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The Effects of PMS or HCG on the Ovulation in Female Immature Rats

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Summary

As a means of approaching the mechanism of the onset of puberty, the alteration of ovarian responsibility to exogenous gonadotropin as related to ovulation was studied in immature rats with advanced age by using PMS alone or HCG alone (PMS: 30 IU, 3 IU and 1.5 IU, HCG: 50 IU, 10 IU and 2 IU).

In the case of the treatment of 3 IU PMS, the ovulation was initiated at age of 21 days and not different in the period of initiating ovulation comparing with 30 IU treatment. The ovulation in 3 IU PMS had simular tendency all through the period of the treatment from 21 to 31 days concerning ovulation rate, number of tubal ova and ovarian weight. These responses were not different from the records of the first ovulation at vaginal opening concerning ovulation rate, number of ova and ovarian weight.

In the case of the treatment of 10 IU HCG, immature rats did not show sufficient ovulation in comparison with full response in adults.

From these results, it is suggested that ovarian follicles had not yet fully developed to ovulate by administration of exogenous HCG until near puberty and that sudden growth of follicles activated by gonadotropin were regarded as one factor initiating puberty.

It has been confirmed from recent studies that a single injection of pregnant mare serum (PMS) induces ovulation in immature rats (1). This treatment can also be used to investigate the ovarian responsibility to exogenous gonadotropin. This ovarian responsibility seems to be part of the mechanism of the onset of puberty. In ovulation induced by the PMS treatment, earlier workers have used 30 IU and over which was enough to induce superovulation.

It is a problem whether the use of such a large dose is proper when the ovarian response to gonadotropin is considered on the physiological level. Accordingly, this investigation was undertaken to obtain the proper dose of hormone for the investigation of the ovarian response to exogenous gonadotropin.

In addition, the induction of ovulation in immature rats by a HCG treatment has not yet been reported, in spite of many works in PMS treatment. With

the HCG treatment it seems to be important to know the time when the ovarian follicles complete sufficient maturation to ovulate, because this treatment will be regard as an index of ovarian responsibility in immature rats. This investigation was also designed to resolve the ovarian responsibility to various doses of PMS or HCG with advanced age before puberty.

Materials and Methods

Female immature rats of Wistar strain were used. The litter size of new born rats was adjusted to 8 on the next day after parturition. All rats were weaned at the age of 21 day. Afterwards, they were fed with a stock diet of dry pellet including 20 per cent crude protein and water ad lib. The room was kept at a temperature of 25±1°C. The light was automatically turned on at 6:00 AM and off at 6:00 PM.

Applied gonadotropin was PMS (Serotropin from Teikoku-Zoki Co.) and HCG (Puberogen from Tomoda-Seiyaku Co.). Each gonadotropin was dissolved in a volume of 0.1 ml of 0.85 per cent saline water. The induction of ovulation in response to exogenous gonadotropin was regarded as an index of ovarian response.

In the first experiment, the effect of PMS on the ovulation was studied. The schedule of the treatment followed the method of Zarrow et al (1) except for the dose of PMS. The PMS was injected at 9:30–10:00 AM. The rats were killed 76 hours after the injection of PMS to examine the presence of tubal ova under a dissecting microscope. The weight of ovaries and uteri were measured simultaneously at autopsy.

The PMS was applied in three different doses of 1.5, 3.0 and 30 IU. These doses were also investigated with advanced age from 19 to 31 days. In the second experiment, the effect of HCG treatment on ovulation was studied. HCG was injected in the amounts of 2, 10 and 50 IU at 4:00–6:00 PM in immature rats. In another series, virgin adult rats indicating late diestrous in smear after the recognition of 4 day's cycle for more than two times were injected with 10 IU HCG at the same time (2). They were autopsied at 20–24 hours after the injection of HCG, and the presence of ova and the weight of organs were checked in the same way as with the PMS treatment. The record of the age of immature rats indicates the day of injection with gonadotropins in these experiments.

Results

The Influence of the Dose of PMS on the Ovulation

As a preliminary experiment, three kinds of doses (1.5, 3.0 and 30 IU) were injected into rats at 25 days in order to know the minimum doses necessary to induce a physiological ovulation.

In the case of the treatment with 30 IU PMS, superovulation was observed

in all rats with the mean number of 36 in tubal ova. When 3.0 IU PMS was used, ovulation was recognised in 8/10 rats with mean ova count of 7.2. However, no ovulation response was observed in the case of the 1.5 IU PMS treatment. Accordingly, 3.0 IU PMS was used to induce the ovulation afterwards with advanced age. 1.5 IU PMS was applied to check the absence of ovulation in pararell with the treatment of 3.0 IU PMS.

