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Histological Changes in Rabbit Ovaries during Estrous and Post-Ovulatory Phases after Successive Administrations of Charcoal-Treated Porcine Follicular Fluid

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INTRODUCTION

Follicular fluid seemed to repress morphological differentiation of ovarian granulosa cells in the rat, since cells cultured *in vitro* did not enlarge and their nuclei contained only 1 small nucleolus or a fragmented and pale nucleolus (BERNARD, 1975)[1]. Injection of human chorionic gonadotrophin (hCG) soon after administration of charcoal-treated porcine follicular fluid (CTPFF) in rabbits resulted in abnormal function and morphology of the corpus luteum at day 7 of pseudopregnancy (YOSHIDA *et al.*,1986)[2]. Administrations of 5, 10 or 20 μg of a highly purified preparation of inhibin daily for 10 days starting from day 11 postnatum in immature rats resulted in atresia of preantral and antral ovarian follicles, hyperplasia of the theca interna, and degeneration of the granulosa cells (CHARI *et al.*,1981)[3]. Thus, it has been suggested that deteriorative effects on the ovarian follicle and/or corpus luteum are exerted by the follicular fluid; but few reports have examined ovarian morphology in detail after treatment with follicular fluid.

In the present study, histological changes of rabbit ovaries affected by the successive administrations in CTPFF during the estrous or post-ovulatory phase were examined.

MATERIALS AND METHODS

Animals, Follicular Fluid and Experimental Design

Twenty-two adult virgin female Japanese White rabbits out of the fifty-five does which were used in the previous study[4], were used for histological observations. Therefore, treatments of the does were those previously reported[4], briefly as follows.

(Experiment 1) Three does in the treatment group were injected subcutaneously with 4 mℓ of CTPFF at 12 hour intervals for about 5 days (10 times). Three does in the control group received 4 mℓ of sterile physiological saline in same manner as the treatment group. The does were laparotomized immediately after the final injection of CTPFF or saline.

(Experiment 2) In all following all treatment groups, 4 mℓ of CTPFF was injected in the same manner as the experiment 1. To induce ovulation the does received 15 IU of human chorionic gonadotropin (hCG)[5]. Eleven does were divided into 4 groups according to the time elapsed between the final administration of CTPFF and hCG injection. In group 0F (5 does), hCG was given simultaneously with the final injection of CTPFF. In groups 12, 36, and 96 (2 does in each group), injections of hCG were given 12, 36, and 96 hours after the final injection of CTPFF, respectively. Five does in the control group (group 0S) received 4 mℓ of sterile

physiological saline and an injection of hCG in same manner as the group 0F. Does of all groups were laparotomized four days after hCG injection to observe the formation of corpora lutea. Then, unilateral ovary of each doe was fixed in Bouin's fluid, and serially sectioned at 6 μm. All sections were stained with haematoxylin and eosin (H.E.) or azan for histological observations.

RESULTS

(Experiment 1)

There was no prominent difference between groups of treatment and control groups in numbers of preantral follicles (primary and secondary follicles) (Plate 1, Fig. 1). Developing antral follicles were classified into the following two types. Type A; small antral follicle in which the antrum shows a cressent-like cleft (Plate 1, Figs. 2, 3). Type B; large antral follicle in which an ovum is suspended in the large follicular cavity by long strands composed of granulosa cells, or in which granulosa cells surrounding the cumulus oophorus form a large cell mass and the large follicular cavity appears peripherally (Plate 1, Figs. 4, 5).

Lightly atretic antral follicles were defined as follicles which showed pyknosis or dissolution in many granulosa cells and shrinkage of the ovum (Plate 1, Figs. 6-9). Normal non-atretic antral follicles are designated as healthy follicles in the present study. Numbers of antral follicles of both types per ovary and ratios (%) of number of healthy follicles to total number of antral follicles of each type (H/T) are shown in Table 1. In the treatment group, the number of healthy follicles of Type B (0.7 ± 0.6) was obviously fewer than that in the control (10.0 ± 5.3). The H/T for Type B (4.3 ± 4.2%) was significantly lower than in the control (79.1 ± 3.8%), while no significant difference appeared between the H/T for Type A in the treatment group (44.2 ± 2.2%) and the control (46.0 ± 1.7%).

