

Morphological Studies of the Uterine and Oviductal Mucosa after Successive Administrations of Charcoal-Treated Porcine Follicular Fluid in Rabbits

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Abstract The morphological changes of uterine and oviductal mucosae after successive administrations of charcoal-treated porcine follicular fluid (CTPFF) in rabbits were examined. In Experiment 1, mucus secretion in uteri of four does which were injected with 4 ml of CTPFF 10 times at 12-hour-intervals was not so active as that of controls (three does). In Experiment 2, thirteen does were injected with 4 ml of CTPFF in the same manner as in Experiment 1, and were divided into 4 groups according to the number of hours — 0 (5 does, group 0F), 12 (4 does, group 12), 36 (2 does, group 36) and 96 (2 does, group 96) hours, respectively — from the final injection of CTPFF to human chorionic gonadotropin injection. The proliferation of uterine mucosa in does of groups 0F, 36 and 96 was repressed in comparison with that in the control group. The numbers of mitoses in the glandular epithelium of groups 12, 36 and 96 were significantly lower ($p < 0.01$) than those of groups 0F and 0S. The ratio of ciliated cells in the mucosal surface epithelium of group 0F was significantly higher ($p < 0.01$) than in groups 0S and 12. In Experiments 1 and 2, swelling of epithelial cells and production and release of mucus in the ampulla were slightly repressed in does of treatment groups. It seems that administration of CTPFF represses the proliferation of endometrium indirectly through the ovary, but the effect of CTPFF on the oviduct is slight.

INTRODUCTION

The progestational change in the genital mucosa of the rabbit is characterized by fold-branching, proliferation of uterine mucosa¹⁻⁴, and increase of uterine and oviductal mucus-secretion^{5,6}. This mucosal transformation is known to establish a favorable environment for fertility and blastocyst implantation, and to be regulated by a hormonal milieu, especially pre-ovulatory estrogen and post-ovulatory progesterone^{2,4,5,6,7,13}.

In our previous studies^{8,9}, effects of administration of charcoal-treated porcine follicular fluid (CTPFF) on ovulation rate, plasma concentration of progesterone and ovarian morphology in rabbits were examined, with the following results. 1) The ovulation rate was significantly reduced⁸. 2) The vulvas became smaller and faded within 2 days⁸. 3) Post-ovulatory progesterone levels in peripheral plasma remained at lower levels than in controls⁸. 4) The mean proportion of number of healthy follicles to number of antral follicles became significantly low, and abnormal interstitial tissue and corpus luteum cells increased⁹.

Therefore, it is speculated that administration of follicular fluid affects not only the ovary but also the uterine and oviductal mucosa, due to changes of hormonal events, especially in estrogen and progesterone levels. But only a few studies have been reported on the effect of

follicular fluid on morphological changes of the uterine and oviductal mucosa. The present study examined morphological changes of the uterine and oviductal mucosa after administration of CTPFF in rabbits.

MATERIALS AND METHODS

Animals, Follicular Fluid and Experimental Design

Twenty-five does out of fifty-five female Japanese White rabbits which were used in previous studies⁸⁾ for observation of ovulation and of ovarian histology were selected at random, and three does which received bilateral ovariectomy were additionally used in the present study.

(Experiment 1) Four does were injected subcutaneously with 4 ml of CTPFF 10 times at 12 hour intervals (group F). Three does received 4 ml of sterile physiological saline in the same manner as the treatment group (group S). Three does were ovariectomized bilaterally (group C). The does of groups F and S were sacrificed by over-dose of sodium pentobarbital (Somnopentyl) immediately after injection of CTPFF or saline, and the does of group C were sacrificed 108 hours after ovariectomy to obtain the genital tracts for histology.

(Experiment 2) In all treatment groups, 4 ml of CTPFF were injected in the same manner as in Experiment 1. To induce ovulation, the does were given 15 IU of human chorionic gonadotropin (hCG). Thirteen does were divided into 4 groups according to time of hCG injection after the final administration of CTPFF. Five does were injected with hCG simultaneously with the final injection of CTPFF (group 0F). Four does were injected with hCG at 12 hours after the final injection of CTPFF (group 12), two does at 36 hours (group 36), and two does at 96 hours (group 96). Five does received 4 ml of sterile physiological saline and 15 IU of hCG in the same manner as group 0F (group 0S). The does were sacrificed four days after hCG injection to obtain the genital tract.

