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Effects of Treatments with Human Chorionic Gonadotropin after Successive Administrations of Porcine Follicular Fluid on the Ovulation Rate and Plasma Concentration of Progesterone in Rabbits

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INTRODUCTION

Follicular fluid contains inhibin, which acts on the pituitary to suppress the secretion of follicle stimulating hormone (FSH) in several animal models, including rabbits[1-11]. Suppression of ovarian follicular development was consequently obtained in rats[4], rabbits[12,13], rhesus monkeys[2] and mares[1].

Our previous reports[12-14] showed that subcutaneous successive injections of charcoal-treated porcine follicular fluid (CTPFF) for 5 days completely blocked ovulation in mated rabbits or reduced the ovulation rate in does treated with human chorionic gonadotropin (hCG), and that average weights of each corpora lutea and levels of plasma progesterone were reduced during 7 days after administration of hCG. According to STOUFFER *et al.* (1980)[15], the administration of CTPFF during the early follicular phase in rhesus monkeys resulted in reduction of the wet weight of corpora lutea, and of progesterone levels at mid-luteal phase, and the gonadotropin sensitivity of luteal cells from treated monkeys was significantly decreased.

MILLS and COPLAND (1982)[16] demonstrated that intraperitoneal injections of CTPFF at 6, 12 and 18 hours *post coitum (p.c.)* in rabbits completely blocked the second FSH surge, which was delayed 36 hours from the usual time of FSH surge, and that FSH levels were nearly 3 times greater than those measured in the usual FSH surge. This phenomenon has been known as a rebound[10]. Administrations of charcoal-treated bovine follicular fluid to ewes resulted in an increase in plasma FSH levels to 3–4 fold over those of controls within 24–48 hours after the end of treatments[8, 10, 17, 18] and a significant increase in ovulation rates following cloprostenol-induced lutiolysis[17,18].

If the rebound in FSH levels is realized in some degree after administration of CTPFF in rabbits, it may increase the ovulation rate after treatment with CTPFF, followed by some changes in plasma concentration of progesterone.

In the present study, effects of treatment with hCG during 96 hours after administrations of CTPFF on the ovulation rate and plasma concentration of progesterone in rabbits were examined

to provide more fundamental information on the phenomenon of FSH rebound for possible application in superovulation.

MATERIALS AND METHODS

Animals Fifty-five adult, virgin, female Japanese White rabbits, weighing 3.1–4.9 kg, were used. Animals were reared in individual cages for over one month after purchase from a local commercial rabbit breeder with food and water provided *ad libiturm*.

Follicular fluid Porcine ovaries were collected at a local meat-packing plant in Hiroshima city and the method used for preparation of CTPFF was the same as that described by Yoshida *et al.* (1985)[12].

Experimental design (Experiment 1) Four does in the treatment group were injected subcutaneously with 4 mℓ of CTPFF at 12 hour intervals for 5 days (10 times). Three does in the control group received 4 mℓ of sterile physiological saline in the same manner as the treatment group. The does were laparotomized immediately after the final injection of CTPFF or saline and the size and numbers of ovarian follicles (≥ 1.0 mm in diameter) were determined (Table 1).

(Experiment 2) In all of the following treatment groups, 4 mℓ of CTPFF were injected in the same manner as in experiment 1. To induce ovulation, the does received 15 IU of hCG, based on a previous study[12] in which some does did not ovulate after an injection with 5 or 10 IU of hCG, but in which all does ovulated after receiving 15 IU of hCG. Forty-eight does were divided into 8 groups. In group 0F or 0S (Table 2), hCG was given simultaneously with the final infection of CTPFF or saline. In groups 12, 24, 36, 48, 72 and 96, injections of hCG were given after 12, 24, 36, 48, 72 and 96 hours after the final injection of CTPFF, respectively. Laparotomy was performed at four days after hCG injection to allow counting of ovulations (corpora lutea) and macroscopic measurement of follicle diameters in both ovaries.

Appearance of vulva Appearance of vulvae of all does used in the present study was ovserved once a day at midday, from one day before injection of CTPFF to one day before laparotomy. The criteria for scoring the appearance of vulvae were the same as those used in previous studies in this laboratory (Yoshida *et al.*, 1985)[12, 13].