Although the distinctive feature in heavily atretic follicles of the control group was massive degeneration of granulosa cells surrounding the degenerated ova (Plate 1, Fig. 10), heavily luteinized atretic follicles appeared more frequently in the treatment group than in controls (Plate 2, Fig. 11). In contrast, interstitial cells showed no significant difference in morphology as seen in antral follicles after CTPFF treatment.

(Experiment 2)

There was no noticeable difference in number of preantral follicles among all groups.

Table 1. Number of antral follicles per ovary and ratio (%) of number of healthy antral follicles to total number of antral follicles (Experiment 1)

Group*	Follicular type*	Number of antral follicles		Ratio H/T (%)
		Healthy (H)	Total (T)	
Treatment (n=3)	A	38.7 ± 14.6	88.3 ± 34.9	44.2 ± 2.2
	B	0.7 ± 0.6	11.7 ± 10.5	4.3 ± 4.2[a]
	A+B	39.3 ± 15.1	100.0 ± 44.5	40.3 ± 4.0[c]
Control (n=3)	A	36.7 ± 4.6	80.0 ± 12.3	46.0 ± 1.7
	B	10.0 ± 5.3	12.7 ± 6.7	79.1 ± 3.8[b]
	A+B	46.7 ± 8.1	93.3 ± 17.0	50.0 ± 1.0[d]

* See text. Statistical analysis (Student's t test) was performed only between same follicular types, and significant differences ($p<0.01$) are seen between a and b, c and d. Total means total number of healthy and atretic follicles.

Numbers of antral follicles classified as in experiment 1, are shown in Table 2. In group 0F, the H/T for Type A (36.1±2.0%) was not significantly different from that in group 0S (35.3±6.9%), and the ratio for Type B (16.4±1.3%) was slightly lower than in group 0S (20.9±5.6%), though the difference was not significant. In groups 12, 36 and 96, the H/T's for both Types A and B were higher than those in groups 0F and 0S, and were significantly different from that in group 0F. The H/T for Type A of group 36 was the highest (60.2±5.9%), and the ratio for Type B reached a maximum (33.3±0.0%) in group 12. In group 0F, however, the H/T for Type A and B (32.1±2.2%) was no different from that in group 0S (32.8±6.8%). In groups 12, 36 and 96, all ratios (Type A, B and A+B) in each group were higher than those in groups 0F and 0S, and significantly differed from those in group 0F. As in experiment 1, many heavily atretic follicles appeared in all groups. The scars of heavily atretic follicles were frequently observed in all groups, except for group 0S (Plate 2, Fig. 12).

In group 0F, it was conspicuous that many round vacuoles of various size, due to degeneration of interstitial cells, appeared dispersedly in interstitial tissue (Plate 2, Figs. 13,16), and that a few masses of degenerated interstitial cells of low cytoplasmic stainability appeared (Plate 2, Fig. 14), except in the ovary of one doe which did not ovulate. The degeneration of the cells was classified as cyto-destruction and nuclear-degeneration to various degree, and this may be due to fatty-degeneration of the interstitial cells for the vacuolation. Does which ovulated in other groups showed normal ovarian morphology (Plate 2, Figs. 15,17-20), although vacuolation in interstitial tissue was evident to some degree. Table 3 shows average numbers of large luteal cells

Table 2. Number of antral follicles per ovary and ratio (%) of number of healthy antral follicles to total number of antral follicles (Experiment 2)

Group*	Follicular type*	Number of antral follicles		Ratio H/T (%)
		Healthy (H)	Total (T)	
0F (n=3)	A	26.3 ± 3.2	73.0 ± 7.5	36.1 ± 2.0[a]
	B	3.0 ± 0.0	18.3 ± 1.5	16.4 ± 1.3[c]
	A+B	29.3 ± 3.2	91.3 ± 7.4	32.1 ± 2.2[e]
12 (n=2)	A	36.0 ± 0.0	73.5 ± 7.8	49.3 ± 5.2[b]
	B	3.7 ± 1.2	12.0 ± 4.2	33.3 ± 0.0[d]
	A+B	40.0 ± 1.4	85.5 ± 3.5	48.1 ± 3.9[f]
36 (n=2)	A	33.0 ± 7.1	54.5 ± 6.4	60.2 ± 5.9[b]
	B	3.0 ± 0.0	12.0 ± 2.8	25.7 ± 6.1
	A+B	36.0 ± 7.1	66.5 ± 9.2	54.0 ± 3.2[f]
96 (n=2)	A	54.5 ± 3.5	102.5 ± 7.8	53.2 ± 0.6[b]
	B	3.0 ± 0.0	11.5 ± 0.7	26.2 ± 1.6[d]
	A+B	57.5 ± 3.5	114.5 ± 7.8	50.3 ± 0.4[f]
0S (n=3)	A	27.7 ± 13.6	75.0 ± 27.0	35.3 ± 6.9
	B	3.0 ± 0.0	15.0 ± 3.6	20.9 ± 5.6
	A+B	30.7 ± 13.6	90.0 ± 26.0	32.8 ± 6.8

