

Dose-Dependent Pregnant Mare's Serum-Induced Ovulation in Immature Rats* (33483)

SHAO-YAO YING AND ROLAND K. MEYER

Department of Zoology, University of Wisconsin, Madison, Wisconsin

It has been reported that a single injection of pregnant mare's serum (PMS) produces ovulation in the immature rat (1-5). Williams (3) demonstrated that one injection of 13 IU of PMS does not cause ovulation, whereas doses of one half or twice this amount were effective. This bimodal dose-response curve has also been described for corpora lutea formation by Cartland and Nelson (6) and Hamburger (2).

In experiments in which ovulation was inhibited by carefully timed hypophysectomy or administration of barbiturate, McCormack and Meyer (7) found in immature rats a "critical period" similar to that described by Everett and Sawyer (8) for adult rats. They have suggested that the neural activating system for pituitary release of ovulating hormone (OH) occurring during the critical period is already operative, or can be made operative by PMS administration, in the 22-day-old rat (7). The administration of human chorionic gonadotropin (HCG) or luteinizing hormone (LH) (9, 10) and/or the secretion of endogenous gonadotropin during this critical period causes ovulation in PMS-treated immature rats.

The dose-response relationship between administration of PMS or PMS-HCG and ovulation has been investigated (3, 6, 11). However, there is little in the literature pertaining to the dose-response relationship in 22-day-old rats. The present study was undertaken to determine the incidence of ovulation caused by a single injection of various doses of PMS.

Materials and Methods. Twenty-one-day-old female rats weighing 45-50 g were obtained from The Badger Research Corp. and The Holtzman Company, Madison, Wisconsin.

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On day 22, between 7:00 and 8:00 a.m., central standard time (CST), a single injection of various doses of PMS (Antex Leo) from 1 to 80 IU in 0.25 ml of 0.9% NaCl was given subcutaneously except in the experiment in Table II in which 3 IU of PMS in 0.03 ml 0.9% NaCl was given. Each group consisted of 5 animals, and to obtain large numbers per dose, the experiment was run in duplicate or triplicate and the data were pooled. The animals were killed between 7:30 a.m. and 12:00 noon on day 25. The ovaries were removed and weighed to the nearest 0.1 mg on a Mettler analytical balance. The oviducts were dissected, separated mechanically with fine forceps, and compressed between two glass slides by binding the ends of the slides with adhesive tape. The ova were counted with the aid of a light microscope.

The rats were maintained on Rockland rat chow and water *ad libitum* and were kept in a room between 75-80°F. The lights were automatically turned on at 5:00 a.m. and off at 7:00 p.m. CST, thus producing a 14-hr light and a 10-hr dark period. Time in this report is designated as "Colony Time" using the nomenclature of Everett and Sawyer (8).

Twenty-seven rats were divided into 2 groups: one group was treated with 3 IU of PMS dissolved in 0.03 ml of 0.9% NaCl; the other group was given 3 IU of PMS in 0.25 ml. To determine whether or not 3 IU PMS-induced ovulation was specific for the Badger strain of rats, 24 Holtzman rats were divided into two groups and treated as above.

The Student's *t* test for significance of differences was used for statistical analysis of the experiments.

Results. Ovulation was induced in 16.67% of the rats with 2 IU of PMS. The percentage increased to 84.6 when 3 IU of PMS were given and then a sharp decline was observed when the dose of PMS was further increased. When the amount reached 12-15

TABLE I. Spontaneous Ovulation Induced by Various Doses of PMS in the Immature Rat.

Doses of PMS (IU)	Body wt. (g)	No. of rats ovul.	Ovul. (%)	Av no. of ova/ovul. rat (\pm SE)	Av ov. wt. (mg \pm SE)
1	58.8	0/20	0	—	14.07 \pm 1.44
2	58.8	3/20	16.67	4.50 \pm 0.50	17.04 \pm 1.27
3	58.7	22/26	84.60	5.00 \pm 0.40	23.70 \pm 2.77
6	60.6	10/18	55.55	5.00 \pm 0.816	30.00 \pm 4.37
8	55.6	4/10	40.00	2.50 \pm 2.50	33.10 \pm 2.47
10	57.3	1/8	12.50	13.0	34.70 \pm 2.96
12	60.0	0/7	0	—	47.50 \pm 2.41
15	59.68	0/35	0	—	65.65 \pm 1.54
20	58.5	4/7	57.14	13.8 \pm 4.5	71.70 \pm 6.71
30	57.0	16/18	88.88	18.0 \pm 2.66	124.90 \pm 8.20
40	61.6	4/6	66.67	24.0 \pm 2.38	131.60 \pm 7.36
50	63.5	0/6	0	—	141.70 \pm 9.81
60	58.5	0/8	0	—	165.31 \pm 7.92
80	57.6	0/5	0	—	166.20 \pm 5.88