Histological Study

In both experiments, the reproductive tracts were excised and trimmed free of fat and extraneous tissue. The two uterine horns were separated at the median septum, and each uterine tube was divided by cutting the utero-tubal junction and the ampulla-isthmic junction. The uterine horns and oviducts were pinned on a narrow board and immediately fixed in Bouin's solution. After a few hours, 5-mm segments of the uterine horn and oviduct were taken by cutting the central portions of the uterine horn and the isthmic and ampullary portions of oviduct. The segments were refixed for about 15 hours, then embedded in paraffin wax. Paraffin sections of 4 μ m were stained with hematoxylin and eosin (H.E.) and Azan, or by the periodic acid-Shiff (PAS) method. The proliferation index in the endometrium, number of mitoses, proportion of ciliated cells of the mucosal surface and glandular epithelium, thickness of epithelium and mucus-secretion activity in the uterus and oviduct were examined microscopically.

Proliferation of the endometrium was evaluated into six grades according to McPHAIL's index (1934)⁷⁾. One tissue sample was taken at random from the uterine horn and both oviductal segments, respectively, and the number of mitoses in the surface and/or glandular epithelia were counted in 4 microscopic fields ($\times 100$). Numbers of ciliated cells per 100 epithelial cells of uterine surface and gland were counted at random on 4 points of a uterine section stained by H.E. Thickness of epithelia of the uterine surface and glands was measured at 5 points of the specimen. The number of oviductal secretory cells per 100 epithelial cells was counted on 4 points of a section stained by Azan, and the thickness of the oviductal epithelium was measured simultaneously. All data recorded were analyzed using Student's *t* test.

Table 1. Features of uterine mucosa after successive injections of charcoal-treated porcine follicular fluid or saline solution and after castration (Experiment 1)

Treatment	Proliferation index	Number of mitoses in epithelium		Proportion of ciliated cells (%)	Thickness of epithelium (μm)	
		Mucosal surface	Uterine gland		Mucosal surface	Uterine gland
Saline (S)	2.38 \pm 0.74 ^a	1.44 \pm 1.46 ^a	1.50 \pm 1.76 ^a	20.53 \pm 11.22 ^a	19.09 \pm 3.81 ^a	15.37 \pm 2.84 ^a
CTPFF (F)	2.50 \pm 0.55 ^a	1.04 \pm 1.33 ^a	0.75 \pm 0.86 ^{ab}	20.66 \pm 13.36 ^a	17.61 \pm 3.58 ^{ab}	13.63 \pm 2.96 ^b
Castration (C)	1.50 \pm 0.54 ^b	0.55 \pm 0.59 ^b	0.21 \pm 2.17 ^b	2.25 \pm 2.91 ^b	13.92 \pm 2.09 ^b	12.21 \pm 2.49 ^c

Data are expressed as mean \pm S.D. Significant differences are seen between different letters in the same column ($p < 0.05$).

Table 2. Features of oviductal mucosa after successive injections of charcoal-treated porcine follicular fluid or saline solution and after castration (Experiment 1)

Treatment	Proportion of secretory cells (%)		Thickness of epithelium (μm)	
	Ampulla	Isthmus	Ampulla	Isthmus
Saline (S)	49.88 \pm 3.14 ^a	56.94 \pm 2.65 ^a	31.20 \pm 4.98 ^a	36.64 \pm 5.53 ^a
CTPFF (F)	49.63 \pm 4.12 ^a	57.54 \pm 3.98 ^a	28.76 \pm 5.74 ^a	34.72 \pm 5.68 ^{ab}
Castration (C)	49.33 \pm 2.66 ^a	53.39 \pm 3.78 ^a	28.49 \pm 5.25 ^a	32.02 \pm 4.58 ^b

Data are expressed as mean \pm S.D. Significant differences are seen between different letters in the same column ($p < 0.05$).

