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Effect of Age on the Changes of Pituitary and Serum Gonadotrophin Levels during PMS-Induced Ovulation in Prepubertal Rats

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Summary

The ovulatory response was investigated in immature rats with advancing age (25, 27, 29, 31 days after birth) by low doses of PMS (1, 3 and 5 IU) which were injected per head or per body weight (b. w.).

Also, the levels of serum and pituitary gonadotrophin during PMS-induced ovulation were measured to know the relation between the pattern of gonadotrophin and the age of the treatment.

One IU PMS per head or per b.w. resulted in a uniformly low ovulation rate (0~25%) at each age treated, but 3 or 5 IU PMS per both treatment showed a full response indicating 87.5~100% ovulation.

The number of ova obtained from ovulated rats, regardless of the dose and age of PMS treatment, was similar to that observed at natural puberty.

Thus it was reconfirmed that 3 IU PMS is the minimal dose to induce ovulation effectively in prepubertal rats.

Pituitary LH and FSH showed a decline in the afternoon of the day before ovulation, but the pattern of the decreasing levels was affected by the age of PMS treatment indicating that both hormones had a tendency to decrease at a faster rate during 52 to 60 hr and reached the lowest levels at 72 hr as rats got older (approached puberty).

The elevation of serum LH and FSH was observed 52 to 60hr after PMS treatment and returned to the initial levels at 72hr, although the secreting pattern did not appear to be the same for the age of PMS treatment.

While ovarian responsibility to PMS did not tend to change through the prepubertal age as judged by an ovulation rate and the number of ova, the pattern of the endogenous gonadotrophin by the stimulation of PMS changed with age. So, it might be suggested that the sensitivity of the hypothalamo-pituitary axis to the PMS stimulation changes near puberty.

Ovulation can be induced by a single low dose injection of pregnant mare serum (PMS) on the third day morning after treatment (1, 2). Also, it has been reported that ovarian responsibility to PMS in rats changes little from 3 weeks of age until puberty when ovulation is used as the index of the responsibility (3).

Moreover many investigators (1, 4, 5, 6, 7) have indicated that ovulation induced with PMS depends on the release of endogenous pituitary ovulating hormone.

Wilson et al (8) showed that the timing and pattern in plasma and pituitary hormone in immature rats during PMS-induced ovulation were very similar to those in adults during natural ovulation.

On the other hand, *Umezu* et al (9) observed that the degree of the decrease in pituitary ovulating hormone just after ovulation induced with PMS became greater as the animal approached puberty.

Therefore there might be a possibility that the endogenous condition in rats to induce the decrease of pituitary ovulating hormone in response to PMS was different through the prepubertal age.

The purpose of this work is to further investigate in detail the ovarian responsibility to different dose of PMS with advancing age in immature rats and to survey the pattern of pituitary and blood gonadotrophin by using the method of radioimmunoassay during PMS-induced ovulation in connection with the prepubertal age.

Materials and Methods

Immature female rats of the Wistar strain inbred in our laboratory were used in this experiment. All animals were weaned at 21 days of age and kept in an air-conditioned room with controlled illumination of 12 hr day and 12 hr night and provided food and water *ad libitum*. Our rats usually attained vaginal opening at 36.9±0.3 (M±S.E.) days after birth when more than 50% of them ovulated (10).

At 25, 27, 29 and 31 days of age (between 09.00 and 10.00 hours) the animals were given a subcutaneous injection of 1, 3 and 5 IU PMS (P-mex Sankyo-zoki) per head in 0.1 ml saline, or per 60 g body weight in 0.4 ml saline. The animals were killed to examine the presence of tubal ova on the third morning after treatment and the number of tubal ova was counted under a dissecting microscope by gently pressing the oviduct between two slides.

Because 3 IU PMS was confirmed to be a minimal dose to induce ovulation in immature rats by the observation discribed above, the animals were treated with 3 IU PMS per head to measure serum and pituitary gonadotrophin during ovulation at 25, 27, 29 and 31 days of age. Blood was collected under light ether anesthesia from a jugular vein and centrifuged at 3,000 rpm for 20 min. The serum was collected at 48, 52, 56, 60 and 72 hr after treatment and stored at −60°C until assayed. Each pituitary was removed and weighed to homogenize in 1 ml physiological saline. After centrifugation the supernatants were stored at −60°C until assayed. At sacrifice ovaries and uteri were removed and weighed on a torsion balance.