Sampling of blood and assay of progesterone in plasma In goups 0F, 0S, 24, 72 and 96, blood samples for progesterone (P) assay were collected from the marginal ear vein every other day from one day before CTPFF injection to the day of laparotomy and 4 hours after hCG injection. In groups 12 and 36, blood samples were collected 4 hours atfer hCG injection and then every other day until laparotomy.

Progesterone concentrations in peripheral plasma were determined by radioimmunoassay as described by Makino (1973)[19]. Peripheral plasma (0.1–0.2 mℓ) was extracted with ethyl ether. The range of the water blank was estimated to be 0 to 0.013 ng/mℓ, the recovery rate was 90.0 ± 10.4 percent (mean ± S.D.), and a good dose-response line was obtained in the range between 0 and 1 ng as the standard curve. Means of within-assay variation and between-assay variation were 0.4270 ng/mℓ (C.V., 8.5%) and 0.5067 ng/mℓ (C.V., 4.0%), respectively.

Statistical analysis Number of ovulations, number of follicles, score of vulval appearance and P levels were analyzed using Student's *t* test.

RESULTS

In experiment 1 (Table 1), when follicles were classified as 1.0, 1.5 and ≥ 2.0 mm in diameter, no large follicles (≥ 2.0 mm in diameter) appeared in ovaries of the treatment group, in comparison with those of the control. However, the total number of follicles in CTPFF-treated does was the same as in control does.

In experiment 2 (Table 2), the mean number of ovulations (corpora lutea) in does which received hCG 12 hours after the final injection of CTPFF (group 12) was significantly higher than that in does which received hCG simultaneously with the final injection of CTPFF ($p < 0.01$, group 0F). However, the mean number of corpora lutea in group 12 was significantly lower than that in groups 36, 48, 72, 96 and 0S ($p < 0.05$). Although no significant difference in mean numbers of corpora lutea were evident among groups 24, 36, 48, 72 and 96, the mean number of corpora lutea in does of group 36 was the largest (Table 2). The largest number of corpora lutea in a doe of this group was 18.

The number of large ovarian follicles (≥2.0 mm in diameter) in does of group 12 was significantly higher than that of any other treatment groups, and the number of mid-size ovarian follicles (1.5 mm in diameter) of group 0F was significantly lower than in groups 12 and 24. The total number of ovarian follicles and corpora lutea in group 0F was smaller than that of any other group.

Table 1. Number of ovarian follicles (mean ± S. D.) of different diameters at laparotomy after successive injections of charcoal-treated porcine follicular fluid (CTPFF) or saline solution for 5 days

Treatment	Follicular diameter (mm)			Total
	1.0	1.5	≥2.0	
CTPFF	12.3 ± 5.3	7.3 ± 7.2	0[a]	19.5 ± 10.2
Saline	9.3 ± 4.7	5.0 ± 4.6	5.0 ± 4.6[b]	19.3 ± 4.7

Significant difference between a and b ($P < 0.05$).

Table 2. Number of ovarian follicles (mean ± S. D.) of different diameters and number of ovulations (copora lutea) in ovulated does 4 days after hCG injection

Group	Follicular diameter (mm)			No. of ovulation	Total*
	1.0	1.5	≥2.0		
0S	9.3 ± 1.9[a]	2.0 ± 1.8[ab]	1.3 ± 2.3[ab]	10.2 ± 1.5[a]	22.8 ± 2.9[a]
0F	11.3 ± 4.2[a]	1.0 ± 1.3[a]	0.2 ± 0.4[a]	2.7 ± 1.6[b]	15.7 ± 4.5[b]
12	10.2 ± 2.0[a]	4.5 ± 3.3[b]	3.3 ± 3.0[b]	6.7 ± 2.4[c]	24.7 ± 7.0[ab]
24	9.5 ± 5.2[a]	4.5 ± 3.0[b]	0.3 ± 0.5[a]	9.5 ± 4.3[ac]	23.8 ± 5.6[ab]
36	11.0 ± 1.4[a]	2.5 ± 2.3[ab]	0.3 ± 0.5[a]	12.0 ± 3.9[a]	26.5 ± 3.8[a]
48	14.0 ± 4.4[a]	3.2 ± 2.6[ab]	0.3 ± 0.5[ab]	11.5 ± 2.6[a]	29.0 ± 6.7[a]
72	11.2 ± 6.8[a]	1.7 ± 3.1[ab]	0.5 ± 0.8[ab]	11.8 ± 1.5[a]	25.2 ± 6.3[a]
96	10.5 ± 1.5[a]	1.3 ± 2.3[ab]	0.2 ± 0.4[ab]	10.8 ± 2.4[a]	22.8 ± 4.1[a]