RESULTS

Tables 1 and 2 show changes in histological features of the uterine and oviductal epithelium after successive injections of CTPFF or saline, and after castration, respectively (Experiment 1). Endometrial changes were most remarkable in castrated does (group C), and all measured values of group C were significantly lower than those of groups F and S. In the comparison of values between groups F and S, there were no significant differences in proliferation index, number of mitotic figure in the uterine surface and glandular epithelia, percentage of ciliated cells, or thickness of the uterine epithelium, except for a significantly reduced thickness of the glandular epithelium in group F ($p < 0.01$) (Plate 1, Fig. 1). Mucus secretion activity in group S was much higher (Plate 1, Fig. 2) than that of group F (Plate 1, Fig. 3), and no mucus secretion was noted in group C.

Although the effect of castration on the oviductal epithelium was not so clear as that in the uterus, the thickness of the isthmic epithelium did decrease slightly after castration. All measured values showed no significant differences between groups F and S. In both the ampullary and isthmic portions, mucus secretion of group F (Plate 1, Figs. 4 & 5) was weakened slightly in comparison with that of group S (Plate 1, Figs. 6 & 7). The activity in group C was much lower than that in groups F and S.

Changes in features of uterine and oviductal mucosae 4 days after hCG dosage which was given at the end of successive injections of CTPFF or saline solution are shown in Tables 3 and 4 (Experiment 2). The proliferation indices of the endometrium in all groups of Experiment 2 were higher than those of non-ovulatory does in groups F and S (Experiment 1). The indices of groups 0S and 12 were significantly higher ($p < 0.05$) than those of groups 0F, 36 and 96 (Plate 1, Figs. 8 & 9). Numbers of mitoses of all groups increased after ovulation, and the numbers in both uterine mucosal and glandular epithelia in groups 0F and 0S were significantly higher than those of other groups (Plate 1, Fig. 10; Plate 2, Fig. 11). Although the number in the mucosal surface in group 0F was significantly higher ($p < 0.05$) than that of group 0S, there was no signifi-

Table 3. Features of uterine mucosa in does with hCG treatment after successive injections of CTPFF or saline solution (Experiment 2)

Group	Proliferation index	Number of mitoses in epithelium		Proportion of ciliated cells (%)	Thickness of epithelium (μm)	
		Mucosal surface	Uterine gland		Mucosal surface	Uterine gland
0S	5.40 \pm 0.69 ^a	3.25 \pm 1.70 ^a	23.10 \pm 8.33 ^a	0.93 \pm 4.41 ^a	28.83 \pm 3.81 ^{ad}	22.45 \pm 3.61 ^a
0F	4.40 \pm 0.55 ^b	4.45 \pm 1.33 ^b	20.43 \pm 15.65 ^a	4.75 \pm 4.41 ^b	27.32 \pm 3.58 ^{cd}	20.80 \pm 3.18 ^b
12	5.25 \pm 0.71 ^{ac}	1.05 \pm 1.09 ^c	5.80 \pm 4.01 ^b	1.31 \pm 2.21 ^{ac}	27.49 \pm 3.01 ^d	20.19 \pm 3.51 ^b
36	4.50 \pm 0.58 ^{bcd}	1.13 \pm 1.20 ^c	2.25 \pm 1.57 ^c	2.50 \pm 3.44 ^{bc}	25.19 \pm 5.60 ^{cb}	20.00 \pm 2.70 ^b
96	4.25 \pm 0.50 ^{bd}	0.19 \pm 0.40 ^d	1.90 \pm 2.17 ^c	4.56 \pm 5.34 ^{bd}	24.81 \pm 2.54 ^b	21.00 \pm 2.49 ^{ab}

Data are expressed as mean \pm S.D. Significant differences are seen between different letters in the same column ($p < 0.05$). Does of groups 0F, 12, 36 and 96 were ovulated by an administration of 15 IU of hCG at the end of CTPFF injection (0F) and 12, 36 and 96 hours after the final injection of CTPFF, respectively. Group 0S was given saline and 15 IU of hCG in same manner as group 0F.