Pituitary and serum gonadotrophin were measured by double antibody radioimmunoassay using reagents provided by the NIAMDD Rat Pituitary Hormone Program. The results were expressed in terms of the RP–1 rats pituitary reference preparations.

Results

Effect of age on the ovulatory respose by PMS treatment

The results are shown in Table 1. The treatment of 1 IU PMS was not effective to induce ovulation in either treatment groups, namely the ovulating rate was below 25% in every age. In the rats treated with 3 IU and 5 IU PMS the ovulatory response was stable regardless of the age in both treatments, indicating that the ovulation rate was about 87.5%.

The average number of ova did not change with age and dose of PMS in case that the ovulation was recognized. In the rats treated with 1 IU PMS per head and b.w., the average number of ova per ovulated rat was 7.4 and 10.7, respectively, although the rat in this case was very few. In the rats treated with 3 IU and 5 IU PMS the number of ova ranged from 6 to 14 in both treatment groups as a whole. Ovulation in all the control rats was not observed at all the ages studied (Table 2).

TABLE 1. *Effect of Age on Ovulation in Immature Rats Treated with PMS*

Age at treatment (Age at autopsy)	Dose of PMS (IU)	PMS treatment per head			PMS treatment per 60 g b.w.		
		No. of rats	Ovulating (%)	No. of ova	No. of rats	Ovulating (%)	No. of ova
25(28)	1.0	8	25.0	7.5	8	12.5	12
27(30)	1.0	7	0	0	7	0	0
29(32)	1.0	8	12.5	7.0	8	12.5	10
31(34)	1.0	8	25.0	7.5	8	12.5	10
25(28)	3.0	8	87.5	8.7±0.2*	8	87.5	8.4±0.5*
27(30)	3.0	8	87.5	8.6±0.4	8	87.5	6.6±0.6
29(32)	3.0	8	100	8.5±0.6	8	100	8.3±0.4
31(34)	3.0	6	100	8.1±0.6	8	100	9.5±0.3
25(28)	5.0	8	87.5	9.6±0.8	8	100	8.0±0.3
27(30)	5.0	8	100	6.9±0.2	7	100	8.3±0.3
29(32)	5.0	6	100	8.2±0.7	8	100	8.1±0.6
31(34)	5.0	8	100	7.5±0.8	8	100	9.3±0.6

* Mean±S.E.

TABLE 2. *Ovulating Rate and Organ Weight in Non-Treated Control Rats*

Age at autopsy	No. of rats	Ovulating (%)	Ovarian weight (mg)	Uterine weight (mg)
28	6	0	14.5±1.0*	38.1± 3.2
30	6	0	10.8±0.6	44.5± 2.7
32	6	0	15.0±1.3	68.0±12.2
34	6	0	17.5±1.2	64.5± 5.3

* Mean±S.E.

Control rats were killed at same age to compare with PMS-treated rats.

Effect of age on pituitary and serum gonadotrophin levels during PMS induced-ovulation

1) Changes of ovarian and uterine weight during ovulation

The ovarian weight of each age had already increased 48 hr after treatment compared with control (Table 2, Fig. 1). The ovarian weight in the rats of each age, reached to a maximum at 60 hr and decreased slightly by 72 hr (Fig. 1). The rats treated at 31 days of age showed a heavier ovarian weight at each time from 56 h to 72hr compared with those of other ages.

The change of uterine weight was shown in Fig. 1. Although a maximal uterine weight in the rats of each age was observed at 56 hr or 60 hr after injection, it decreased slowly thereafter. The uterine weight tended to have great variation through the experimental period, but the weights in the rats of 29 and 31 days were heavier 48, 60 and 72 hr and 48, 56, 60 and 72hr after treatment respectively than in the rats of 25 days.

