

Differences of Effects Between Successive Administration of Whole and Charcoal-Treated Porcine Follicular Fluid on the Ovarian Follicles, Ovulations and Plasma Sex Steroids in the Rabbits

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Abstract Differences of effects between successive administration of whole porcine follicular fluid (WPF) and charcoal-treated porcine follicular fluid (CTPF) on the ovary, pituitary and sex steroids of rabbits were investigated. Does of control and each treated group were injected with 4 ml of WPF, CTPF or saline, respectively, at 12 hour intervals during 5 days. At the end of the treatment the mean number of all ovarian follicles (≥ 1.0 mm in diameter) in WPF-treated does was significantly fewer than those of the other groups. A few large follicles (≥ 2.5 mm) appeared in this group while we had no large follicles in the other groups. Some of the large follicles were cystic (≥ 3.0 mm), they were hemorrhagic. The injection of 15 IU hCG for induced ovulation at the end of the WPF treatment produced no ovulation in 2 does out of the 6 by inspection four days after the hCG injection, while all does ovulated in both the CTPF- and saline-treated groups. The mean numbers of ovulation by count of corpora lutea in WPF- and CTPF-treated does were significantly lower than those in controlled does. Only in non-ovulated does of the WPF-treated group, large cystic and/or blood follicles appeared. The number of luteal cells per unit area and plasma progesterone levels 4 days after hCG treatment was lower in WPF-treated does than in the other groups. Vulval swelling and coloration receded 1 day after beginning of WPF- or CTPF-treatment, and estradiol levels in WPF- or CTPF-treated does were low during the treatment period. From these results we may conclude that WPF-treatment exerts more suppressive effect on the female reproductive activity than CTPF-treatment. In histology of anterior pituitary, basophilic cells which were filled with granules appeared frequently at the end of CTPF-treatment, moreover degranulation and vacuolization in basophilic cells occurred 36 hours after the end of CTPF-treatment.

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INTRODUCTION

Administrations of charcoal-treated follicular fluid can suppress the peripheral follicle-stimulating hormone (FSH) levels during the treatment period in several mammalian species, including rabbits (MILLS and COPLAND, 1982). Charcoal-treated porcine follicular fluid (CTPFF) inhibits FSH release in a dose-dependent manner from the cultured female rabbit pituitary cells, in the same way as it inhibits in other species (GOODMAN, 1984). When the follicular fluid administration is finished, the suppression of the peripheral FSH concentrations is followed by a "rebound" increase or a transient rise in FSH levels in rats (DEPAOLO *et al.*, 1979; HIRSHFIELD and DEPAOLO, 1981; PHILLIPS *et al.*, 1982), rabbits (MILLS and COPLAND, 1982), ewes (MILLER *et al.*, 1982; MCNEILLY, 1984, 1985; WALLACE and MCNEILLY, 1985; WALLACE *et al.*, 1985; HENDERSON *et al.*, 1986; KIND *et al.*, 1988), heifers (LEFÈVRE and CAILLOL, 1978; JOHNSON and SMITH, 1985), mares (BERGFELT and GINTHER, 1985), and monkeys (CHANNING *et al.*, 1981; STOFFER *et al.*, 1981). In our previous reports (YOSHIDA *et al.*, 1985a, b), although successive administrations of CTPFF to female rabbits reduced the ovulation rate induced by human chorionic gonadotropin (hCG) at the end of the CTPFF treatment, by its injection the ovulation rate had improved slightly 36 hours later after the end of the treatment (NAGAHAMA *et al.*, 1987). This might point to the rebound phenomenon in FSH levels. MILLS and COPLAND (1982) mentioned that administration of porcine follicular fluid to copulatory rabbits blocked completely the second release of FSH at 24 hr. and delayed in until 60 hr. *post coitum*.