The Influence of PMS on the Ovaries

i) The Rate of Ovulation

As shown in Table 1, the ovulation was initiated at 21 days in 3/22 rats when injected with 3 IU PMS and the initiation of ovulation was not so different comparing with 30 IU treatment at 21 and 23 days respectively ($P>0.05$ by X^2). Afterwards, ovulation occurred with high ovulation rate until 31 days. There was no difference in the rate all through the period ($P>0.05$ by X^2). In the case of treatment with 1.5 IU PMS, ovulation was not observed except for a positive example at 23 days.

ii) The Number of Tubal Ova

The mean number of tubal ova of rats which the presence of ova was recognised, were 7.0, 6.9, 7.2, 8.5 and 9.1 at the age of 21, 23, 25, 29 and 31 day respectively as shown in Table 1. When the number of ova was compared in each period there was no significant difference. Also there was no significant difference when comparing the number of ova at the vaginal opening (Table 2).

iii) The Weight of Ovaries

In the case of the treatment with 3 IU PMS, the ovarian weights increased rapidly at 23 days when the ovulation rate became higher (Table 1).

A significant difference was recognized at each period from 21 to 31 days between the ovaries with ovulation and those without. The rats injected with 1.5 IU PMS through each period showed signifiicantly smaller ovarian weight compared with ovarian weight of rats injected with 3 IU PMS ($P<0.01$). In the case of comparing the ovarian weight of rats injected by 3.0 IU PMS with rats ovulated at vaginal opening, the former indicated a slightly smaller weight than the latter at 21 and 23 days, but there was no difference in 25 to 31 days ($P>0.05$).

The Influence of HCG on the Ovaries

i) The Rate of Ovulation

When 10 IU HCG was injected at 27 days, ovulation was not observed (Table 3). Ovulation was observed in 3/15 and 2/10 at ages 31 and 33 days respectively.

Considering the effects of the same dose of HCG on adult rats, they indicated a high rate of ovulation with 7/8. When this adult rat value was compared with that of each immature group, there was a significant difference at 31 days ($P<0.01$ by X^2) and 33 days ($P<0.025$ by X^2). Accordingly, it was suggested that the follicles

TABLE 1. *Influence of Age on Ovulation by Some Dose of PMS*

Age of day at PMS injection	PMS (IU)	Number of rats	Body weight g	Ovulation rate	Number of ova	Ovarian weight mg	Uterine weight mg
19	3.0	10	49.2±3.3*	0/10	0	14.9±2.8*	117.0±24.3*
21	3.0	22	46.5±5.0	3/22	7.0±0.9*	13.0±5.4	92.1±34.1
23	3.0	10	50.2±6.0	8/10	6.9±2.3	23.6±5.4	96.4±16.9
25	3.0	10	73.3±9.3	8/10	7.2±1.6	27.9±6.7	132.5±31.1
29	3.0	12	67.3±4.4	12/12	8.5±1.7	27.5±5.2	112.9±22.8
31	3.0	7	82.1±7.5	7/ 7	9.1±1.6	31.8±5.8	130.2±16.9
21	1.5	5	48.0± 2.0*	0/5	0	15.0±2.5*	32.2± 4.2*
23	1.5	6	53.3±10.5	1/6	6	12.2±3.1	84.8±39.6
25	1.5	5	54.2± 2.5	0/5	0	19.2±3.1	71.6±35.0
29	1.5	4	74.8± 2.9	0/4	0	15.2±2.9	65.0± 8.9
31	1.5	3	76.0± 7.1	0/3	0	17.6±5.2	123.3±32.4
21	30.0	21	49.0± 4.0*	0/21	0	57.9±27.6*	133.9±19.1*
23	30.0	12	52.1± 6.1	7/12	6.9± 5.2*	112.3±30.0	145.3±15.5
25	30.0	9	57.0± 4.2	9/ 9	36.0±24.3	130.7±21.3	123.0±27.8
27	30.0	10	61.8± 3.9	10/10	31.6± 9.2	100.9±23.1	113.4±14.0
29	30.0	11	55.1± 8.2	11/11	14.7± 7.1	98.1±28.1	132.3±13.0
31	30.0	6	84.2± 6.2	6/10	28.7±10.5	101.1±29.8	161.7±25.2

* Mean±s.d.

TABLE 2. *Ova and Ovarian Weight of the First Ovulation at Vaginal Opening*

Day at vaginal opening	Number of rats	Body weight g	Number of ova	Ovarian weight mg	Uterine weight mg
34.7±2.1*	14	93.9±14.9*	9.7±3.1*	29.7±4.8*	140.5±14.0*

* Mean±s.d.