Table 4. Features of oviductal mucosa in does with hCG treatment after successive injections of CTPFF or saline solution (Experiment 2)

Group	Proportion of secretory cells (%)		Thickness of epithelium (μm)	
	Ampulla	Isthmus	Ampulla	Isthmus
0S	50.70 \pm 3.20 ^a	56.60 \pm 2.21 ^a	29.16 \pm 3.63 ^a	34.43 \pm 3.95 ^a
0F	51.64 \pm 2.74 ^a	57.43 \pm 2.14 ^a	26.09 \pm 3.42 ^b	35.09 \pm 3.53 ^a

Data are expressed as mean \pm S.D. Significant differences are seen between different letters in the same column ($p < 0.05$). Does of group 0F were ovulated by an administration of 15 IU of hCG at the end of CTPFF injection, and does of group 0S were given saline and 15 IU of hCG in same manner as group 0F.

cant difference in the number of glandular mitoses between them.

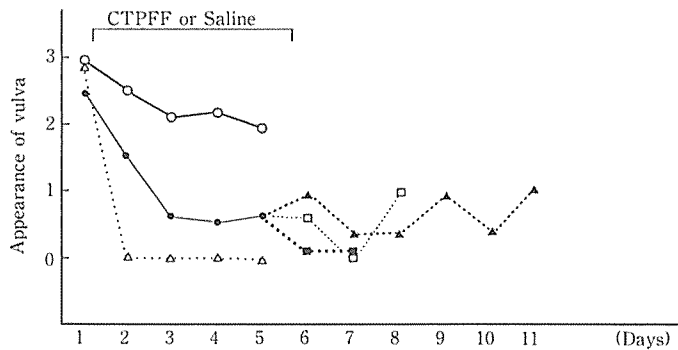
Numbers of ciliated cells in the endometrial epithelium significantly decreased after ovulation. The ratio of the number of ciliated cells in group 0F was significantly higher ($p < 0.01$) than in groups 0S and 12 (Plate 1, Fig. 3; Plate 2, Figs. 12 & 13). The proportions of the number of ciliated cells progressively increased with the time elapsed between the last injection of CTPFF and hCG treatment (Plate 2, Fig. 14).

Thickness of the epithelia in the both mucosal surface and the uterine gland increased after ovulation, and the epithelial thickness in group 0S was the highest among all groups. Although many secretory cells which were filled with secretory granules were scattered in the mucosal epithelia in groups 36 and 96 (Plate 2, Fig. 14), mucus secretory activity was scant in does of other groups.

Although the ratio of number of secretory cells in oviductal epithelium showed a tendency to increase in the isthmus portion, there was no significant difference between Experiments 1 and 2, nor between groups 0F and 0S. Thickness of epithelium in the ampullae, only of group 0S was significantly less ($p < 0.05$) than that in group 0F. Other measured values didn't show any significant differences. Secretory cells in both ampulla (Plate 2, Figs. 15 & 16) and isthmus (Plate 2, Figs. 17 & 18) became small after ovulation. In group 0F, the amount of secretory substance in the secretory cells was slightly more than that in group 0S.

DISCUSSION

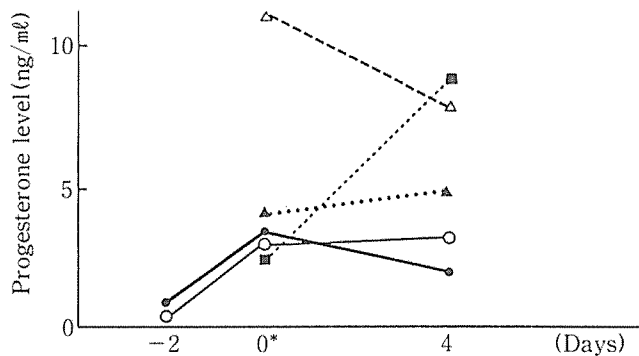
When rabbits show swollen and flushed vulvas and have large follicles in their ovaries, estrogen (E) levels in blood plasma are elevated^{10, 11}). The proliferation of the uterine mucosa is induced by post-ovulatory progesterone after the action of pre-ovulatory estrogen^{2, 7}).