Until recent documentations concerning inhibin in the follicular fluid were developed, the role of negative feedback of estrogen secreted from ovarian follicles to pituitary had been emphasized as a main way to elucidate the initiation of FSH secretion from the pituitary. In order to evaluate the follicular inhibin, most studies used charcoal-treated follicular fluid to avoid the effects of steroid hormones. In fact, the charcoal treatment removed over 99% of estradiol-17 β (E₂) and progesterone from the follicular fluid (WELSHEN *et al.*, 1977, 1980; CHANNING *et al.*, 1979, 1981; CHAPPEL and SELKER, 1979; WISE *et al.*, 1979; MCNEILLY, 1984; BERGFELT and GINTHER 1985; JOHNSON and SMITH, 1985; WALLACE and MCNEILLY, 1985; HENDERSON *et al.*, 1986). Our previous study (YOSHIDA *et al.*, 1985b) showed moreover that percentages of residual steroids by charcoal treatment of porcine follicular fluid were approximately 0.6% for E₂ and 0.3% for progesterone. Then, the follicular inhibin seemed to be one of the powerful regulators to FSH secretion from pituitary. Some investigations (MILLER *et al.*, 1979b; MCNEILLY, 1984; BERGFELT and GINTHER, 1985; REDMER *et al.*, 1985) treated the whole follicular fluid instead of using the charcoal-treated follicular fluid for elucidation of follicular inhibin. However, CUMMING *et al.* (1974) described that baseline levels of FSH in the ewe are regulated by E₂, that a negative feedback system operates, and that this system requires long (2 - to 3 - week) period for full effect, because continuous E₂ administration immediately following ovariectomy holds FSH basal levels below those found in ovariectomized ewes, and treatments of E₂ in anestrus ewes fall unto the basal levels of FSH about 4 to 6 hours. Then, they rise above basal levels. Although BRONSON and CHANNING (1978) detected totally suppressed serum FSH levels in intact female mice by E₂ administration, they supported a contention that follicular fluid contains a nonsteroidal factor which is capable of acting additively with E₂ to regulate FSH secretion. In a progressed study by WILLIAMS and LIPNER (1981), neither E₂ nor CTPFF alone were capable of suppressing serum FSH in rats to diestrus levels but the combination of E₂ and CTPFF treatment was capable of suppressing

serum FSH unto diestrous levels. In ewes, E_2 rise could not account for the low basal level of FSH typical of the follicular phase, whereas basal luteinizing hormone (LH) was normal (GOODMAN *et al.*, 1981). In rabbits, an injection of 2 μg E_2 /kg body weight prevented the post-castration rise of FSH, but those of E_2 (0.5 to 20 $\mu\text{g}/\text{kg}$) could not fully restore the pattern of the second release of FSH that occurred after ovulation. This suggests that some ovarian factor (inhibin) other than E_2 was required for the second release of FSH (MILLS and COPLAND, 1982, 1983). It was demonstrated also that in the ewe E_2 alone cannot account for the negative feedback control of the hypothalamic/pituitary gland function, in particular for the FSH secretion, and that other ovarian factors must have an effect on the secretion of gonadotrophins (WEBB *et al.*, 1985). Recently, MARTIN *et al.* (1988) emphasized that FSH secretion appears to be primarily controlled by the synergistic action of E_2 and inhibin on the anterior pituitary gland in the ewe, while LH secretion is inhibited during the follicular phase by an effect of estrogen at pituitary level and is affected during the luteal phase by the synergistic of E_2 and progesterone, at the hypothalamic level.

Furthermore, MILLER *et al.* (1979) showed that ovulation was inhibited in the bovine after treatment with whole bovine follicular fluid, but was not inhibited after treatment with either steroid free or protein denatured follicular fluid. Treatment with a combination of steroid free follicular fluid and protein denatured follicular fluid was as effective as whole follicular fluid in inhibiting ovulation.

The documents mentioned above suggest that significant differences in reproductive physiology might be induced by administration of the whole follicular fluid and the charcoal-treated follicular fluid. Therefore, the present study dealt with the effects of successive administrations of whole porcine follicular fluid (WPF) or charcoal-treated porcine follicular fluid (CTPFF) on the ovary and plasma sex steroids in rabbits. Histological observations on the anterior pituitary cells were made in some does given CTPFF, additionally.