* See text. Statistical analysis (Student's *t* test) was performed only between same follicular types, and significant differences ($p < 0.01$) are seen between a and b, c and d, e and f. Total means total number of healthy and atretic antral follicles.

Table 3. Average number of large luteal cells counted from 3 microscopic fields of 0.024 mm² (× 580)

Group*	No. of luteal cells	No. of copora lutea observed
0F	20.3 ± 2.9[a]	15
12	19.4 ± 2.8[a]	7
36	20.1 ± 2.6[a]	9
96	21.6 ± 3.0	17
0S	23.2 ± 3.6[b]	16

* See text. Statistical analysis (Student's *t* test) was performed and significant differences ($p < 0.05$) are seen between a and b.

(blossom cells) counted from 3 microscopic fields of 0.024 mm² (× 580). In groups 0F, 12 and 36, numbers of blossom cells counted were significantly fewer than in group 0S. Ovaries in groups 0F and 12 showed similar luteal structure, in which luteal cells were not so developed and infiltration of fibroblast-like cells appeared frequently in the luteal tissue (Plate 2, Figs. 16,17). In contrast, in groups 0S, 36 and 96, well developed blossom cells were demonstrated, and large blood vessels were located in the corpora lutea and at their periphery, and definite connective tissue bands showed clearly between lobes of interstitial tissue (Plate 2, Figs. 18–20).

DISCUSSION

Several investigators have proposed that the administration of follicular fluid suppresses FSH secretion and thereby leads to the inhibition of follicular development in various animals[5–8]. Without exposure to sufficient FSH, granulosa cells of small antral follicles are unable to maintain their growth and undergo atresia[9]. In experiment 1, although the H/T in small antral follicles (Type A) in CTPFF treated does showed no difference to that of control does, H/T in large antral follicles (Type B) in CTPFF treated does was significantly lower than that in control does. Therefore, it is considered that decreased FSH secretion from the pituitary in the treated does suppresses the development of small antral follicles to large antral follicles. This is supported by our previous study[4], in which a possibly similar decrease in FSH secretion from the pituitary, caused by administration of CTPFF, inhibited follicular development and reduced the number of large follicles in rabbits. Chari *et al.* (1981)[3] reported that the immature rat treated with 20 μg of inhibin extracted from human follicular fluid showed a high degree of damage to the granulosa cells.

It is evident that a rebound in FSH levels occurs after follicular fluid treatment in the ewe [10–12], and rabbit [13]. According to Mills *et al.* (1982)[14], injections of CTPFF into rabbits at 6, 12 and 18 hours *post coitum* completely blocked the second release of FSH (FSH-II), and the FSH levels at 60 hours *post coitum* were nearly 3 times greater than those measured at 24 hours for FSH-II. When groups 0F and 0S were compared in our experiment 2, the H/T for small follicles (Type A) and large follicles (Type B) showed no significant differences between two groups. However, in groups 12, 36 and 96, H/T's for small and large follicles were consistently higher than those in groups 0F and 0S. This suggests that FSH secretion remains at low levels within 12 hours after the end of CTPFF treatment, and that antral follicular growth is restored by 4 days after hCG injection. Furthermore, there is a possibility that the rebound of FSH levels occurs from about 12 hours after the end of CTPFF treatment, or during to the time elapsed (4 days) from the hCG injection. This consequently may give rise to restoration of secondary and antral follicles, since Peters (1975)[15] reported that PMSG injection restored the large atretic follicles in the mouse.