Text-fig. 1. Effect of successive administratins of charcoal-treated porcine follicular fluid (CTPFF) to 13 does on appearance of vulva in groups 0F, 12, 36 and 96, which were ovulated by administration of 15 IU of hCG at the end of treatment (0F) and 12 (■), 36 (□) and 96 (▲) hours after treatment, and mean score of treatment groups before ovulation (●), in comparison with 8 does (○) which were given saline in the same manner, and 3 does which were ovariectomized (△).

Criteria for scoring the appearance of vulva are from 0 (non-swollen, small and whitish vulva) to 3 (largely swollen, congested, and purple or dark red vulva).

Scores of vulva of does which were used for the present histological study are shown.



Text-fig. 2. Progesterone levels in peripheral blood plasma in groups 0S (○), 0F (●), 12 (■), 36 (△) and 96 (▲) in Experiment 2.

Progesterone levels of does which were used for the present histological study are shown.

* 0 means 4 hours after hCG injection and a criterion for day.

Changes in vulva scores and in progesterone (P) levels in peripheral blood plasma in does used in the present histological studies, drawn from our previous study⁸⁾, are shown in Text-figs. 1 and 2. Vulvas became small and faded after and during CTPFF treatment, and this may be due to lower E levels caused by CTPFF treatment (Text-fig. 1). Although P levels before ovulation showed only a small difference between the groups treated with CTPFF (0F, 12, 36 and 96) and the saline control (0S), P levels rose on and after ovulation in all groups (Text-fig. 2). Although the proliferation index for the uterine mucosa in group F showed no significant difference from that in group S (Table 1), the value in group 0S was significantly higher in comparison with group 0F (Table 3) ($p < 0.01$). It may be reasonable to speculate that the difference in proliferation index in Experiment 1 was amplified by elevated P levels at and after ovulation (Text-fig. 2). Higher proliferation indices in groups 12 and 0S than in other groups (Table 3) may be explained as follows: E levels before ovulation in does of group 12 must have been lower than in group 0S, because the appearance of vulvas was much more faded than in control does (Text-fig. 1). However, P levels in does of group 12 at 4 days after ovulation were remarkably higher than those of group 0S (Text-fig. 2). The low proliferation index of group 96, similar to that in group 0F (Table 3), may be attributed to presumably low E levels (indicated by the low-grade appearance of vulvas) during CTPFF treatment to laparotomy (Text-fig. 1) and to low P levels at 4 days after ovulation in contrast to high P levels in groups 12 and 36 (Text-fig. 2).

According to LEE and DUKELOW (1972)²⁾, cell division in the uterine mucosal epithelium is induced by post-ovulatory progesterone together with earlier pre-ovulatory estrogen, and the cell division reaches a peak at 3 day of pregnancy in rabbits. In our results, the numbers of mitoses in glandular epithelia of groups 12, 36, and 96 were significantly lower ($p < 0.05$) than those in groups 0S and 0F, and were similar to those in groups F and S (Tables 1 & 3) in spite of induced ovulation. The small number of mitoses in group 12 may be explained by consideration that the peak in cell division has passed, as indicated by the most remarkable branching of mucosal folds. On the other hand, cell division in does in groups 36 and 96 may not have yet attained the peak, judging from modest branching of the endometrium.

Estrogen has dual effects on the ciliated cells of the uterus and cervix — that is, vanishment and reappearance of cilia — while progesterone induces a only decrease of ciliated cells in the endometrial and cervical epithelia of the rabbit ovariectomized for a long time^{12, 13)}. An increase in number of ciliated cells in the uterine epithelium has been shown to occur in the uterus 2 days after ovulatory stimulus, and at that time E levels in ovarian venous plasma should be at its nadir¹³⁾. Therefore changes in E levels must affect the number of ciliated cells in the uterus. But in the present study, ratios of number of ciliated cells in the mucosal surface epithelium in does of groups F and S were both about 20%, and a significant difference was not shown between these groups; and these values are significantly larger than those of any other groups, including castration (Table 1). Thus, it seems that decline in E levels caused by CTPFF treatment doesn't affect numbers of ciliated cells. The decrease in the number of ciliated cells in mucosal surface epithelium after ovulation in the present study agrees with previous reports^{1, 3, 4)} that many ciliated cells exist during estrus, with decreased ciliated cells after ovulation. The ratios of number of ciliated cells in ovulatory groups were various (Table 3). These results of Experiment 2 indicate that groups which had high P levels did not always have a low ratio. The reasons for this are not clear.