MATERIALS AND METHODS

Animals Thirty-three 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 libitum*. Experiments were started on confirmation of estrus by judging the vulval coloration and swelling.

Follicular Fluid Porcine ovaries were collected at a local meat-packing plant in Hiroshima city and the method for preparation of CTPFF was the same as that described by YOSHIDA *et al.* (1985a). Whole porcine follicular fluid (WPF) means intact porcine follicular fluid without extraction by active charcoal to remove steroids.

Experimental Design (Experiment 1) Nine does were divided into three groups according to injection materials, which were CTPFF, WPF or physiological saline solution (PSS). Does received subcutaneously each fluid at 12 hour intervals for 5 days (10 times) were laparotomized immediately after the final injection of fluids and the size and numbers of ovarian follicles (≥ 1.0 mm in diameter) were estimated. Then, an unilateral ovary of each doe was resected to fix in Bouin's fluid, and serially sectioned at 6 μm . All sections were stained with hematoxylin and eosin (H.E.) or azan for histological observations.

(Experiment 2) Eighteen does were divided into three groups, they were injected with CTPFF (group OF), WPF (group OW) and PSS (group OS), respectively, according to the same schedule as in Experiment 1. All does were injected with 15 IU of hCG in order to induce ovulation at the end of the fluid's treatment. Laparotomy was performed 4 days after the

hCG injection in order to count the number of ovulations (corpora lutea) and to measure the follicular diameter macroscopically in both side of ovaries. An unilateral ovary of each doe was taken for histological sections as in Experiment 1.

(Experiment 3) Four does were treated with CTPFF in the same order as in Experiment 1, then two does of them were sacrificed in order to collect the pituitary at the end of the CTPFF treatment (group F). Two other ones were slaughtered in order to collect the pituitary, 36 hours after the CTPFF treatment (group 36). Two does were injected with PSS and the pituitary was collected in the same manner as in group F (group S). These pituitaries were fixed in Bouin's fluid, and sectioned at 3 μm . All sections were stained with azan for histological observations of the anterior pituitary.

Appearance of Vulva Appearance of vulvae of all does was observed once a day at noon, from one day before the injection of porcine follicular fluid until one day before laparotomy. The criteria for scoring the appearance of the vulva were the same as those used in our previous studies (YOSHIDA *et al.*, 1985a).

Sampling of Blood and Assay of Sex Steroids in Plasma Blood samples for the sex steroid assay were collected from the marginal ear vein of does used in Experiment 2: before injection of CTPFF or PSS; 2 days before hCG injection; 4 hours after hCG injection and 4 days after hCG injection.

The radioimmunoassay methods described by MAKINO (1973) with some modifications were used for the measurements of estrogen and progesterone concentrations. Sephadex LH-20 microcolumn chromatography was used to separate the fractions eluted with benzene:methylalcohol (85:15) as estrone and estradiol 17 β , from 3.0–5.0 *ml* samples of plasma. Progesterone was extracted from aliquots of 0.05–0.2 *ml* plasma with 10 volumes of diethyl ether and the evaporated extracts under nitrogen were then subjected to radioimmunoassay without chromatography. Antiserum to progesterone-11 α -hemisuccinate-BSA, estrone-6 (O-carboxymethyl) oxime-BSA, and estradiol-17 β -6 (O-carboxymethyl)-oxime-BSA raised in rabbits was obtained by Dr. A. KAMBEGAWA or Teikoku Hormone Mfg. Co. Ltd., Tokyo. Extraction losses in assays were measured by the use of tritiated hormones. The mean recovery rates were 90.0% for progesterone; 83% for estrone, and 72% for estradiol-17 β . Results of all samples were corrected for extraction and other procedural losses. The antiserum was diluted with borate buffer containing 0.06% bovine serum albumin and 0.05% bovine serum globulin. Dilution rate of each steroid antiserum was as follows; 1:25,000 dilution for progesterone, 1:30,000 for estrone, and 1:40,000 for estradio-17 β , respectively. The reproducibility was examined by measurements of steroids of the same pool in the same assay and in different assays. Of ten replicate assays on the same day, the coefficient of variation was 8.5% for progesterone, 5.0% estrone and 9.0% for estradiol-17 β . The coefficient of variation on 50 determinations assayed on different days was 13.1% for progesterone, 13.4% for estrone and 15.9% for estradiol-17 β , respectively. Blank values, determined by the extraction, chromatography and radioimmunoassay of appropriate aliquots of water, were equal to or slightly greater than 0 pg. The results for each steroids were 0–13.0 pg for progesterone; 0–4.0 pg for estrone; and 0–8.0 pg for estradiol-17 β .