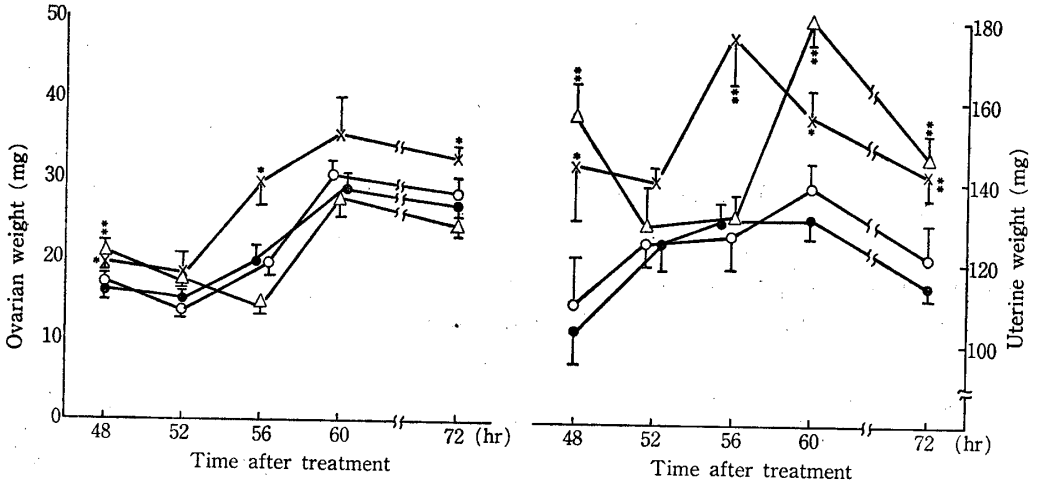

Fig. 1. Ovarian and uterine weights during PMS-induced ovulation in immature rats. The rats were treated with 3IU PMS at 25(●), 27(○), 29(△) and 31(×) days of age and killed at each time after treatment. The vertical bars represent standard error of the mean. The asterisk represents significance by *t* test as compared with the rats treated at 25 days of age. *; $P<0.05$, **; $P<0.01$

2) Changes of pituitary and serum LH levels during PMS-induced ovulation

Pituitary LH levels decreased in the afternoon of the day before ovulation, showing a different pattern in the rats of each age (Fig. 2-a, b). Pituitary LH levels started to decrease from 56, 52, 56 and 48 hr (646.4, 538.9, 564.6 and 603.0 μg/gland respectively) at 25, 27, 29 and 31 days of age respectively after PMS treatment followed by a rapid and significant decline to 392.3, 339.7, 304.7 and 178.5 μg/gland at 60 hr. The levels then remained low until 72 hr. When compared groups of each age, the pituitary levels of 25 days were significantly higher at 56 hr ($P<0.01$) and 60 hr ($P<0.05$) after injection than those of the rats of 31 days. However no

significant difference in the level which depends on age was recognised 72 hr after treatment. When pituitary LH levels were given as μg per mg (Fig. 2-b), a significant difference between the levels of the rats of each age was still more apparent at each time after injection. The level of the rats of 25 days was significantly higher at 56 hr than that of the rats of 27 ($P<0.05$) and 31 days ($P<0.01$). Also at 60 hr after injection, the rats of 25 days had a significant higher level compared with those of 29 and 31 days and even at 72 hr showed significantly higher level as compared with those of 29 and 31 days ($P<0.01$).