The ovarian interstitial tissue has been suggested as a site of estrogen production; and it has been postulated that in rabbits LH acts directly on the interstitial cells, stimulating secretion of estrogen, which in turn maintains the corpus luteum[16]. In the rat, estrogens are produced from the ovary by an interplay between granulosa cells and theca interna cells or interstitial cells under the influence of LH and FSH[17]. In rabbits, hCG has been reported to mobilize lipid droplets from the interstitial tissue within two hours post injection[18]. In our present experiment, vacuolation in interstitial cells was evident in ovulatory does of all groups in experiment 2. However, the vacuolation in the degenerated interstitial cells and formation of a few lobuli of degenerated interstitial cells with faded cytoplasm were prominently demonstrated only in group 0F, while such a phenomenon could not be found in all non-ovulatory does. Therefore, it seems that some factors in CTPFF enhance the degeneration of interstitial cells in ovulatory does, and its defective action has vanished within 12 hours after CTPFF treatment. In groups 0F and 12, numbers of large luteal cells (blossom cells)[19] were significantly lower than in controls, and infiltration of fibroblast-like cells appeared frequently in the luteal tissue. This might be related to the decreased number of normal granulosa cells caused by CTPFF treatment. YOSHIDA *et al.* (1986)[2] also stated the same result.

The ovarian interstitial tissue has been known as a source of preovulatory progestin (20α-hydroxypregn-4-en-3-one; 20α-OH), playing a definitive role in the reflex ovulation of the rabbit by prolonged LH discharge following copulation[8]. HILLIARD *et al.* (1968)[21] reported that the ovary released substantial preovulatory amounts of 20α-OH during the 8 hours, and that just prior to ovulation, ovarian steroid output dropped and remained at a very low level for 3-4 days. Thereafter, progesterone could be detected gradually in the ovarian venous effluent. Thus they hypothesized that just prior to ovulation and for a brief period thereafter, the ovary might secrete a gonadotrophin-inhibiting substance into the circulation which counteracts the effects of LH. In the present study, the does of group 0F showed morphological damage in ovarian interstitial cells, and functional properties of interstitial cells might have been affected also. Therefore, there is a possibility that hCG (or LH)-inhibitor in CTPFF affected on the interstitial cells, and the morphological defects found in the corpus luteum occurred in does receiving hCG simultanously with the final administration of CTPFF. This suppressive effect on the formation of the corpus luteum seems to persist until 12 hours after the final administration of CTPFF.

SUMMARY

Mature rabbits were investigated to examine the changes in ovarian histology after successive administrations of charcoal-treated porcine follicular fluid (CTPFF).

In experiment 1, treatment groups were injected with CTPFF 10 times at 12-hour intervals. Control group received saline in the same manner as the treatment group. In experiment 2, four groups were injected with CTPFF in the same manner as in experiment 1, and each group received hCG 0 (simultaneously with final injection of CTPFF), 12, 36 and 96 hours after the final injection of CTPFF, respectively. One group received saline and hCG simultaneously with the final injection of saline. All does were laparotomized 4 days after hCG injection.

In ovaries of does which had received CTPFF treatment, the mean ratio of number of healthy large antral follicles to total number of all large antral follicles (H/T) was significantly lower than in controls.

In ovaries of does which received hCG simultanuously with the final injection of CTPFF (experiment 2), the H/T was lower than that of any other groups, and was significantly lower than the H/T in groups which received hCG 12, 36, and 96 hours after the final administration of CTPFF. Interstitial cells with cytoplasmic vacuolation were found in all ovulated does to some degree. However, degeneration and destruction of interstitial cells were markedly prominent only in does receiving hCG simultanously with the final administration of CTPFF (Group 0F). No vacuolation in interstitial cells was evident in non-ovulatory does. In does receiving hCG simultanously with or 12 and 36 hours after CTPFF treatment, number of luteal cells was significantly fewer than in controls. In does which received hCG simultanously with or 12 hours after CTPFF treatment, fibroblast-like cells appeared frequently among luteal tissues.

These results indicate that successive administrations of CTPFF give rise to defects in ovarian morphology directly or indirectly in rabbits. It is suggested that these suppressive effects have been restored within 36 hours after the final injection of CTPFF.

