Absence of Bimodal Ovulatory Effect of PMS in the 30-Day-Old Rat[1] (36702)

B. N. Sridharan and Roland K. Meyer

Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706

Cole (1) and Cartland and Nelson (2) reported ovulation in immature rats after administering 2 IU of PMS. Some investigators (3, 4) reported that PMS was ineffective in causing ovulation after a single dose and used PMS followed by HCG as a procedure for consistently inducing ovulation (3). Since purified serum gonadotropins became available commercially (5), PMS has been used routinely to cause ovulation in immature rats (6, 7).

Strauss and Meyer (8, 9) showed evidence for the existence of neural control of ovulation in immature rats and used 8 IU of PMS in 30-day-old rats to cause ovulation on the morning of day 33. Zarrow and Wilson (10) studied the influence of age on ovulation induced by gonadotropins and reported consistent ovulation at the age of 28 to 32 days.

An interesting observation was made by Ying and Meyer (11) who studied dose-dependent effects of PMS on 22-day-old rats and observed a bimodal effect. A dose of 3 IU caused ovulation in 80% of the rats but with increasing dosage, the ovulation rate was lower. With 12 or 15 IU of PMS, the ovulation was reduced to zero. However, 30 IU of PMS caused ovulation in a large percentage of animals and still higher doses were ineffective and caused cystic follicles. Khan and Meyer (12, 13) showed that while 3 IU-treated 22-day-old rats ovulated consistently, they were not able to mate or implant or maintain pregnancy without further steroid treatment. Strauss (14) demonstrated that 30-day-old 8 IU-treated rats not only ovulated but mated, implanted and maintained pregnancy without exogenous steroids. It was interesting, therefore, to study the dose-dependent effect of PMS on ovulation in the 30-day-old rat.

Materials and Methods. Twenty-six-day-old female rats weighing 65–70 g were received from the Holtzman Co., Madison, WI. On day 30, between 8:00 a.m. and 10:00 a.m. Central Standard Time (CST), a single subcutaneous injection of various doses of PMS (Equinex) from 1 to 120 IU in 0.5 ml of 0.9% sodium chloride was given. Controls were injected with the vehicle. The animals were autopsied on day 33 between 11:00 a.m. and 3:00 p.m. The oviducts were carefully dissected and were gently pressed between two glass slides which were bound by adhesive tape. The ova were observed and counted with the aid of a light microscope under low power. The ovaries were weighed on a Mettler analytical balance.

The rats were housed in an air-conditioned room (75–80°F) and maintained on Rockland rat diet and water *ad libitum*. The lights were turned on at 5:00 a.m. and off at 7:00 p.m. CST, providing a 14-hr light and a 10-hr dark period.

Results. Ovulation was caused in some of the treated animals with doses as little as 2 IU (Fig. 1, Table I). PMS within a wide range of dosage from 3 to 67 IU caused ovulation in 100% of animals treated. The number of ova found, however, was proportionately higher. The average number of ova per animal was 7.0 with 3 IU and increased steadily with dosage up to 55 IU where an average of 68.7 ova was found. With 67 IU the percentage of ovulation was also 100% but the average number of ova was reduced to 17.6. The incidence of ovulation with doses higher than 67 IU was reduced to 50% and remained so with the largest dose used, 120 IU (Fig. 1).

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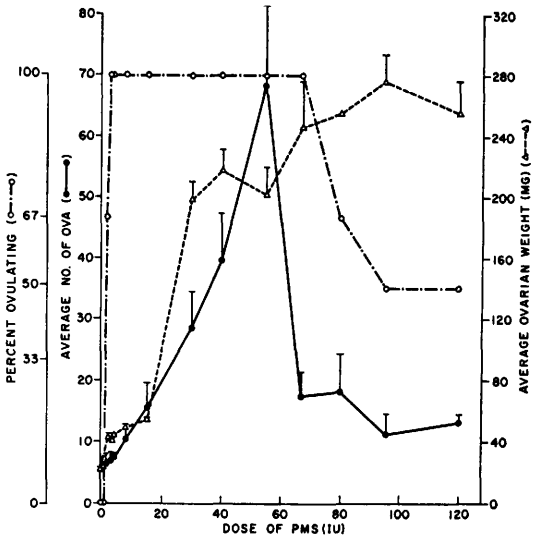

FIG. 1. Induction of ovulation in the 30-day rat by PMS.