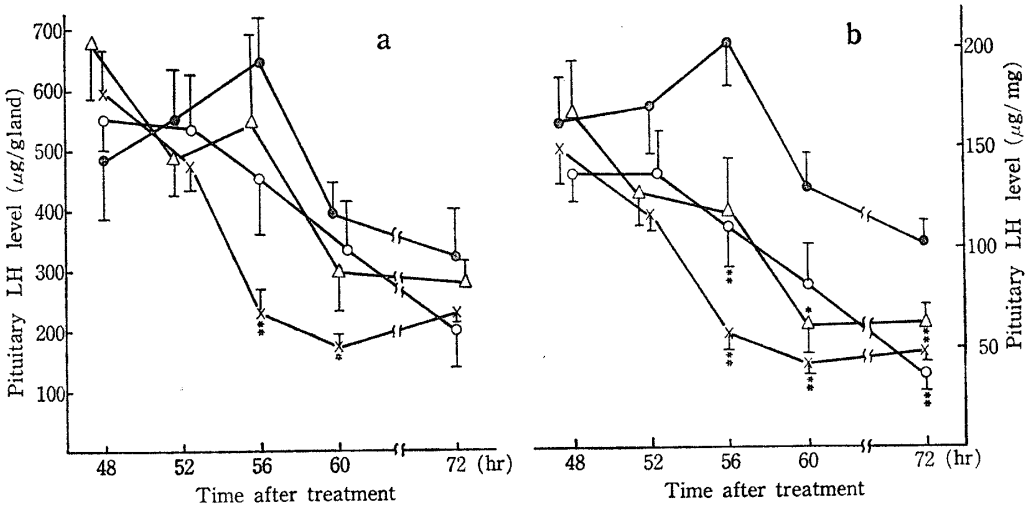

Fig. 2. Pituitary LH levels during PMS-induced ovulation in immature rats.
a); LH level given as μg/gland. b); LH level given as μg/mg. † See Fig. 1.

Individual change in serum LH levels caused great variation of standard error through experimental period (Fig. 3). Serum LH of the rats treated at 25, 27, 29 and 31 days of age began to rise from 52, 52, 48, and 48 hr (627.3, 731.8, 190.5 and 223.0 ng/ml) after injection and reached to peaks of 4548.0 ng at 60 hr, 2192.1 ng at 56 hr, 2732.8 ng at 56 hr and 4142.3 ng at 52 hr respectively and by 72 hr after treatment serum LH levels returned to initial level.

3) Changes of pituitary and serum FSH levels during PMS-induced ovulation

Pituitary FSH levels of the rats treated with PMS at each age also decreased in the afternoon of the day before ovulaton (Fig. 4-a, b). Pituitary FSH of the rats treated at 25 and 29 days of age slightly increased from 48 hr to 52 hr and from 48 hr to 56 hr respectively, but in the rats treated at other ages this increase was not observed. The levels of the rats treated at 25, 27 and 29 days of age began to decrease 56 hr after treatment (189.8, 121.7 and 169.4 μg/gland respectively), except for the rats treated at 31 days of age in which the levels began to decrease 52 hr (66.9 μg/gland) after treatment, then decreased to 137.8, 60.4, 33.5 and 21.7 μg/gland at 72 hr respectively. Among pituitary FSH levels of the

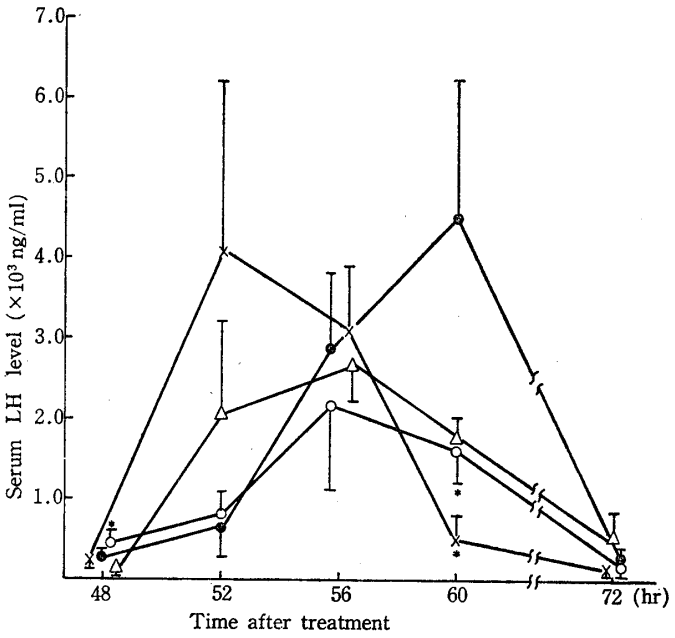

Fig. 3. Serum LH levels during PMS-induced ovulation in immature rats.
† See Fig. 1.