According to SUZUKI and TSUTSUMI (1980)^{3, 13)}, cervical epithelial cells which had abundant secretory granules were taller than those which had only few granules, and estrogen given to ovariectomized rabbits resulted in restoration of secretory activity and an increase in thickness

of epithelial cells in the uterus. When sections of group F are compared with group S, using Azan stain and PAS, it is clear that mucus secretion in group S was more active than that in group F. E levels in does of group S were presumably higher than in group F (Text-fig. 1); and differences in E levels probably account for both differences in mucus production and thickness of glandular epithelium (Table 1) between groups F and S. Mucosal surfaces and glandular epithelia of does in Experiment 2 were thicker after induced ovulation as compared with those in Experiment 1 (Tables 1 and 3), probably owing to elevated P levels after ovulation (Text-Fig. 2).

In the human, estrogen plays an important role in maintenance and growth of the oviductal ciliated cell^{14, 15}, and reappearance of ciliated cells after a remarkable decrease of such cells in fimbriae of oviducts of ovariectomized does has occurred following estrogen administration¹⁶. Therefore differences in E levels may be reflected in numbers of ciliated cells and/or secretory cells. In the present study, although there were differences in E levels between does of treatment groups and those of control groups, as judged from the appearance of vulvas (Text-fig. 1), the ratio of number of secretory cells to number of ciliated cells showed no difference between them (Tables 2 and 4). It isn't clear why cell proportion was not directly related to E levels.

Estrogen plays an important role in the production of the secretory material and progesterone causes release of these materials in the oviduct^{5, 7}. Thickness of ampullar epithelium in groups 0F and 0S decreased compared with groups F and S, respectively (Tables 2 and 4). These results imply that the mucus in secretory cells was reduced as a consequence of rising P levels (Text-fig. 2) after ovulation. Nevertheless, P levels in group 0S were higher than in group 0F, and the epithelial thickness in group 0S was significantly higher ($p < 0.05$) than in group 0F. These results suggest that CTPFF may have some repressing effect on swelling of the oviductal epithelium.

It seems that the proliferation index is the most useful one in assessing uterine activity — more so than the other criteria which were used in the present study. Based on the indices, the effect of CTPFF on uterine epithelium is clear, and the effect may continue to 96 hours or more after the final injection of CTPFF. But the effect of CTPFF on oviductal epithelium may be slight during a short period of administration.

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活性炭処理豚卵胞液投与が家兎子宮及び卵管 の粘膜組織に及ぼす影響

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活性炭で処理した豚卵胞液 (CTPFF) を家兎に投与し，投与終了時より時間を追って hCG 処理による排卵を誘起し，それが子宮及び卵管の粘膜組織に及ぼす影響について観察した。

1. CTPFF を 4 ml ずつ12時間おきに投与し，その直後に開腹して得られた子宮及び卵管を組織学的に検討したところ，粘液の分泌が子宮で抑制されており，また卵管膨大部にもその傾向が見られた。
2. 前項1と同様に CTPFF を投与し，投与終了直後 (0F-区)，12時間後 (12-区)，36時間後 (36-区)，96時間後 (96-区)，に hCG 15 IU を静注し，4日目に開腹して得られた子宮及び卵管を組織学的に観察した。子宮では，0F-区，36-区，96-区，で内膜の分岐，腺の発達が対照区 (生理的食塩水投与直後に，hCG を投与し4日目に開腹：0S区) より劣っていた。12-区，36-区，96-区の子宮腺上皮での細胞分裂数が0F-区，0S-区と比較して有意に少なかった。0F-区の子宮粘膜嚢の表面上皮における線毛細胞数は0S-区のものほど減少しておらず，0S-区との間には有意差が認められた。また卵管は0F-区で膨大部の粘液分泌が僅かに抑制されていた。