Statistical Analysis Number of ovulations, number of follicles and score of vulval appearance were analyzed using Student's *t* test, and the ratio of ovulated does was analyzed using χ^2 test.

Table 1. Mean numbers of ovarian follicles (mean \pm S.D.) in different diameters at laparotomy after successive injections of whole porcine follicular fluid (WPFf), charcoal-treated porcine follicular fluid (CTPFF) or physiological saline solution (PSS) for 5 days (Experiment 1)

Treatment	Mean no. of follicles in different diameter (mm)						Mean no. of blood follicles
	1.0	1.5	2.0	2.5	≥ 3.0	Total	
WPFf	5.0 \pm 1.0 ^a	2.3 \pm 1.7 ^a	1.3 \pm 1.2 ^a	2.3 \pm 1.7	0.7 \pm 1.2	8.0 \pm 2.2 ^a	2.0 \pm 2.0 ^a
CTPFF	12.3 \pm 5.3 ^b	7.8 \pm 7.2 ^a				19.5 \pm 10.2 ^b	4.7 \pm 7.2 ^a
PSS	9.3 \pm 4.7 ^{ab}	6.0 \pm 4.6 ^a	5.7 \pm 4.9 ^a			19.3 \pm 4.7 ^b	1.4 \pm 3.3 ^a

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

RESULTS

Macroscopic Observation In Experiment 1 (Table 1), ovarian follicles except blood follicles were classified as 1.0, 1.5, 2.0, 2.5 and ≥ 3.0 mm in diameter. No large follicles (≥ 2.0 mm in diameter) appeared in the ovaries of CTPFF-treated does. The mean number of all follicles, except blood follicles, in WPFf-treated does was significantly smaller than those of other groups (CTPFF- and saline-treated). Opaque follicles in large size (> 2.0 mm in diameter) were observed only in WPFf-treated does. These follicles were flatly located on the ovarian surface. Especially, the large follicles over 3.0 mm were cystic.

In Experiment 2 (Table 2), the ratio of ovulated does in group OW was significantly lower than the ones of other groups (OF and OS) ($p < 0.05$). Though the large cystic follicles (3.0–4.0 mm) were observed only in non-ovulated does of group OW, these cystic follicles did not appear in the ovulated does of this group. The mean numbers of ovulations (corpora lutea) in groups OF and OW were significantly lower ($p < 0.01$) than those of control does (group OS).

Microscopic Observation In histology of the does in Experiment 1, it was noted that follicular fluid of cystic follicles was stained red by azan stain in WPFf-treated does, while the color of follicular fluid in healthy follicles was blue. Granulosa cells in blood follicles luteinized in WPFf-treated does.

In Experiment 2, most cystic follicles were hemorrhagic in various degree, and granulosa cells in half of these cystic follicles luteinized partially. The mean numbers of large luteal cells in blossom stage in groups OW and OF, counted 4 microscopic fields of 0.024 mm² ($\times 580$). They were significantly fewer than those in group OS.