IU of PMS, none of the rats ovulated (Fig. 1). Ovulation occurred with higher doses of PMS (Fig. 1), starting with 57.14% ovulation with 20 IU, reaching a maximum of 88.88% at 30 IU, and declining to 66.67% with 40 IU. Doses from 50 to 80 IU did not induce ovulation, but cystic follicles were observed in the ovaries (Table I).

The average number of ova shed with 2–6 IU of PMS was 5. However, the number of ova ovulated from 20 to 40 IU showed a steady increase as the doses of PMS increased; 20 IU of PMS gave 13.8 \pm 4.50 ova; 30 IU, 18.0 \pm 2.66; and 40 IU, 24.0 \pm 2.38.

Data from 36 experiments performed during 1 year showed that 3 IU of PMS injected on day 22 produced ovulation in 153 of 186 rats with an average of 5.0 \pm 0.06 ova (Table II). No significant differences were noted between strains of rats or the volume of vehicle administered. An average of 86.4% of the Badger rats ovulated 5.08 \pm 0.20 ova

with 3 IU of PMS in 0.03 ml of 0.9% NaCl and 85.7% ovulated an average of 4.70 \pm 0.45 ova with the same dose of PMS in 0.25 ml of 0.9% NaCl. In Holtzman rats, 83.33% ovulated with 4.33 \pm 1.57 ova with 3 IU of PMS in 0.03 ml of 0.9% NaCl, and 91.77% ovulated 4.63 \pm 0.68 ova with the same dose of PMS in 0.25 ml of 0.9% NaCl.

Discussion. The results show that a single injection of from 1 to 80 IU of PMS produced a biphasic dose-response curve with two peaks, at 3 and 30 IU. No ovulatory response was observed with doses higher than 40 IU or between 10 and 20 IU. The average number of ova shed plotted per ovulating rat or per rat fluctuated coincidentally with the bimodal curve of ovulation.

Stimulation of ovulation by PMS in the immature rat appears to be through both direct and indirect mechanisms (5). Initially, PMS causes ovarian follicular development. Subsequently, the increased estrogen and/or progesterone produces either a positive or a

TABLE II. Effect of Different Volumes of 3 IU PMS on Immature Badger and Holtzman Rats.

Treatment	No. of rats ovul.	Ovul. (%)	Av no. of ova/ovul. rat (\pm SE)	Av ov. wt. (mg \pm SE)
Control (Badger rats from Sept. 1965 to Sept. 1966)	153/186	80.95	5.007 \pm 0.06	25.05 \pm 0.48
PMS (0.03 ml) Badger rats	11/13	84.6	5.08 \pm 0.20	25.40 \pm 1.40
PMS (0.03 ml) Holtzman rats	10/12	83.33	4.33 \pm 1.57	28.60 \pm 1.70
PMS (0.25 ml) Badger rats	12/14	85.7	4.70 \pm 0.45	23.70 \pm 1.51
PMS (0.25 ml) Holtzman rats	11/12	91.77	4.63 \pm 0.68	29.30 \pm 0.90