以上の結果から CTPFF 投与は，排卵前の子宮粘液の分泌，排卵後の子宮内膜の増殖を抑制し，その効果は投与終了96時間後でも認められ，また卵管への影響はあまりなく，子宮への影響は卵巣機能の低下による間接的影響によるものと判断された。

PLATE 1
EXPLANATION OF FIGURES

1. Uterine mucosal folds of a doe received of successive injections of saline solution (group S). Scanty branching of the fold is noted. Fold-branching in does of group S showed like as this figure. H.E. $\times 29$
2. Uterine mucosa of a doe received successive injections of saline solution (group S). Activity of the mucus secretion is very high, and the secretory material is abundant in the lumen. Azan. $\times 170$
3. Uterine mucosa of a doe received successive injections of CTPFF (group F). The mucus secretion is weak and very few secretory materials is attached on the surface of the epithelial cells. Many ciliated cells are located on the surface of epithelium. Azan. $\times 170$
4. Ampulla epithelium of a doe received successive injections of CTPFF (group F). Active mucus secretion and narrow secretory cells are marked. Azan. $\times 170$
5. Isthmic epithelium of a doe received successive injections of CTPFF (group F). Secretory cells are filled with secretory products which expanded to lumen. Azan. $\times 170$
6. Ampullar epithelium of a doe received successive injections of saline solution (group S). Secretory cells are filled with secretory granules, and granules are occasionaly observed even at the bottom of the cells. Azan. $\times 170$
7. Isthmic epithelium of a doe received successive injections of saline solution (group S). Mucus secretion is active like as ampulla, and the activity is slightly higher than that of group F. Azan. $\times 170$
8. Uterine mucosa of a doe received hCG treatment with the final injection of CTPFF (group 0F). Remarkable fold branching is observed, but the glands don't reach the deepest part of the stroma. H.E. $\times 29$
9. Uterine mucosa of a doe received hCG treatment 12 hours after the final injection of CTPFF (group 12). Fold branching is remarkable, and the glands have reached into the deepest part of the stroma. Fold branching in does of group 0S was showed like as this figure. H.E. $\times 39$
10. Uterine glandular epithelium of a doe received hCG treatment with the final injection of CTPFF (group 0F). Many mitoses are found in the glandular epithelium. In group 0S, many mitoses were observed in glandular epithelium, also. H.E. $\times 170$