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REFERENCES

1) Bernard, J.: *J. Reprod. Fert.*, **43**, 453-460 (1975).
2) Yoshida, Y., Nagahama, S., Ito, Y., Maeda, T., Terada, T., Tsutsumi, Y., Okuda, K., Sumi, N., and Sato, K.: *Jpn. J. Zootec. Sci.*, **57**, 1010-1021 (1986).
3) Chari, S., Aumüller, G., Daume, E., Sturm, G., and Hopkinson, C.: *Arch. Gynecol.*, **230**, 239-249 (1981).
4) Nagahama, S., Fukunari, A., Maniwa, J., Alfonso, N.F., Maeda, T., Tsutsumi, Y., Tozuka, A., and Sato, K.: *J. Fac. Appl. Biol. Sci., Hiroshima Univ.*, **26**, 23-30 (1987).
5) Yoshida, Y., Ohara, N., Samori, T., Maeda, T., Terada, T., and Tsutsumi, Y.: *Jpn. J. Zootec. Sci.*, **56**, 971-979 (1985).
6) Bergfelt, D.R. and Ginther, O.J.: *Theriogenology*, **24**, 99-108 (1985).
7) Channing, C.P., Anderson, L.D., and Hodgen, G.D.: in "Ovarian Follicular and Corpus Luteum Function" (Channing, C.P., Marsh, J., and Sadler, W.A., ed.), pp.407-415, Plenum Press, New York and London (1979).
8) Yoshida, Y., Maeda, T., Terada, T., Tsutsumi, Y., Hirata, T., and Kanematsu, S.: *J. Fac. Appl. Biol. Sci., Hiroshima Univ.*, **24**, 15-24 (1985).
9) Peters, H., and McNatty, K.P.: in "The Ovary" pp.75-84, Granada Publishing Ltd., London (1980).
10) McNeilly, A.S.: *J. Reprod. Fert.*, **74**, 661-668 (1985).
11) Miller, K.F., Critser, J.K. and Ginther, O.J.: *Theriogenology*, **18**, 45-53 (1982).
12) Wallance, J.M. and McNeilly, A.S.: *J. Reprod. Fert.*, **73**, 505-515 (1985).
13) Mills, T.M. and Copland, J.A.: Program of the Annual Meeting of the Society for the Study of Reproduction, 15th, p.154 A, Madison, Wisconcin (1982).
14) Mills, T., Stopper, V., Setty, S., and Sorrell, M.: *Biol. Reprod.*, **34**, 336-343 (1986).
15) Peters, H., Byskov, A.G., Himelstein-Braw, R., and Faber, M.: *J. Reprod. Fert.*, **45**, 559-566 (1975).
16) Everett, J.W.: in "Sex and Internal Secretion" (Young, W.C., ed.), Vol.1, pp.530-539, The Williams and Wilkins Co., Baltimore (1961).

17) Dorrington, J.H., Moon, J.S., and Armstrong, D.T.: *Endocrinology*, 97, 1328-1331 (1975).
18) Guraya, S.S. and Greenwald, G.S.: *Am. J. Anat.*, 114, 495-501 (1964).
19) Spies, H.G., Coon, L.L., and Gier, H.T.: *Endocrinology*, 78, 67-74 (1966).
20) Hilliard, J., Penardi, R., and Sawyer, C.H.: *Endocrinology*, 80, 901-909 (1967).
21) Hilliard, J., Spies, H.G., Lucas, L., and Sawyer, C.H.: *Endocrinology*, 82, 122-131 (1968).

活性炭処理豚卵胞液連続投与に伴う家兎卵巣の組織学的変化について

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活性炭で処理した豚卵胞液(CTPFF)の投与後からの時間経過に伴ってhCGを投与し，その時間経過が家兎の卵巣組織に及ぼす影響について検討した。

1．CTPFF を4 mℓずつ12時間おきに10回連続投与し，その直後に開腹して得た卵巣を組織学的に観察したところ，閉鎖進行中の卵胞が目立ち，胞状卵胞数中に占める正常卵胞数の割合は対照の生理的食塩水投与区に比べ有意に低かった。