* Total number of ovarian follicles and corpora lutea.
Significant differences exist among figures with different superscripts in the same column ($p < 0.05$).

During the period of CTPFF treatment, two days before the final injection of CTPFF, there appered a significant difference in P levels between the treatment and control groups (Fig. 1). Although P levels in treatment groups from the final injection of CTPFF to hCG administration are not shown in Fig. 1, they remained at about the same levels as found two days before the final injection of CTPFF.

P levels in all groups rose markedly at 4 hours after hCG injection and fell the next day, except in group 12. At 4 hours after hCG injection, P levels in groups 24, 36, 48 and 72 were significantly higher than those of groups 0S, 0F, 12 and 96. Although P levels in all groups at 4 days after hCG injection were higher than those at 2 days, the P level in group 12 was the highest

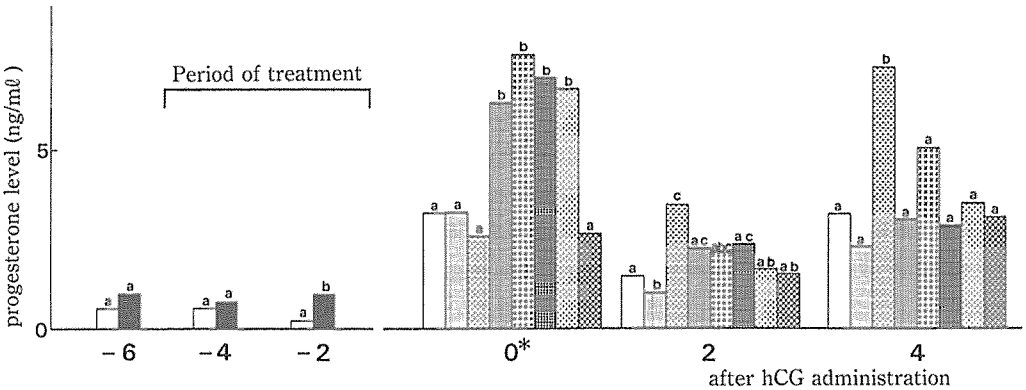

Fig. 1 Progesterone levels in pheripheral plasma in experiment 2.
Group; □ 0S, ■ Treatment, ▨ 0F, ▩ 12, ▩ 24, ▩ 36, ▩ 48, ▩ 72, and ▩ 96.
* Day 0 means 4 hours after hCG injection. Differences among progesterone levels with different superscripts (a, b, c), on the same day, are significant ($P<0.05$).

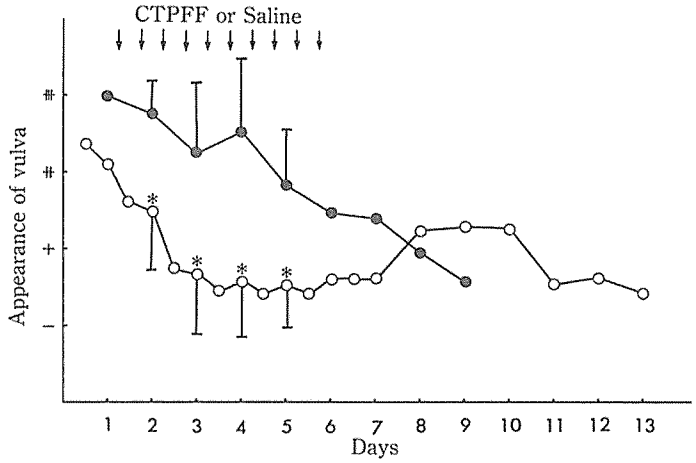

Fig. 2. Effect of successive administrations of charcoal-treated porcine follicular fluid (CTPFF) on vulval appearance in 42 does (○, mean ± S. D.), in comparison with 6 does (●, mean ± S. D.) which were given saline in the same manner. The appearance of the vulva was scored 1 (non-swollen, small and whitish vulva) to 4 (largely swollen, congested, and purple or dark red vulva). ↓, dose of CTPFF or saline solution; *, $P<0.01$

of any groups.