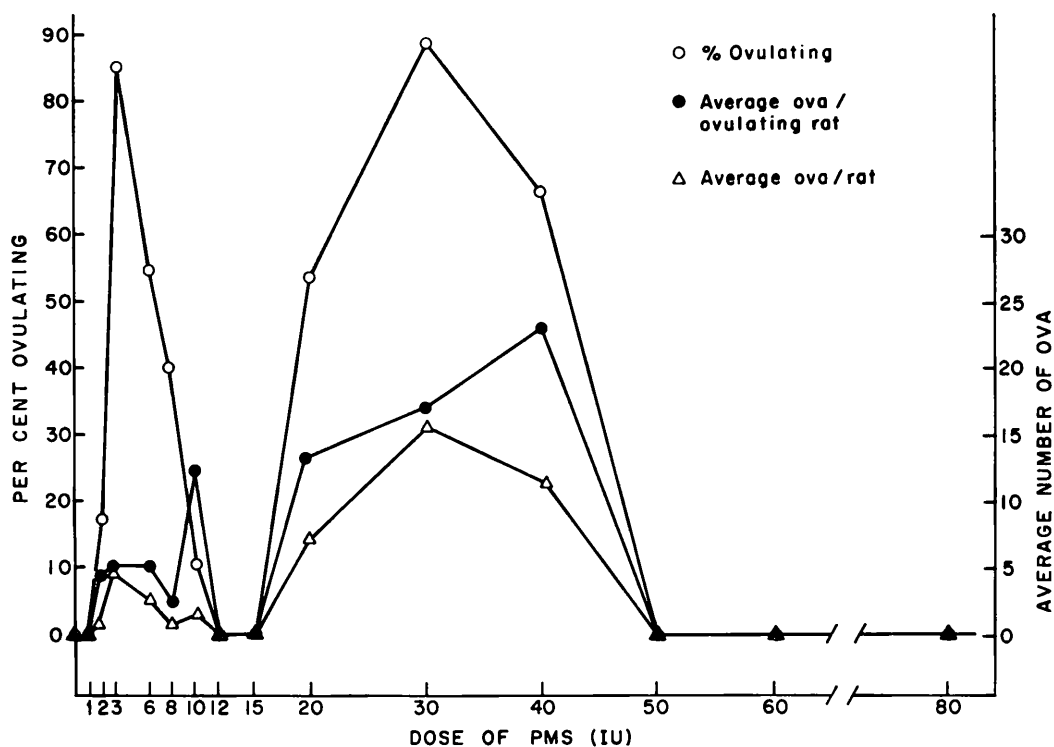


FIG. 1. Induction of ovulation by PMS in the immature rat.

negative feedback on the endogenous ovulatory mechanism, which depends upon the different steroid environments stimulated by different amounts of PMS. The number of ova shed and the ovulating capacity depend on both the maturation of follicles and the endogenous release of LH. Presumably, the dosage of PMS administered is the primary factor influencing levels of estrogen and/or progesterone, and the quantity of LH and FSH. Any change in these factors affects ovulation.

The administration of small doses of PMS (2–6 IU) stimulated the development of a few follicles and led to an optimal environment which resulted in the first peak of ovulation in the bimodal curve (Fig. 1). It is well known that small amounts of estrogen stimulate the growth of follicles (12–14) and induce ovulation (5, 15–18) as the result of a positive feedback which accelerates the synthesis and release of FSH and LH. With the synergism or potentiation of FSH by LH, greater stimulation of follicles would be expected (19), an optimal level of estrogen and progesterone might be produced, which leads

to the spontaneous surge of endogenous OH during the critical period and consequently promotes ovulation. With doses of 12–15 IU of PMS more follicles were stimulated to develop as a result of an increase in the amount of estrogen secretion and thus a decrease in release of FSH and LH (19). This deficiency can be corrected by administration of exogenous LH or by the injection of progesterone prior to the critical period (5).

Animals treated with doses from 20 to 40 IU showed another peak of ovulation. Recently, unpublished data in this laboratory have demonstrated that the progesterone level on day 24 in 30 IU PMS-treated animals is higher than that in 15 IU PMS-treated animals (20). Zarrow and his co-workers (21) reported that unilateral ovariectomy in 15 IU PMS-treated rats ovulated the same number of ova as the intact controls, but 30 IU PMS-treated unilaterally ovariectomized rats ovulated only half the number of ova as the controls. They postulated that the number of follicles stimulated per ovary is maximum in 30 IU

PMS-treated animals. Although a large amount of estrogen is produced in animals treated with from 20 to 40 IU of PMS, the increasing level of progesterone may play an important role in knocking out the inhibition of LH release either by forming an optimal steroid environment which triggers LH release, or by acting directly on the hypothalamo-hypophysis. The administration of high doses of PMS (50–80 IU) led to the production of cystic follicles and no ovulation was observed. It is possible that the high dose of PMS acts directly on the follicles and/or the high level of steroid inhibits the release of LH. Additional experiments are being conducted to test these possibilities.

Summary. The incidence of ovulation in 25-day-old animals after various doses of a single injection of PMS reached its maximum at 3 and 30 IU. Doses between 10 and 20 IU or higher than 40 IU did not induce ovulation. In 3 IU PMS-primed rats no significant differences were observed between strains, or with the volume of vehicle used. The mechanisms by which a single injection of various doses of PMS induce ovulation are discussed.

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