The incidence of fragmented ova increased with doses of 30 IU or greater. The fragmentation did not appear as an irregular degenerative type but the egg was neatly broken in 8–16 equal segments and took on an appearance more like that of an 8-celled or 16-celled fertilized ovum (Fig. 2).

To test the possibility that the phenomenon observed might be due to a parthenogenic stimulus, a group of 30-day-old rats were treated with 60 IU PMS and on the morning of ovulation, the vaginae were stimulated with a glass rod (a procedure that consistently caused pseudopregnancy in adult or 8 IU PMS-treated immature rats). Examination of the uteri of animals, 4 or 9 days later showed no evidence of blastocysts or implantation sites, respectively.

TABLE I. Induction of Ovulation by Various Doses of PMS in the 30-Day-Old Immature Rat.[a]

Dose of PMS (IU)	Av body wt (g)	No. of rats ovul.	Ovul. (%)	Av no. of ova/ovul. rat	Av no. of ova/treated rat	Av ov. wt (mg)
0 (saline)	101 ± 1.8	0/6	0			22.4 ± 0.8
1	99 ± 1.6	0/5	0			24.1 ± 1.2
2	106 ± 3.8	4/6	67	6.5 ± 1.5	4.3 ± 1.7	42.2 ± 3.3
3	99 ± 2.8	5/5	100	7.0 ± 1.2	7.0 ± 1.2	40.8 ± 2.6
4	108 ± 3.0	6/6	100	7.5 ± 0.6	7.5 ± 0.6	44.8 ± 1.7
8	100 ± 1.1	6/6	100	10.3 ± 1.3	10.3 ± 1.3	49.8 ± 2.3
15	110 ± 2.6	5/5	100	15.6 ± 4.0	15.6 ± 4.0	55.9 ± 1.6
30	99 ± 3.1	5/5	100	28.6 ± 6.1[b]	28.6 ± 6.1	199.1 ± 11.6
40	105 ± 2.5	6/6	100	39.7 ± 8.1[b]	39.7 ± 8.1	218.0 ± 14.2
55	103 ± 1.0	6/6	100	68.7 ± 13.2[b]	68.7 ± 13.2	202.6 ± 18.0
67	96 ± 2.3	5/5	100	17.6 ± 3.6[b]	17.6 ± 3.6	246.9 ± 29.5
80	102 ± 1.6	4/6	67	18.3 ± 5.8[b]	12.1 ± 5.3	254.2 ± 7.8
95	105 ± 2.0	3/6	50	11.3 ± 2.9[b]	5.7 ± 2.8	276.6 ± 13.6
120	104 ± 0.9	3/6	50	13.3 ± 1.2[b]	6.7 ± 3.0	258.0 ± 21.3

[a] All values ± SE of mean.

[b] Many of the ova seen were segmented in 2–16 equal parts.

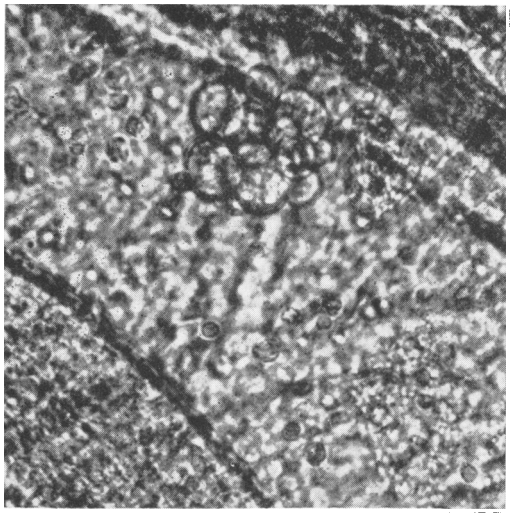

FIG. 2. Segmented ovum seen with high doses of PMS. This egg was observed on day 33, in an animal treated with 60 IU of PMS on day 30.