Values for appearance of vulvae decreased significantly from 2 to 5 days after the beginning of CTPFF injections, followed by slight recovery after the final administration of CTPFF (Fig. 2). In all treatment groups, the vulval swelling decreased and color faded during the period from hCG injection to laparotomy. After hCG injection, no difference in scores of vulval appearance was evident in all treated groups.

DISCUSSION

As shown in table 1, the total number of all ovarian follicles of various sizes in CTPFF-treated does showed no significant difference from that of control does, but follicles of large size (≥2.0 mm in diameter) were absent in ovaries of CTPFF-treated does. This may be due to decreased secretion of FSH from the pituitary in the treatment group, causing inhibition of follicular development and/or reduction in number of large follicles.

The mean number of ovulations in does which received hCG simultaneously with the final administration of CTPFF (group 0F) was significantly lower than in any other group (Table 2). It is speculated from the results of experiment 1 that the fewer ovulations in group 0F were due to a smaller number of follicles which were mature enough to ovulate. The number of ovulations increased with increasig time from the final administration of CTPFF to hCG injection, up to 36 hours. Thereafter, the numbers of ovulations did not differ significantly among treated groups. Therefore, it is considered that the suppressive effect of CTPFF on follicular development diminishes up to 24 hours after the end of CTPFF dosage, and has disappeared by 36 hours.

The rebound in FSH levels occurs 24–48 hours after the end of follicular fluid treatment in rabbits[16] and ewes[8,10,18]. There is a possibility that FSH levels rebound within 36 hours after end of CTPFF treatment, because the number of ovulations of does in group 36 was the largest. The result suggests that the phenomenon of rebound in FSH levels may be useful for induction of superovulation.

In rabbits, the post-ovulatory FSH surge (the second FSH surge) occurs 24 hours after mating or hCG injection[20]. Although differences in the number of small follicles (1.0 mm in diameter) were not significant among treatment groups, the number of large follicles (≥ 2.0 mm in diameter) in group 12 was larger than those of any other treatment groups (Table 2). This suggests that large follicles in does which receive hCG 12 hours after the end of CTPFF treatment can survive and/or develop owing to the stimulation by the second FSH surge.

In the present study, P levels during CTPFF treatment were slightly higher than those of the control (Fig. 2). This result is similar to a previous report by Anderson *et al* (1979)[21] who observed CTPFF stimulated P secretion by cultured granulosa cells in the presence of FSH and androgens. Preovulatory FSH and LH surges lead to elevation of P levels which are secreted by granulosa cells[22]. During the pre-ovulatory period, higher P levels in groups 24, 36, 48 and 72 may indicate some possibility that many large ovarian follicles grow in ovaries of these groups in a short time before ovulation, and that these large follicles may be ovulated by high does of gonadotropin.

Henderson *et al.* (1986)[23] demonstrated that the large follicles of ewes treated with bovine follicular fluid had lower concentrations of estradiol-17ß in follicular fluid and contained fewer granulosa cells and that the granulosa cells had a reduced capacity to aromatize testosterone to

estradiol-17ß and produced cyclic AMP when challenged with FSH or LH. In our group 12, P levels were slightly higher at 2 days and significantly higher at 4 days after hCG injection as compared with any other groups. This means that many large ovarian follicles may secret P because of inhibition of synthesis of estrogen from P by stimulation of FSH.

Estrus in does is diagnosed from the state of vulval swelling and/or coloration[24,25]. When does are in heat, the appearance of the vulva is characterized by deep color and swelling, caused by high levels of estrogen from large ovarian follicles[24-26]. In all of our CTPFF-treated groups, the score of appearance of the vulva significantly decreased, then recovered slightly after the end of CTPFF treatment and also decreased after hCG injection in all does. These fluctuations may be caused by the decrease in estrogen secretion from the ovaries due to reduction of FSH levels, and subsequent rebound in the FSH levels after the final injection of CTPFF. Although there were many large follicles in group 12, the score of appearance of vulva was low after hCG injection. It seems that many large follicles effectively secreted P, also.