In the observations of the anterior pituitary cells (Experiment 3), no apparent difference

Table 2. Mean numbers of ovarian follicles (mean \pm S.D.) in different diameters and mean numbers of ovulations (copora lutea) in each group of does at 4 days after hCG injection

Group	Mean no. of follicles in different diameter (mm)						No. of ovulated does vs. no. of total does	Mean no. of ovulations	Mean no. of luteal cells*
	1.0	1.5	2.0	2.5	3.0	≥ 3.5			
OW	11.3 \pm 7.7 ^a	6.2 \pm 3.2 ^a	3.5 \pm 3.7 ^a	0.7 \pm 0.8 ^a	1.3 \pm 2.3 ^a	0.3 \pm 0.8	2/6 ^a	3.0 \pm 2.0 ^a	18.4 \pm 1.4 ^a
OF	11.3 \pm 4.2 ^a	1.0 \pm 1.3 ^b	0.6 \pm 1.4 ^a		0.3 \pm 0.5 ^a		6/6 ^b	2.7 \pm 1.6 ^a	20.3 \pm 2.9 ^{ab}
OS	9.3 \pm 1.9 ^a	2.0 \pm 1.8 ^b	0.3 \pm 0.5 ^a	0.1 \pm 0.3 ^a	0.2 \pm 0.4 ^a		6/6 ^b	10.2 \pm 1.5 ^b	23.2 \pm 3.6 ^b

*Number of large luteal cells counted on 4 microscopic fields of 0.024 mm² ($\times 580$).

Significant differences exist among figures with different superscripts in the same column ($p < 0.05$).

was detected in acidophilic and chromophobic cells among all groups. However, basophilic cells in does of group F appeared in bigger number than those of the other groups. They filled with blue and slightly expanded granules. Many basophilic cells in group 36 showed degranulation and vacuolization in most of cytoplasm.

Concentrations of Sex Steroids Because only 2 does out of 6 ovulated in group OW, the ovulated does were designated as OW+ and the non-ovulated ones were designated as OW- in Figures 1-3. Estrone (E_1) levels in group OW+ rose during the treatment period and after hCG injection, while E_1 levels in other groups during the same periods remained roughly at the same levels as found before treatment. Low levels in groups OW+ and OW- (group OW) 4 days after hCG injection (Fig. 1) were an exception to this fact. E_2 levels in groups OF and OW lowered during the treatment period in comparison to those in group OS. Four hours after hCG injection E_2 levels rose only in group OW+. At laparotomy, E_2 levels in group OW were lower than those in groups OS and OF (Fig. 2). Progesterone levels in all groups increased markedly 4 hours after hCG injection. At laparotomy, progesterone levels in group OW fell remarkably compared to those of groups OF and OS (Fig. 3).

Appearance of Vulva The values in appearance of the vulvae decreased significantly 2 days after the starting of CTPFF or WPPF injections in comparison with those in the control group (Fig. 4).

DISCUSSION

In our previous studies (FUKUNARI *et al.*, 1987; NAGAHAMA *et al.*, 1987), no large follicles (≥ 2.0 mm in diameter) appeared in ovaries of the CTPFF-treated does despite their presence in those of the control (administration of saline solution). The mean ratio of the number of healthy antral follicles in histology was significantly lower than the total number of antral follicles in the CTPFF-treatment. Ovulation rates were significantly reduced when ovulation was induced by the administration of hCG immediately after the end of the CTPFF-treatment, also (YOSHIDA *et al.*, 1985a, b, 1986; NAGAHAMA *et al.*, 1987). The present study in the CTPFF-

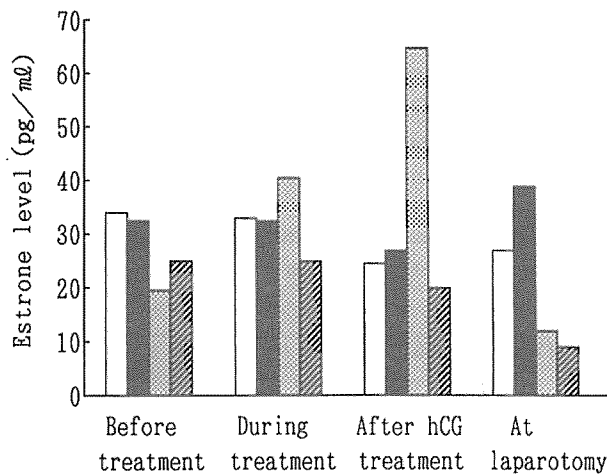


Fig. 1 Estrone levels in peripheral plasma in Experiment 2.
Group; □ OS, ■ OF, ▨ OW+, ▩ OW-.