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日目に開腹して得た卵巣を組織学的に観察した。0F-区は対照区（生理的食塩水投与直後に hCG を静注して4日目に開腹；0S-区）に比べて胞状卵胞数中に占める正常卵胞数の割合はわずかに低かったが有意差は認められず，その他の区と比べると有意に低い値を示した。間質細胞の空胞化が全区でところどころで観察されたが，特に 0F-区では，脂肪変性様の変性，核変性，細胞崩壊を呈する著しい変化が認められ，卵巣間質全体に散在する空胞化のものが著しく多く，その他に細胞質の染色性が劣って白色化した間質細胞が塊を形成して出現している2種類が確認された。しかし排卵させなかった（黄体形成がない）0F-区の卵巣ではこのような変化は見られなかった。発達した黄体細胞数は，0F，12，36-区では 0S-区に比べて有意に少なく，0F，12-区では黄体組織内に多くの繊維芽細胞様細胞の侵入が認められ，血管の形成不全も観察された。

以上の結果から，CTPFF の連続投与が，家兎卵巣の胞状卵胞の発達を抑制すること，hCG の静注による黄体形成に伴い間質細胞に著しい変性をもたらすこと，黄体形成不全を誘起することが示唆され，これらの影響は CTPFF 投与後36時間までに回復しているものと推察された。

Plate 1

EXPLANATION OF FIGURES

1. Ovarian preantral follicles of various size in the CTPFF treatment group. (Experiment 1) H.E. (× 29)
2. Follicle of type A with small antrum. (Experiment 1) H.E. (× 90)
3. Follicle of type A with larger antrum in control group. (Experiment 1) H.E. (× 38)
4. Follicle of type B in which ovum is suspended with long strands composed of granulosa cells in the follicular cavity. (Group 0S) H.E. (× 36)
5. Follicle of type B in which granulosa cells surrounding the cumulus oophorus form a large cell mass and a large follicular cavity appears peripherally. (control group) (Experiment 1) H.E. (× 36)
6. Atretic follicle of type A (larger one). The ovum and granulosa cells are degenerated. (Group 0F) Azan (× 90)
7. Atretic follicle of type A. A shrunken ovum and granulosa cells encroaching on the zone pellucida. (control group) (Experiment 1) H.E. (× 90)
8. Atretic follicle of type B. An ovum is degenerated with vacuolation in its cytoplasma in CTPFF treated group. (Experiment 1) Azan (× 36)
9. Atretic follicle of type B. A shrunken ovum; granulosa cells are dissolved into the antrum in CTPFF treatment group. (Experiment 1) Azan (× 36)
10. Heavily atretic follicle with the degenerated granulosa cells in the control group. (Experiment 1) Azan (× 90)

