Compensatory Ovulation After Unilateral Ovariectomy in Immature Rats Primed with Gonadotropins[1] (37518)

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Following unilateral ovariectomy in animals which normally bear more than one young at a time, the remaining ovary undergoes excessive enlargement in compensation for the loss of the contralateral gonad. Peppler and Greenwald (1) have reported that following unilateral ovariectomy, the number of follicles undergoing maturation and ultimately rupturing in the remaining ovary is twice that of a single ovary from an intact adult cycling rat. A similar situation occurs in the ovary of the immature, unilaterally ovariectomized rat which responds to 15 IU of PMS by shedding the same number of ova as both ovaries of an intact animal (2). However, in this study, compensatory ovulation was not observed in unilateral-ovariectomized rats pretreated with 30 IU PMS.

It is well known that a single injection of low doses of PMS induces ovulation in immature rats nearing puberty. Female rats given either 3 or 4 IU of PMS on Days 22 or 30 of age, respectively, are expected to ovulate within 72 hr following gonadotropin administration (3). The release of ova approximately 72 hr after PMS injection is due to an endogenous LH surge on the day prior to ovulation. This preovulatory LH release is preceded by an estrogen "surge" which constitutes an essential signal in the chain of events leading to the acute release of LH. The present study has been undertaken in an attempt to see the effect of unilateral ovariectomy on immature rats of different ages which have been primed with ovulatory doses of PMS.

Materials and Methods. Immature rats were obtained from the Holtzman Co., Madison, Wisconsin and maintained on Purina rat chow and tap water *ad libitum*. The daily lighting schedule consisted of 14 hr of light and 10 hr of darkness. Three or four IU of PMS ("Equinex," Ayerst Laboratories) was injected sc in 0.25 ml of physiological saline on either Day 22 or Day 30, respectively. In addition, some rats received 25 IU of PMS on Day 22 or 30. Unilateral ovariectomies were performed under light ether anesthesia at various times on Days 23, 30, 31 and 32. At such times, some of the operated animals, along with intact animals received either or both estradiol benzoate (EB) or progesterone sc at various dose levels in 0.1 ml of sesame oil.

The ovariectomies were performed according to standard surgical procedures. Sham operations involved opening the body cavity, exposing the ovary and uterus and passing a silk thread through the fat adjacent to the uterus without actually performing the ligation. The rats were killed on the third day after PMS injection by an overdose of ether. The oviducts were removed, compressed between two glass slides and examined under a light microscope for the presence of ova in the distended ampullar portion.

Results. Statistically significant compensatory ovulation ($p < 0.001$) was found in rats treated with 4 IU of PMS on Day 30 followed by unilateral ovariectomy on the

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TABLE I. Acute Compensatory Ovulation Following Unilateral Ovariectomy in Rats Treated with 4 IU PMS on Day 30.

Time of unilateral ovariectomy	Rats ovulating	% Ovulating	Ova/ovulating rat	Ova/ovary
None	6/6	100	8.17 ± 0.48	4.08 ± 0.31
9:30–10:00 AM Day 30	4/5	80	9.00 ± 0.58	9.00 ± 0.58
11:00–12 noon Day 31	5/6	83.3	8.40 ± 0.40	8.40 ± 0.40
12–1:30 PM Day 31	4/6	66.7	7.75 ± 1.93	7.75 ± 1.93
1:30–2:30 PM Day 31	6/6	100	7.60 ± 0.51	7.60 ± 0.51

morning and afternoon of the next day (Table I). No compensatory ovulation or inhibition of ovulation followed unilateral ovariectomy in rats treated with 25 IU of PMS on Day 22 or Day 30 (Table II). In rats treated with 3 IU of PMS on Day 22 unilateral ovariectomy inhibited ovulation whereas the sham operation did not (Table III).

Estrogen replacement significantly restored the ovariectomy-inhibited ovulation (Table IV). Progesterone given at the same time as EB was ineffective in restoring the occurrence of ovulation (Table IV).

Discussion. The ability of estrogen but not progesterone to restore ovulation inhibited by unilateral ovariectomy in 3 IU PMS-treated rats points to a positive feedback role for estrogen in triggering the ovulatory surge of LH on the day prior to ovulation. The ability to mimic an endocrine event by exogenously administered hormone is not necessarily conclusive evidence for a physiological role for that hormone. However, the fact that extremely low levels of estrogen were used and the added evidence that the same doses of EB increased the percent ovulation in intact rats when given on Day 23 (Table IV) makes it seem feasible that a prior elevation in estrogen is necessary for positive feedback. Presumably the two ovaries act together to produce just enough estrogen at this age to feed back in a stimulatory manner on the preoptic area of the hypothalamus.