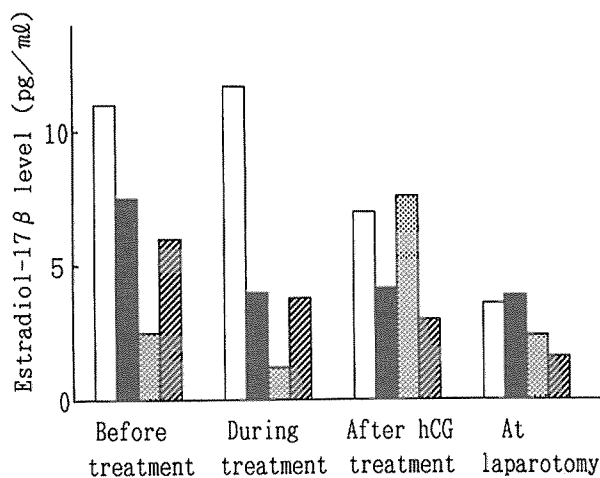


Fig. 2 Estradiol levels in peripheral plasma in Experiment 2.
Group; □ OS, ■ OF, ▨ OW+, ▩ OW-

treatment experiments produced identical results to those described above. However, the state of follicular distribution on the ovarian surface in WPFf-treated does was quite different to that in CTPFF-treated does. In WPFf-treated does, large cystic follicles appeared while no such follicles showed in CTPFF-treated or in saline-treated does. In the histological observations, the staining quality of follicular fluid in the cystic follicles was different from that of healthy follicles. The cystic follicles were hemorrhagic and/or luteinized in various degrees. In the induced ovulation experiments, the rate of ovulated does treated with WPFf was significantly lower than that of does treated CTPFF, though there was no difference in the mean number of ovulations per ovulated doe between CTPFF- and WPFf-treatments. Large cystic follicles remained in WPFf-treated does which were not ovulated by administration of hCG.

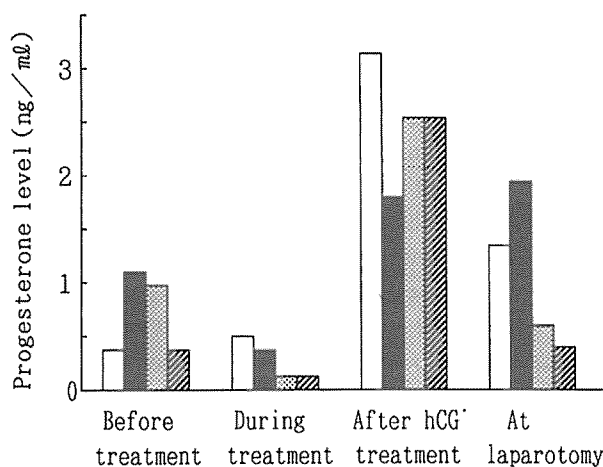


Fig. 3 Progesterone levels in peripheral plasma in Experiment 2.
Group; □ OS, ■ OF, ▨ OW+, ▩ OW-