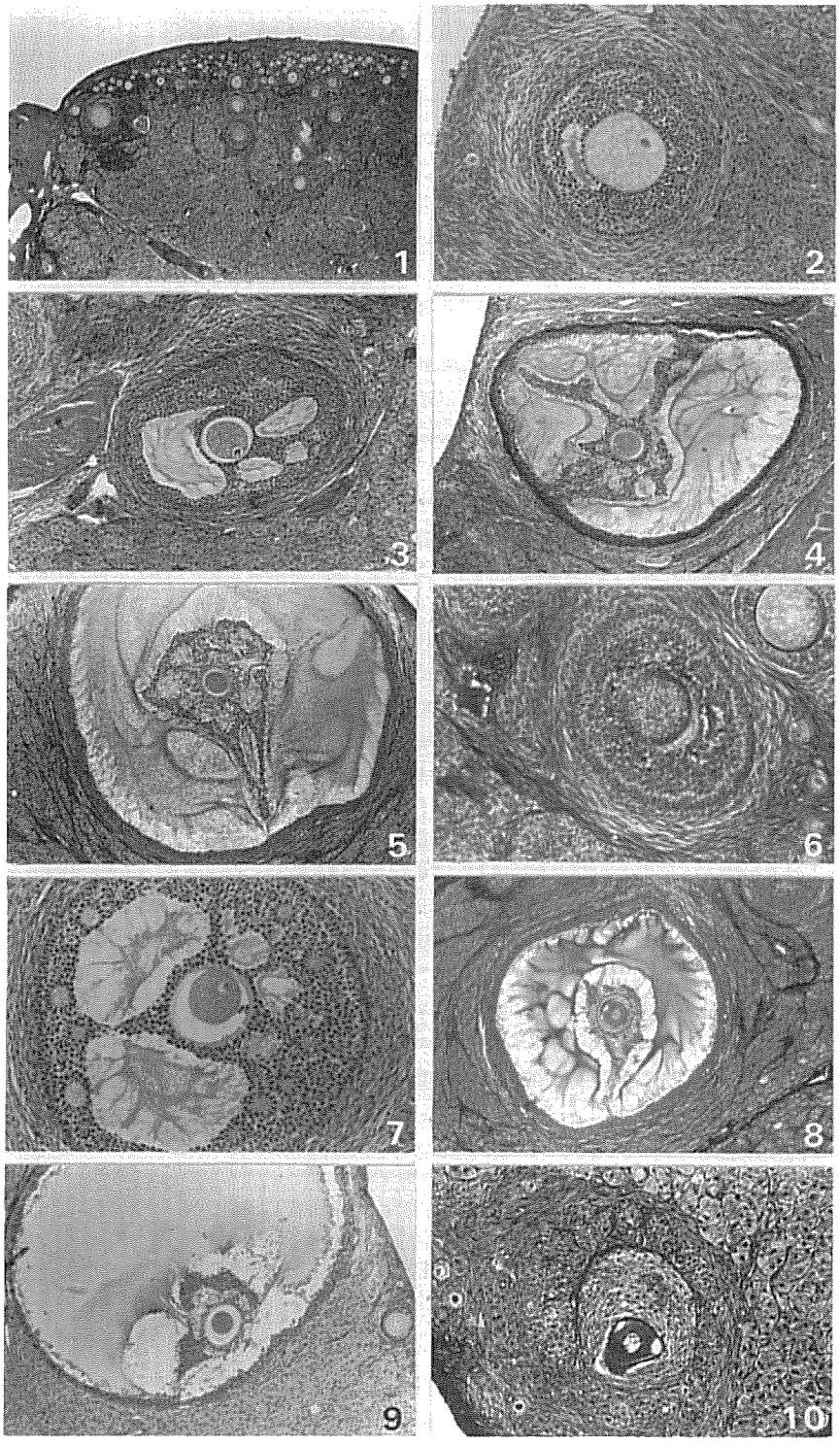
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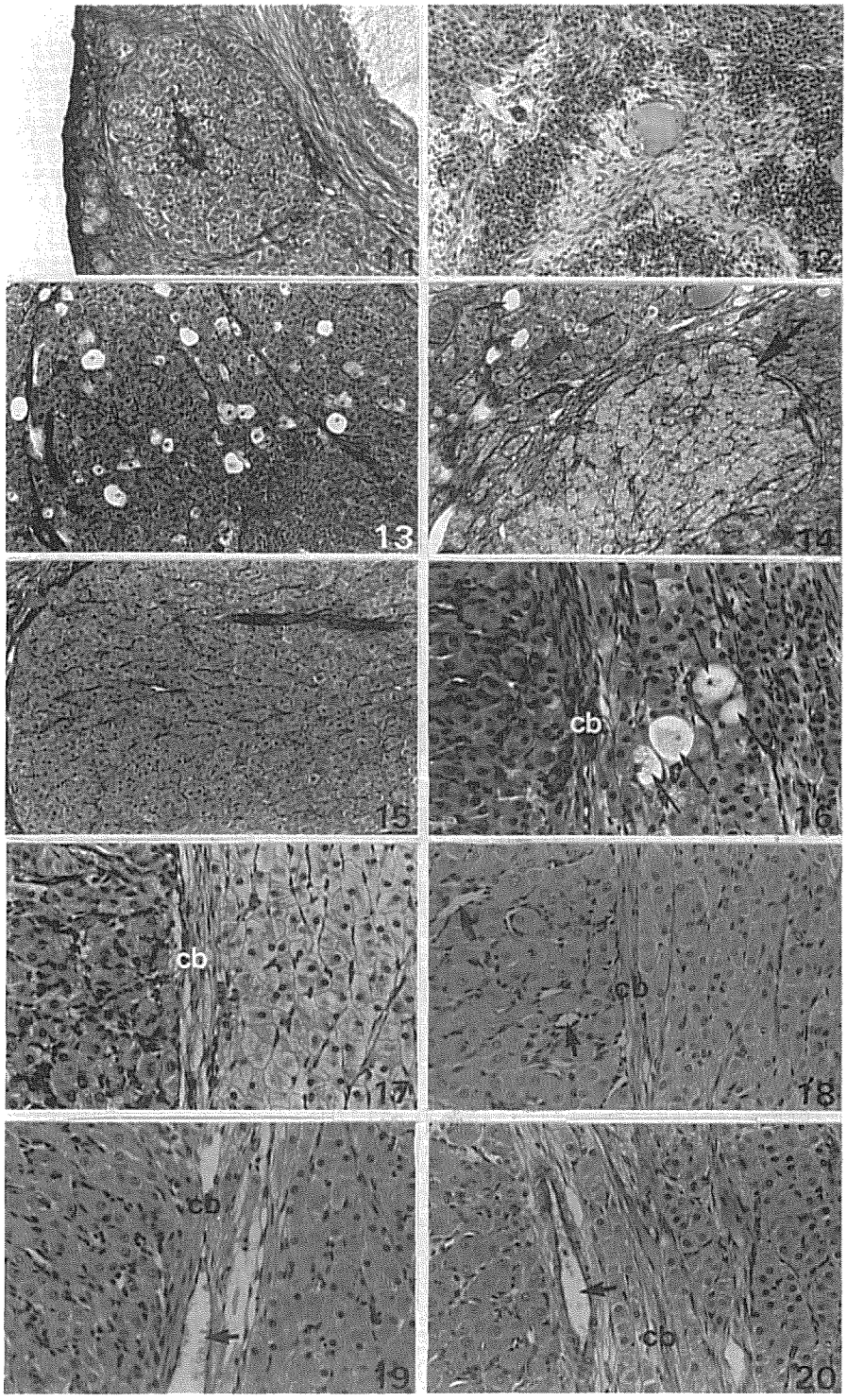
Plate 2