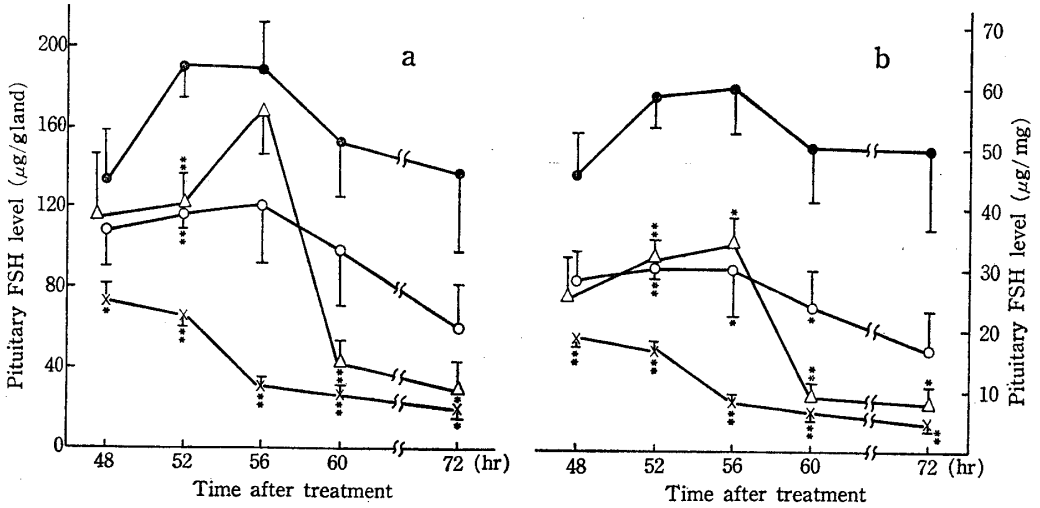

Fig. 4. Pituitary FSH levels during PMS-induced ovulation in immature rats. a; FSH level given as μg/gland, b; FSH level given as μg/mg. † See Fig. 1.

rats treated at each age, the levels of the rats treated at 25 days of age was significantly higher at each time after treatment than those of rats treated at 31 days of age (48, 72 hr; $P<0.05$, 52, 56, 60 hr; $P<0.01$). Also the level of the rats of 25 days at 52 hr was significantly higher than that of rats of 27 and 29 days ($P<0.01$), higher in 25 days at 60 hr than in 29 days ($P<0.01$). A significant defference between the levels of the rats treated at 25 and 29 days of age was remained by

72 hr after treatment ($P<0.05$). When pituitary FSH levels were given as μg per mg (Fig. 4b), a difference between the levels which depends on age became clearer. Therefore the changes of pituitary FSH level, got greater, as the rat got older.

Serum FSH of the rats treated at 25, 27 and 29 days of age increased rapidly from 52 hr after treatment (Fig. 5) and reached to peak of 6,200.0, 4,269.0 and 5,500.0 ng/ml at 60 hr, followed by the decreased level of 1,200.0, 995.0 and 1,193.0 ng/ml at 72 hr. In the rats of 31 days serum FSH began to increase at 52 hr (2537.5 ng/ml) and reached a peak of 3,625.0 ng/ml at 56 hr and then decreased to 802.0 ng/ml at 72 hr.

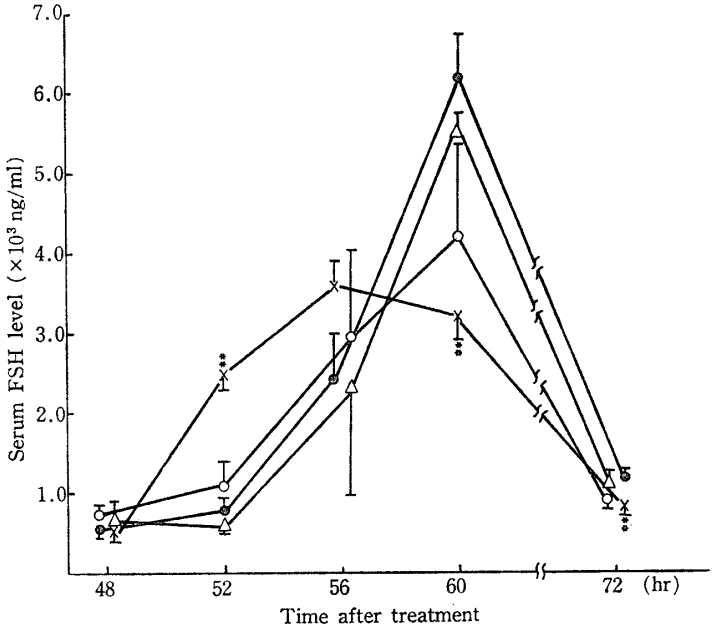

Fig. 5. Serum FSH levels during PMS-induced ovulation in immature rats.
† See Fig. 1.