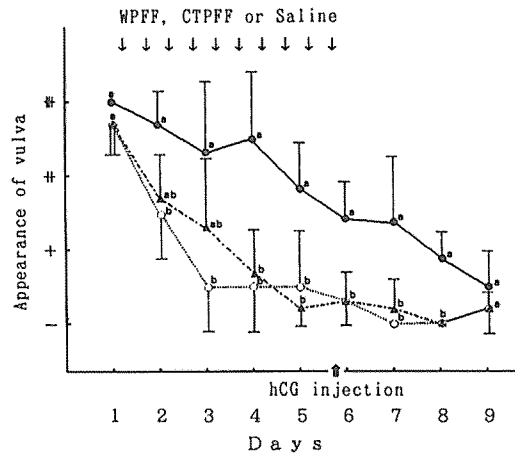


Fig. 4 Effect of successive administrations of whole porcine follicular fluid (WPF) or charcoal-treated porcine follicular fluid (CTPF) on vulval appearance in 12 does (WPF—6 does, ▲, mean \pm S.D.; CTF—6 does, ○, mean \pm S.D.), in comparison with 6 does (●, mean \pm S.D.) which were given saline in 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). ↓, does of CTF or saline solution; ↑, injection of 15 IU of hCG; significant difference between a and b ($p < 0.05$).

These differentiations are caused by differences between CTF and WPF, especially related to the presence of steroid hormones in the follicular fluid. Follicular inhibin can inhibit FSH secretion from the pituitary. Follicular estrogen also suppresses the FSH secretion in the feedback action synergistically. Thus, the reduction in FSH levels in WPF-treated does may be greater than those in CTF-treated does, this may cause a significant decrease of the total number of antral follicles located on the ovarian surface. However, WPF contains not only estrogen but also progesterone and/or testosterone as major sex hormones. For example, the rabbit ovary secretes testosterone 10 times more than E_2 . They are produced by their follicles (YOUNGLAI, 1978). Single intact follicles significantly increased the E_2 production during incubation in medium added FSH alone or in combination with anti-LH serum. The addition of LH or LH plus FSH did not increase E_2 production, this suggests that FSH may be important in the aromatisation of testosterone in the rabbit follicle (YOUNGLAI, 1976). On the other hand, it is known that granulosa cells have receptors for testosterone and testosterone that regulate the progesterone production in the granulosa cells (YOUNGLAI, 1978). It is also probable that testosterone may stimulate the formation of atretic follicles (YOUNGLAI, 1978).

When does are in heat, the appearance of the vulva is characterized by a deep color and swelling in most cases, caused by high levels of estrogen delivered from large follicles (LEFÈVRE and CAILLOL, 1978; PLÁ *et al.*, 1986). In CTF- or WPF-treated does, the scores of vulval appearances markedly decreased 2 days after the beginning of these follicular fluid treatments,

suggesting reduced estrogen levels due to suppression of FSH secretion which bring about a reduction of large follicles in the ovaries as shown in Table 1. There was no significant difference in the vulval scoring between CTPFF- and WPFf-treatment notwithstanding the great differences in their contents. This seems quite natural because the scores decreased to reach a lowest level in both treatments. It is not comprehensive that administrations of WPFf with rich estrogen do not markedly elevate the estrogen levels in does more than administrations of CTPFF-treatment do, except in case of 4 hours after hCG injection. Although plasma progesterone concentrations were lower in does that received WPFf than in those of control or CTPFF-treated, progesterone levels markedly elevated 4 hours after hCG injection in all does. A striking fall in progesterone levels was evident in does, ovulated or non-ovulated does treated with WPFf 4 days after hCG injection, but progesterone levels in does treated with CTPFF remained in high levels. YOSHIDA *et al.* (1986) reported that progesterone levels in does treated with CTPFF for 5 days remained at lower levels from 3 to 7 days after ovulation than those in controls, and that some regressive changes of its levels appeared in luteal cells 7 days after ovulation. A similar trend of them was realized in histology of corpora lutea 4 days after hCG injection in the present study. Furthermore, lower progesterone levels in WPFf-treated does seem to suggest that administrations of WPFf have more suppressive corpus luteum function than administrations of CTPFF. The reason for formation of the large cystic follicles in WPFf administrations only remains obscure. According to YOUNGLAI (1978), immunization of testosterone in rabbits caused abnormal growth of follicles including large cystic or hemorrhagic follicles. Thus, the effects of WPFf on the ovary are very complicated and difficult to elucidate only by inhibin, progesterone and estrogen. However, the suppressive power of WPFf on the ovarian function seems to be greater than that of CTPFF. Many questions remain unsolved for application of follicular fluid on animal reproduction.