TABLE 3. *Influence of Age on Ovulation by Some Dose of HCG*

Age of day at HCG injection	HCG (IU)	Number of rats	Body weight g	Ovulation rate	Number of ova	Ovarian weight mg	Uterine weight mg
31	2	5	82.0± 7.5*	0/ 5	0	14.4±2.7*	141 2±28.8*
27	10	4	62.0± 3.5*	0/ 4	0	28.0± 2.9*	50.3± 6.5*
31	10	15	79.3± 9.6	3/15	3.3±0.7*	25.3± 7.4	73.4±15.8
33	10	11	88.1±12.5	2/11	5.5±1.5	20.6± 8.6	88.3±34.6
27	50	9	64.2± 4.9*	4/ 9	1.8±0.8*	17.9± 6.6*	55.1±10.7*
31	50	11	84.4±13.7	8/11	5.4±1.4	31.7±10.7	84.8±27.3
adult	10	8	214.5±37.7*	7/ 8	8.3±3.0*	/	/

* Mean±s.d.

of the immature ovaries had not sufficiently matured by this time to respond to exogenous HCG. With an increased dose of 50 IU HCG, the ovulation rate became gradually higher at 31 and 33 days compared to treatment with 10 IU HCG. With

a decreased dose of 2 IU HCG a positive effect was not observed.

ii) *The Number of Tubal Ova*

Mean number of ova was 3.3 and 5.5 at 31 and 33 days with a 10 IU HCG treatment and 1.8, 5.4 at 27, 31 days with 50 IU HCG respectively as shown in Table 3. When these results were compared with the first ovulation count in the vaginal opening, the number of ova obtained by HCG treatment showed a significant lower value at each period ($P<0.01$, 50 IU at 27 days. $P<0.025$, 50 IU at 31 days: $P<0.01$, 10 IU at 31 and 33 days respectively).

iii) *The Weight of Ovaries*

The ovarian weight of the HCG treated and the first ovulation at vaginal opening rats was not observed to be a regular tendency. Most of the ovaries were observed to be red showing atresia of follicles.

Discussion

In this study, a treatment with 3 IU PMS was found to induce ovulation in a high percentage of immature rats, indicating the initiation of response at age of 21 days and a similarity of response in each period from 23 to 31 days. The response of the ovaries to 3 IU PMS injections was not different from that of observed in the first ovulation at vaginal opening. Accordingly, the dose of about 3 IU PMS seems to be a critical dose for inducing "normal ovulation" in immature rats from the standpoint of a high positive rate of ovulation, the number of tubal ova and the ovarian weight.

The period of onset of ovulation with 3 IU PMS treatment was not different than with 30 IU PMS treatment which induce superovulation. The onset of ovulation by treatment of gonadotropin was, therefore, considered to have no relation with the dose of PMS.

Earlier workers have suggested concerning the mechanism of ovulation by PMS alone treatment from the study by neural blocking agent (1, 3, 4, 5), hypophysectomy (3) and brain lesion (6) that the ovarian hormones released by follicles stimulated by PMS activates the central nervous system following by the induced release of LH from the pituitary. Since the ovulation by PMS treatment in this investigation is also considered due to a simular mechanism, it is suggested that the turning point in the function of ovary or central nervous system is at about 21 days. Because of no difference in the responses to 3 IU PMS treatment after initiating ovulation, we assume that the function of ovaries or central nervous system dose not have so remarkable change until near puberty (first ovulation).

In the case of HCG alone treatment, difficulty in inducing ovulation was found in immature rats in spite of easy induction in adults. Even when the ovulation was increased by high dose of HCG, the number of tubal ova was still smaller than that of observed in the first ovulation at vaginal opening. It is suggested,

therefore, that the ovarian follicles have not yet developed enough to ovulate in response to HCG in spite of approaching puberty.

The above results obtained by PMS treatment and HCG treatment appear to indicate an interesting idea concerning the mechanism of the onset of puberty by referring to earlier works as follows. A functional alteration of sensitivity to gonadotropin occurs in the ovaries of immature rats at about 21 days. Also just about this time the central nervous system attains one stage of maturity and initiates FSH secretion (7) in spite of the small amounts. Parlow (8) found a sudden decrease of pituitary FSH near puberty and suggested that the phenomena is the primary remarkable change in the pitutiary at puberty. This phenomena appears to be supported also from another point of view i.e. ovulation by PMS in our records can be obtained by a high dose of FSH alone (9). Also the follicles of the ovaries in immature rats have not yet matured enough to induce ovulation near the stage of puberty under exogenous HCG treatment as they do with LH, although their ovaries are already able to respond to an exogenous PMS like FSH far before puberty.

On approaching puberty, therefore, a sudden stimulation of the follicles by released FSH occurs and the growing follicles begin to secrete an increased level of estrogen which activated the central nervous system on the basis of positive feed back (10) and pituitary LH which induces the first ovulation at puberty may be discharged.

It seems necessary to detect the plasma level of the FSH in order to know further details of the mechanism concerning attainment of puberty.

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