rats on Day 5 ($P<0.001$). In pseudopregnant rat uterus, a few protrusions appeared on Day 4 (Fig. 7). However, the number of protrusions did not increase drastically on Day 5 (Fig. 8) and were fewer than that on Day 5 of pregnancy ($P<0.001$). These cytoplasmic protrusions disappeared and were replaced by a rough luminal surface on Day 6 of pseudopregnancy (Fig. 9) as did those on Day 6 of pregnancy. However, when the uterus was mechanically stimulated by forceps on Day 5 of pseudopregnancy, the number of protrusions dramatically increased at 6 and 12 hr after the stimulation (Fig. 10) to almost the same number as on Day 5 of pregnancy, then subsequently decreased at 24 hr after the stimulation. There was a significant difference between the decidualization and the pseudopregnant rat ($P<0.001$). At 24 hr after the stimulation of the uterus, the uterine epithelial cells, which shapes were rounder than before, were extruded into the uterine lumen and showed a rough surface (Fig. 11). Most of the cytoplasmic protrusions demonstrated in the present experiment had a height of about 0.5–3.0 μm and a width of about 0.5–5.0 μm. They had a morel-like shape with a wrinkled surface (Fig. 12). The interior of the protrusions was dense and rather homogeneous. Granules of the size of ribosomes were present, but these were no other organelles or inclusions. The cytoplasm of the uterine epithelial cell was characterized by many apical vesicles and rough endoplasmic reticulum. The microvilli were regular, with a length of about 0.8 μm and a width of about 0.1 μm (Fig. 13). In some cases, cytoplasmic protrusion with many microvilli was observed.

Discussion

When rats are deprived of the endogenous steroid hormones by combined ovariectomy and adrenalectomy, the height of the luminal epithelial cells becomes shorter, and the densities of the microvilli on the cell membrane are much reduced (9). However, progesterone administered to the ovariectomized-adrenalectomized rat leads to the formation of numerous bleb-like cytoplasmic protrusions on the surface of the luminal epithelial cells, concomitant with a remarkable increase in the density of the surface microvilli (6, 7, 9). In the present experiment, cytoplasmic protrusions abruptly appeared on Day 5 and disappeared on Day 6 of pregnancy or pseudopregnancy, although they were not observed on the luminal surface during the estrous cycle and early progestation. Therefore, it is likely that a significant increase of these protrusions on Day 5 of pregnancy or pseudopregnancy is due to the successive secretion of progesterone after copulation or to the faradic stimulation of the cervix uteri and to the nidatory estrogen surge on Day 4 (11). On Day 5 of normal pregnancy, namely at 102 to 114 hr after fertilization, blastocysts can be recovered from the uterus by saline flushing. The rapid growth of blastocysts is observed in accordance with the disappearence of zona pellucida from 108 hr to 114 hr after fertilization (12). Moreover, it is well known that the sensitivity of uterus for the decidual cell reaction appears on Day 5 of pregnancy or pseudopregnancy (13). In the present experiment, the number of uterine epithelial cytoplasmic protrusions in oviduct-ligated pregnant rats and pseudopregnant rats are significantly fewer than those on Day 5 of normal pregnancy. On the other hand, when the uterus was stimulated on Day 5 of pseudopregnancy, these protrusions rapidly increased in number at 6 hr after the stimulation, and are more numerous than those on Day 5 of oviduct-ligated pregnant rats and pseudopregnant rats. Accordingly, it is conceivable that the formation of cytoplasmic protrusions on the luminal surface of rat uterus is influenced not only by ovarian hormones, but also by the mechanical stimulus to the uterus, i.e. existance of the blastocyst in the lumen of the uterus.

It is reported that the shapes of cytoplasmic protrusion are morel-like with a wrinkled surface (6) and sea anemone-like (7). These different shapes might be due to the preparation of the uterine specimens for electron microscopy or the difference of experimental treatment such a hormone administration. Many of cytoplasmic protrusions had a morel-like shape with a wrinkled surface except for a few rosette formations in the present experiment, in which the critical point drying method was applied.

Although the physiological roles of these protrusions are not clear, Nillson (6) suggested that the protrusions secrete a proteinous material and that they provide an important way of transmitting information to the blastocyst. On the other hand, Enders (1) and Parr and Parr (14) suggested that the protrusions mediate endocytosis, not apocrine secretion, in the rat. Since it is considered that the

blastocysts in the stage just before implantation are closely related to the formation of the cytoplasmic protrusions on the luminal surface of the rat uterus, further studies are needed to elucidate the interrelationship between blastocysts and cytoplasmic protrusions.