PLATE 1

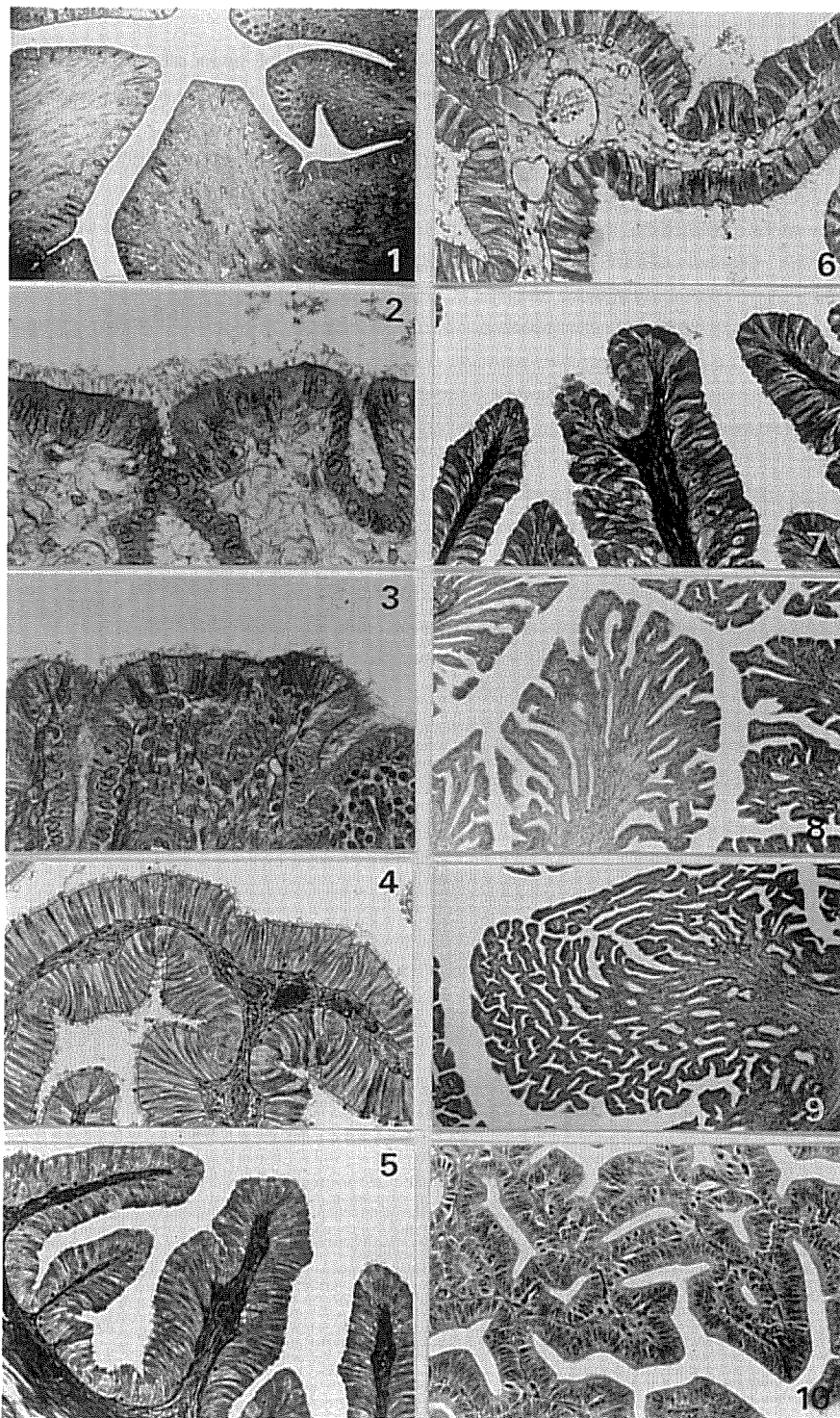


PLATE 2
EXPLANATION OF FIGURES

11. Uterine glandular epithelium of a doe received hCG treatment 36 hours after the final injection of CTPFF (group 36). Glands are proliferated, but mitoses are rare. Does of group 96 showed the same feature like this. H.E. $\times 86$
12. Uterine surface epithelium of a doe received hCG treatment with the final injection of CTPFF (group 0F). Few ciliated cells (\downarrow) are observed. H.E. $\times 345$
13. Uterine surface epithelium of a doe received hCG treatment with the final injection of saline solution (group 0S). Ciliated cells are not recognized. H.E. $\times 170$
14. Uterine surface epithelium of a doe received hCG treatment 96 hours after the final injection of CTPFF (group 96). Mass of ciliated cells appeared. Mucus secretion is very active. Does of group 36 showed similar feature in mucus secretion activity. PAS. $\times 170$
15. Ampullar epithelium of a doe received hCG treatment with the final injection of CTPFF (group 0F). Mucus secretion is active, and bodies of secretory cells are less narrow than those of group F. Azan. $\times 170$
16. Ampullar epithelium of a doe received hCG treatment with the final injection of saline solution (group 0S). Secretory substance in secretory cells is little, and secretory cells become small in size. Azan. $\times 170$
17. Isthmic epithelium of a doe received hCG treatment with the final injection of CTPFF (group 0F). In comparison with group F, secretory matter decreased slightly in the cells. Azan. $\times 170$
18. Isthmic epithelium of a doe received hCG treatment with the final injection of saline solution (group 0S). The mucus in the secretory cells exhibit a slight decrease compared with groups S and 0F. Azan. $\times 170$

PLATE 2