Discussion

In the present study, the aspect of ovulation in prepubertal rats was not so different by PMS treatment by age or injection method. Three and 5 IU PMS were effective to induce normal ovulation regardless of age, but 1 IU was not.

Ying et al. (2) have reported that a low dose of PMS (2–6 IU) stimulates the development of a few follicles and leads to an optimal environment which results in the ovulation. Also in our laboratory (2) it has been observed that the ovulatory response to PMS initiates at 21 days of age and changes little from 23 to 31 days of age. The result in this experiment also indicates that the ovarian responsibility to PMS is nearly the same through all ages studied, as judged by the ovulation rate and the number of ova. Therefore 3 IU PMS was reconfirmed to be a minimal dose to induce ovulation in immature rats.

The ovarian weight by the treatment of 3 IU PMS increased in the afternoon of the day before ovulation. The weight of rats treated at 31 days increased with a faster rate during ovulation than that of rats treated at other days. Also, the uterine weight at 31 days increased during the same period.

Since the increase of ovarian and uterine weight seems to be dependent on the growth of follicles in immature rats (11, 12), it may be possible that the degree of follicle growth accelerated by PMS or endogenous gonadotrophin has a relation with age.

But the number of matured follicle as judged by the number of ova was not apparently affected through age. So, it is assumed that a function of ovary after PMS treatment does not show a remarkable change until near puberty in spite of slightly maturating with advanced age.

Pituitary LH and FSH decreased during critical period in the afternoon before ovulation. The results agrees with the finding of *Klausing* et al (13) and *Zarrow* et al (14) who reported a depletion in pituitary ovulating hormone during the period. While, serum LH and FSH elevated in the afternoon before ovulation. So, our result supports the evidence that the ovulatory surge occurs in the afternoon of the day before ovulation in immature rats with PMS treatment (1, 4, 5, 6, 7, 15).

The pattern of pituitary gonadotrophin changed with advanced age indicating that LH and FSH decreased at a significantly faster rate during critical period and after ovulation.

Umezu et al (9) observed this phenomenon by bioassayable measurement of pituitary ovulating hormone just after ovulation. They also reported that the amount of the hormone decreased in rats just before puberty is quite similar to that observed at natural puberty (the first ovulation). Therefore, the reason why the change in the content of the pituitary hormones occured seems important for understanding the onset of puberty. It is widely accepted that the ovulation with PMS in immature rats result from positive feed-back action of endogenous estrogen through hypothalamo-pituitary axis (16, 17, 18, 19).

Ying et al (20) observed that the increase in ovulation rate and ovarian weight after single injection of estradiol benzoate has the relation with age; both of the responses developed as animals become nearer to puberty. We suppose that the secreted levels after PMS treatment during the critical period for the ovulatory surge was not so different through the age because of the biological observations above mentioned.

So, the sensitivity of the axis to estrogen with advanced age seems to play an important role for the occurrence of puberty. But it seems necessary to estimate the levels of blood estrogen for clarifying this idea because the uterine weight which responds with estrogen slightly increased with advanced age in our observation. Also, the remarkable change in pituitary gonadotrophin during the critical period for the ovulating surge, in particular, at an older age seems to relate with the

change in the level of hypothalamic LH-RH or/and in the sensitivity of pituitary to LH-RH.

It has been reported that hypothalamic LH-RH drastically decreases at natural puberty suggesting the release of the hormone (21, 22), while there is few report on the change of the hypothalamic hormone with connection to age at the PMS treatment.

Also, *Umezu* et al (unpublished data) observed that the ovulatory capacity of the pituitary after PMS treatment in response to exogenous LH-RH was enhanced with age, particularly near puberty. Therefore these factors also seem valuable for the occurrence of puberty (the first ovulation).

About the blood levels of gonadotrophin, the variation was so great and also the samples were taken in a rather long interval (4 hours). So a valuable relationship between the gonadotrophin levels and the prepubertal age was not evident.

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