In conclusion, we suggest that the FSH levels may rebound within 36 hours after the end of CTPFF treatment, and that this may be due to useful in studies of superovulation.

SUMMARY

Effects of treatments for induced ovulation with hCG during 96 hours after successive administrations of charcoal-treated porcine follicular fluid (CTPFF) on the ovulation rate and plasma concentration of progesterone (P) in rabbits were examined.

In experiment 1, four does or three does which were injected with 4 mℓ of CTPFF or saline (control), respectively, at 12-hour intervals for 5 days (10 times) showed no difference in the total number of follices. No large follicles (≥ 2.0 mm in diameter) appeared in ovaries of the treated group, in comparison with their presence in those of the control.

In experiment 2, forty-two does were injected with 4 mℓ of CTPFF in the same manner as in experiment 1. Thirty-six of them were divided into 6 groups according to the number of hours from the final administration of CTPFF to hCG injection-12, 24, 36, 48, 72 and 96 hours. The number of ovulations in six other does which were ovulated by injection of hCG simultaneously with the final administration of CTPFF (group 0F) were significantly lower than those of any other group, and the number increased with interval up to 36 hours from the end of CTPFF treatment to hCG injection. The number of follicles of large size (≥ 2.0 mm in diamter) in ovaries of does in group 12 (12-hour interval) at 4 days after hCG injection was larger than in any other treatment group.

In does of groups 24, 36, 48 and 72, plasma P levels 4 hours after hCG injection were higher than those of the other groups of does. In group 12, P levels at 4 days after hCG injection were higher than those of any other group.

Vulval swelling and coloration receded 1 day after the beginning of CTPFF treatment, and recovered slightly after the end of CTPFF treatment until hCG injection. These results indicate that successive treatments of CTPFF for 5 days during the follicular phase decrease ovulation rate, and the inhibitory effect on ovulation is lost within a short time. Follicle-stimulating hormone levels may rebound within 36 hours after the final administration of CTPFF treatment, because the ovulation rate in group 36 was the highest.

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活性炭処理豚卵胞液投与後の時間経過が，家兎の排卵数及びプロジェテロン濃度に及ぼす影響

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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による排卵の誘起を試み，それが家兎の排卵数および血漿プロジェステロン濃度に与える影響について検討した。

1．CTPFF を4 mℓずつ12時間おきに10回連続皮下投与し，その直後に開腹して卵巣表面を観察すると，大型卵胞（≧2.0㎜直径）が全く存在しなかったが，対照の生理的食塩水投与区には多数存在していた。

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），24時間後（24-区），36時間後（36-区），48時間後（48-区），72時間後（72-区），96時間後（96-区）の各期に hCG 15 IU を投与し，4日目に開腹して卵巣表面を観察した。0F-区の排卵数（2.3個）はその他の処理区（6.7～12.0個）及び0S-区（10.2個）と比較して有意に少なかった。平均排卵数は12-区（6.7個）で0S-区（10.2個）より有意に少なかったのに対し，36-区（12.0個）で最も多く（最高18個），その他の区ではほとんどかわらなかった。また，12-区には大型卵胞（1.5㎜及び≧2.0㎜直径）が多く存在していた。末梢血漿中のプロジェステロン濃度（RIA法）は CTPFF 処理中は対照区よりも僅かに高かった。12-区は hCG 投与後からプロジェステロン濃度が高くなったが，その他の区では hCG 投与後に一度低下し，以後増加した。また，24～72-区は hCG 投与4時間後に高い値を示した。外陰部の腫脹・潮紅の度合には CTPFF 投与翌日から低下し，投与終了後に少し回復した。

以上の結果，CTPFF の連続投与が家兎の排卵に対する抑制的影響は比較的早く失われ，CTPFF 投与終了後36時間までに FSH 濃度にリバウンドが起きていることが推測された。また，プロジェステロン濃度は処理区で高く，ステロイドの生合成に何らかの変化が起きているものと推測された。