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Plate 1

Explanation of Figures

Fig. 2 The cytoplasmic protrusions were not observed on the surface of uterine lumen at diestrous. × 750

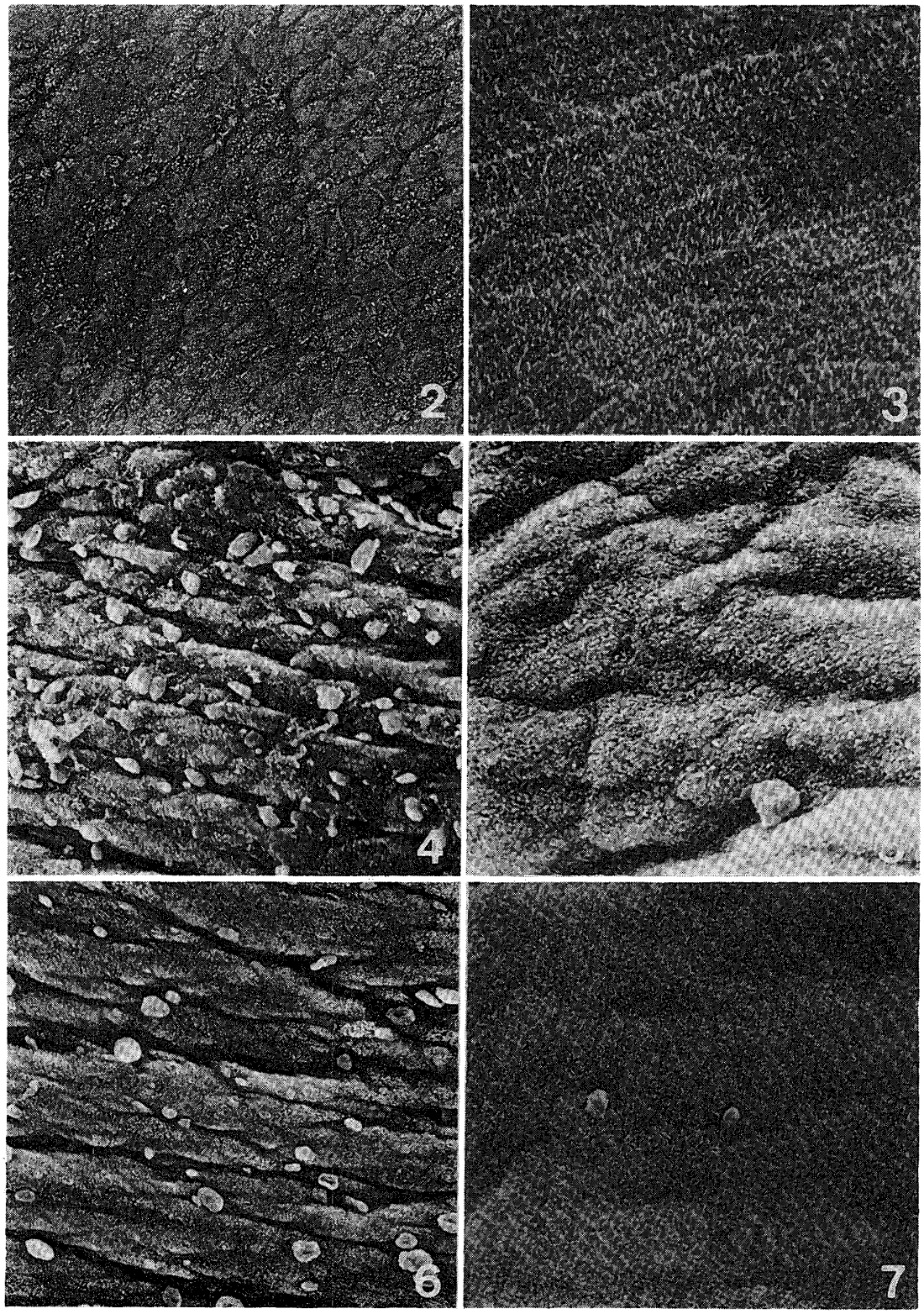
Fig. 3 The cytoplasmic protrusions were not observed on the surface of uterine epithelia on Day 3 of pregnancy. × 750

Fig. 4 The cytoplasmic protrusions increased its number on Day 5 of pregnancy. × 750

Fig. 5 The flattening of luminal surface and the distorted rough microvilli were observed on Day 6 of pregnancy. × 750

Fig. 6 The cytoplasmic protrusions were observed in the oviduct-ligated rat on Day 5 of pregnancy. × 750