EXPLANATION OF FIGURES

11. Heavily atretic luteinized follicle in CTPFF treatment group. (Experiment 1) Azan (× 90)
12. The scar of heavily atretic follicles in the group receiving hCG simultanously with the final administration of CTPFF. (Group 0F) H.E. (× 90)
13. Interstitial tissue in a doe which received hCG simultanously with the final administration of CTPFF. Many vacuoles of various size, due to fatty degeneration-like changes in interstitial cells, appear dispersed in interstitial tissue. (Group 0F) Azan (× 72)
14. Interstitial tissue in a doe receiving hCG simultanously with the final administration of CTPFF. Vacuoles scattered in interstitial tissue due to fatty degeneration-like cytoplasm and nuclear degeneration (↑) and a degenerating lobule of interstitial cells with faded cytoplasm (⬆). (Group 0F) Azan (× 72)
15. Interstitial tissue of normal morphology in a doe receiving hCG simultanously with the final administration of saline. (Group 0S) Azan (× 72)

16–20. Morphology of corpus luteum (left), and interstitial tissue (right).

16. Infiltration of fibroblast-like cells appears frequently among luteal cells in a doe receiving hCG simultanously with the final administration of CTPFF (Group 0F). Tight arrangement of connective cells located between corpus luteum and interstitial tissue is noted (cb). Interstitial cells with cyto-destruction (arrow) are seen. H.E. (× 145)
17. Structure of corpus luteum in a doe receiving hCG 12 hours after the final administration of CTPFF (Group 12) resembles that in group 0F. Tight arrangement of connective cells located between corpus luteum and interstitial tissue is noted (cb). Interstitial tissue shows normal morphology. H.E. (× 145)
18. Large luteal cells show clearly, and blood vessels (arrow) are located in corpus luteum of a doe receiving hCG 36 hours after the final administration of CTPFF (Group 36). Interstitial cells are well developed. Connective tissue band (cb) forms a line between corpus luteum and interstitial tissue. H.E. (× 145)
19. Large luteal cells are well developed, and blood vessels (arrow) are located in corpus luteum and at its periphery in a doe receiving hCG 96 hours after the final administration of CTPFF (Group 96). Interstitial cells are well developed. Connective tissue band (cb) forms a line between corpus luteum and interstitial tissue. H.E. (× 145)
20. Large luteal cells are well developed, and blood vessels (arrow) are located in corpus luteum and at its periphery in a doe receiving hCG simultanously with the final administration of CTPFF (Group 0S). Interstitial cells are well developed. Connective tissue band (cb) forms a line between corpus luteum and interstitial tissue. H.E. (× 145)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