In rats treated on Day 30 with 25 IU of PMS, unilateral ovariectomy does not result in compensatory ovulation, whereas hemicastrated 4 IU rats show acute compensation. This data supports Zarrow's findings in 30-day-old rats pretreated with 30 IU of PMS. Each ovary is presumably ovulating the maximum number of ova possible without the additional stimulation received after unilateral ovariectomy.

Whether the additional stimulation is the

TABLE II. Lack of Acute Compensatory Ovulation Following Unilateral Ovariectomy in Rats Treated with 25 IU PMS on Day 30.

Time of unilateral ovariectomy	Rats ovulating	% Ovulating	Ova/ovulating rat	Ova/ovary
None	6/7	85.7	42.83 ± 9.71	21.42
9–9:30 AM Day 30	9/9	100	19.89 ± 4.09	19.89 ± 4.09
11:30–12:30 PM Day 31	7/9	77.8	21.14 ± 4.78	21.14 ± 4.78
9:30–10:30 AM Day 32	8/9	88.9	20.00 ± 3.86	20.00 ± 3.86

TABLE III. Inhibition of Ovulation Following Unilateral Ovariectomy in Rats Treated with 3 IU PMS on Day 22.

Treatment				
PMS	Time of unilateral ovariectomy	Rats ovulating	% Ovulating	Ova/ovulating rat
3 IU	—	12/17	71	7.37 ± 0.54
3 IU	11 AM–12 noon	1/12	7	5.0
3 IU	12 noon–1:30 PM	0/6	0	—
3 IU	1:30 PM–2:30 PM	1/5	20	7.0
3 IU	4 PM–4:30 PM	0/5	0	—
3 IU	Sham operated	2/3	67	4.5 ± 1.5

result of an increase in gonadotropin secretion due to the partial removal of a source of steroid negative feedback, or whether it is due to an increased "seizure" of gonadotropin by the one ovary with no increase in total gonadotropin release, is still uncertain. More evidence, however, points toward the former hypothesis. A decline in pituitary FSH has been found 24 hr after unilateral ovariectomy of adult rats, but increased serum FSH has not been detected by bioassay to occur until four days after the surgery (4). The inadequacy of the "seizure theory" in explaining acute compensatory ovulation has been pointed out by Greenwald's finding that keeping the level of gonadotropin constant and halving the number of ovaries does not result in compensatory ovulation (5). Sensitive radioimmunoassay procedures need to be applied in an attempt to determine whether FSH increase occurs within 12 hr following hemi-castration.

Summary. Immature rats of 22 or 30 days of age pretreated with an ovulatory dose of pregnant mare serum (PMS) were used. Statistically significant compensatory ovulation was found in rats treated with 4 IU PMS on Day 30 followed by unilateral ovariectomy on Day 31. No compensatory ovulation followed unilateral ovariectomy in rats treated with 25 IU PMS on Day 22 or Day 30. In rats treated with 3 IU PMS on Day 22 and unilaterally ovariectomized on Day 23, ovulation was inhibited, whereas in sham-operated animals it was not. Estradiol benzoate (EB) injected sc at the time of unilateral ovariectomy significantly restored ovulation in immature rats primed with 3 IU PMS; progesterone was ineffective. These findings suggest that the endogenous estrogen level

TABLE IV. Reversal of the Inhibitory Effect of Unilateral Ovariectomy by Estrogen in Immature Rats Pretreated with 3 IU PMS.

PMS	Time of unilateral ovariectomy and treatment	Rats ovulating	% Ovulating	Ova/ovulating rat
3 IU	—	27/45	60	6.34 ± 0.58
3 IU	11–12 noon	1/38	3	5.0
3 IU	11–12 noon + EB[a]			
	0.25 μg	13/27	48	4.25 ± 1.05
	0.1 μg	14/26	54	4.92 ± 0.69
3 IU	— + EB			
	0.25 μg	7/8	88	6.15 ± 0.65
	0.1 μg	4/6	67	6.5 ± 0.43
3 IU	11–12 noon + Prog 1 mg[b]	0/14	0	—
3 IU	— + Prog 1 mg	6/10	60	5.80 ± 0.97

[a] EB = Estradiol benzoate.
[b] Prog = Progesterone.

affects compensatory ovulation, and unilateral ovariectomy inhibits ovulation in the 22-day-old rat model by reducing the quantity of estrogen available for positive feedback.

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