Fig. 7 A few number of protrusions appeared on Day 4 of pseudopregnant rat uterus. × 750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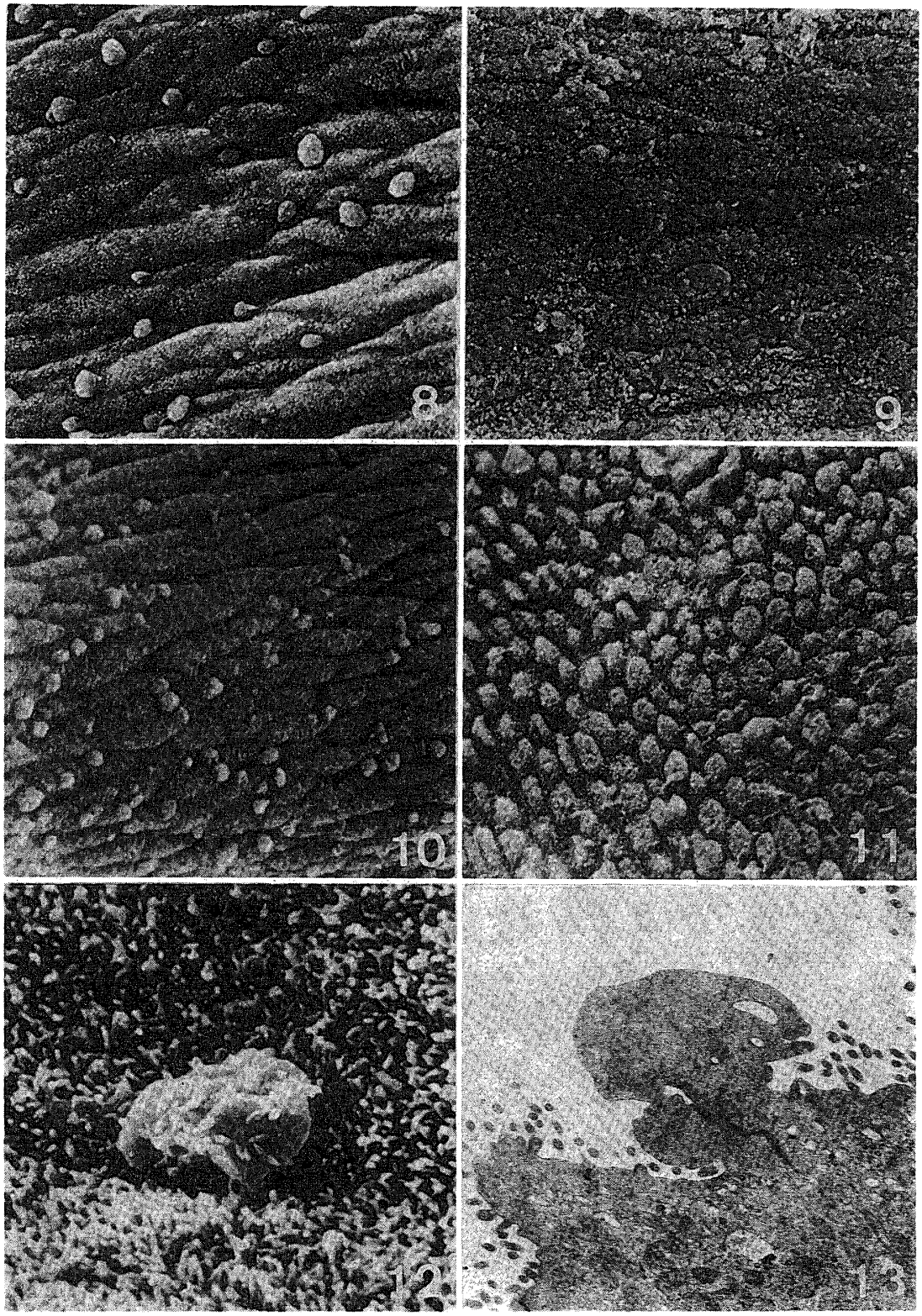
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PLATE II

Explanation of Figures

Fig. 8 The number of protrusions did not increase drastically on Day 5 of pseudopregnancy. × 750

Fig. 9 The cytoplasmic protrusions disappeared and were replaced by rough luminal surface on Day 6 of pseudopregnancy. × 750

Fig. 10 The number of protrusions increased at 12 hr after the stimulation of uterus. × 750

Fig. 11 At 24 hr after the stimulation of uterus, uterine epithelial cells are extruded into the uterine lumen and demonstrated rough surface. ×750

Fig. 12 Most of cytoplasmic protrusions have a morel-like shape with a wrinkled surface. × 750

Fig. 13 The interior of the protrusions was dense and rather homogeneous. × 13,500