Additional histological studies on the anterior pituitary showed that basophilic cells which were filled with granules were observed more frequently at the end of CTPFF-treatment than in control does and that degranulation and vacuolization in the cells appeared 36 hours after the end of CTPFF-treatment. These phenomena may be responsible for FSH accumulation by CTPFF-treatment and its release by liberation from inhibitory action of CTPFF. These evidences agree with the descriptions by NAKAHARA (1962, 1963) and SAEKI (1953), who observed degranulation and vacuolization in the basophilic cells after coitus or castration.

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活性炭処理および未処理豚卵胞液の投与が家兔の排卵、卵巣組織、血漿中の性ステロイドホルモンおよび下垂体組織に及ぼす影響

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活性炭で処理した豚卵胞液 (CTPFF) あるいは未処理の豚卵胞液 (WPF) の投与が家兔の排卵, 卵巣組織, 血漿中の性ステロイドホルモンおよび下垂体組織に及ぼす影響について検討した。

1. CTPFF あるいは WPF を 4 ml ずつ 12 時間おきに 10 回連続皮下投与し, 投与終了直後に開腹して卵巣表面を観察した結果, 直径 1.0mm 以上の卵胞数は CTPFF あるいは生理的食塩水投与区と比較して WPF 投与区で減少していた。また CTPFF 投与区には大型卵胞 (≥ 2.0 mm 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。さらに WPF 投与区には囊腫様大型卵胞が観察された。各処理区の卵巣を組織学的に観察したところ WPF 投与区に見られた囊腫様卵胞の卵胞液はアザン染色で赤色に染まっていた。また WPF 投与区の血球は, 顆粒層細胞が黄体化している黄体化血球が殆どであった。
2. 前項 1 と同様に CTPFF (OF-区), WPF (OW-区) あるいは生理的食塩水 (OS-区) を投与し, 投与終了直後に hCG 15 IU を静注して 4 日目に開腹し, 卵巣表面を観察した。OF-区および OS-区は全例排卵していたが, OF-区の排卵数 (2.3 個) は OS-区 (10.2 個) と比較して有意に少なかった。また OW-区は 6 羽中 2 羽しか排卵しておらず, 全例排卵した他の 2 区と有意な差が認められ, 排卵しなかった個体には前項 1 で見られた様な囊腫様卵胞が存在していた。これらの囊腫様卵胞も前項 1 と同様に顆粒層細胞が黄体化しているものであった。各処理区の単位面積当たりの黄体細胞を数えたところ, OW-区, OF-区, OS-区の順で少なく, 特に OW-区の黄体は黄体形成が不完全であった。末梢血漿中のエストロゲン濃度は, OW-区および OF-区で処理期間中に低く, 更にその時の外陰部の腫脹の程度も低下していた。また, 開腹時のプロゲステロン濃度は OW-区で低い値を示した。
3. 前項 1 と同様に CTPFF を投与し, 投与終了直後あるいは 36 時間後に下垂体を採取して組織学的に観察すると, CTPFF 投与終了直後の下垂体前葉には, 顆粒を蓄積している好塩基性細胞が多く観察されたが, CTPFF 投与終了後 36 時間の下垂体前葉の好塩基性細胞は顆粒を放出しているものが多かった。

以上の結果 WPF 及び CTPFF の投与は卵胞の発育を抑制し, WPF の投与は CTPFF の投与よりも FSH 分泌の抑制効果も強く, 排卵に対する抑制効果は強いが, WPF 中に含まれている多量の非蛋白系物質等の影響により囊腫様卵胞が形成される事が明らかになった。また CTPFF 投与終了後の FSH のリバウンド現象は, CTPFF の投与により下垂体前葉の好塩基性細胞に蓄えられた FSH が放出されて招来されるものと考えられた。