The ovaries were progressively heavier, roughly in proportion to the dose, but there was a sharp increase in weight betweeen 15 and 30 IU (Fig. 1, Table I).

Discussion. In the 30-day-old rats, there was no indication of the bimodal effect that was reported in 22-day-old rats by Ying and Meyer (11). The incidence was 100% with 3 IU and remained so with increasing doses until 80 IU when the incidence fell to 67%. Zarrow, Sundaram and Stob (15) reported that unilateral ovariectomy in 15 IU PMS-treated rats caused ovulation of the same number of ova as the intact controls, but in 30 IU PMS-treated rats unilateral ovariectomy did not increase the number of ova ovulated from the remaining ovary. They suggested that the number of follicles stimulated per ovary is the maximum in 30 IU PMS-treated rats. Our data appear to indicate that the maximum number of eggs is obtained with 55 IU.

Ying and Meyer (11) proposed that the failure of ovulation in 12–15 IU PMS-treated 22-day-old rats was probably due to an increase in the amount of estrogen secretion and thus a decrease in release of FSH and LH. In the present investigation the animals were nearer to maturity by approximately 8 days and it appears that the qualitative response of the follicles to PMS treatment is different (16) and so with high doses of PMS no inhibition of ovulation is seen, except in extremely high doses which cause cysts.

The number of ova released appears to reach a maximum mean of 69. This is in agreement with the findings of Zarrow *et al.* (3) who could obtain 72.6 ova as the maximum number as an average in a group, although individual rats occasionally ovulated as high as 105 ova. In the present data, the greatest number recorded was 101 ova with 55 IU. With larger doses there is no increase in the number.

The segmentation in many eggs with high doses of PMS is interesting. One often finds fragmented ova about 36 hours or later postovulation at which time they are in the posterior part of the oviduct and are naked. In the present experiment, the segmenting ova were seen in the anterior part of the oviduct in the ampullary region and were surrounded by cumulus cells. It seems unlikely that they were ovulated much earlier than the eggs from rats injected with smaller doses. It is not clear whether the division of the egg seen is a degenerative process brought about by high levels of gonadotropins or steroids, or the result of a stimulatory effect leading the ovum to undergo division.

The only treatment given in all these animals was a single dose of PMS and it is probable that the ovulating hormone is of endogenous origin. McCormack and Meyer (17) and Strauss and Meyer (9) demonstrated by timed hypophysectomy and phenobarbital treatment that PMS-treated animals were dependent upon the endogenous pituitary for the ovulating stimulus. Recently Ying and Greep (18) have shown that in the immature rat an estrogenic stimulus could set in motion the sequence of events resulting in ovulation. However, as PMS does possess ability to stimulate ovulation in a developed follicle (3) it is not known whether in the rats treated with high doses, the circulating PMS played any part in ovulation. Sasamoto and Kennan (19), using an anti-PMS serum, have shown that in immature hypophysectomized rats PMS is required for the maintenance of follicles as late as 66 hr after one

injection of PMS. It is therefore, logical to assume in animals treated with high doses of PMS in the present experiment, that PMS is in the system on day 32 and does have some effect on the ovulation.

Summary. The effect of a single injection of varying amounts of PMS on ovulation in 30-day-old rats was investigated. Doses as small as 2 IU caused ovulation in some of the animals. Ova were ovulated in 100% of the animals injected with doses ranging from 3 to 67 IU. The incidence of ovulation declined with higher doses. A mean maximum of 68.7 ova was obtained with 55 IU and declined with larger amounts. A segmentation of ova was observed with 30 IU of PMS and higher.

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