FOLLICULAR DEVELOPMENT IN THE PMSG-TREATED, IMMATURE RAT

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ABSTRACT

The induction of ovulation in immature rats by preg-
nant mare's serum gonadotropin (PMSG) is frequently used as a model for studying the mechanisms in the ovulatory cycle of the adult rat. The present study was designed to compare follicular development in two immature animal model systems (PMSG-treated, intact, immature rat and PMSG-treated, unilaterally ovarecto-
tomized (ULO), immature rat) with that reported for the intact and ULO adult rat (Peppler and Greenwald, 1976a).

Holtzman, female immature rats were bred in our laboratory and allowed to deliver. Average litter size was 7.9 ± 1.4 young per litter with 57.1% being female. Parturition was designated day 1 of age for the off-spring. Males were removed from the litters as day 14 and females were weaned at day 21. Sixty-four females were assigned to either an (a) intact or (b) unilaterally ovarecto-
tomized (ULO) group and housed six per cage under condi-
tions of controlled lighting (lighting dihersion from 5 AM to 7 PM) and temperature (25 ± 2.5°C). Purina lab chow and tap water were available ad libitum.

The surgical procedure (ULO) and injection with pregnant mare serum gonadotropin (PMSG) were performed between 2 and 4 PM at 28 days of age. ULO was accomplished by the dorsal route with the animal under ether anesthesia. Alternate left and right ovaries were removed. Intact and ULO rats re-
ceived 30 IU of PMSG (Sigma, St. Louis, Mo) dissolved in 0.5 ml of 0.9% saline solution and were killed between 3 and 4 PM at 24, 48 or 72 hrs post-injection.

In addition, non-injected intact (N=8) and ULO (N=8) rats were killed at day 0.

At autopsy, body weight was recorded and ovaries were re-
moved and measured. Ovaries from three animals in each group were fixed in Bouin's solution, embedded in paraffin, sectioned serially at 5 µm and stained with hematoxylin and eosin. Each section was examined with the identity of indi-
vidual ovarian structures remaining unknown and unmarked. All follicles were scored as either growing or atretic, with growing consisting of a follicle whose follicular size was calculated by measuring two diameters at right angles to each other, and the follicles were arbitrarily classified into groups: 352 to 394 µm; 395 to 437 µm; 448 to 517 µm; 518 to 570 µm; >571 µm.

Treatments were assigned, perfonned and data were analyzed according to a completely randomized design. Statistical prob-
abilities were determined by analysis of variance or Student's t-test.

RESULTS

The pattern of follicular development in the immatur-
e rat treated with PMSG is shown in Table 1. Since the ovary of a non-treated animal (control) contained only a few (2.0 ± 1.0) follicles >352 µm at day 31, it is assumed that a similar pattern, or less, existed at day 28. (p < 0.05) increase in the total number of follicles had occurred by 24 hrs. after the PMSG injection. This number did not change at 48 hrs.

Post-injection and ovaries of all animals now had fol-
licles 448-517 µm in size. Twenty-four hrs. later, 72 hrs. post-injection, the total number of follicles had doubled and all animals had follicles in each of the five size groups. In addition, all ovaries had corpora lutea at this time period.

The remaining ovary of non-treated animals, ULO for 72 hrs., had more follicles (6.3 ± 1.1; Table II than controls (2.0 ± 1.0); Table I) of intact controls and thus, one can assume that the at 24 or 48 hr. time period (day 29 or 30) the non-stimulated ovary in the ULO animal contained follicles ranging between these two numbers. Twenty-four hrs. following PMSG injec-
tions, a significant increase (p < 0.05) was observed. The number of follicles >352 µm in the remaining ovary of the ULO rat was apparent (Table 2). One day later, 48 hrs. after the injection, the number of follicles was increased (p < 0.05) further but remained unchanged after the subsequent 24 hrs. (72 hrs. post-injection). At this time point, a distinct number of follicular corpora lutea had formed in all size groups with the exception that only 67% of the animals had follicles in the 571± µm group.

TABLE 1: Pattern of follicular development in the immatures treated with PMSG.

| Size of follicles (µm) | Control (Day 31) | Mean number of follicles per ovary ± SEM in rats injected with 30 IU PMSG at day 28 of age and killed post-injection at:
<table>
<thead>
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<tbody>
<tr>
<td>352-394</td>
<td>1.0 (i)</td>
<td>7.3 ± 1.5 (i)</td>
</tr>
<tr>
<td>395-447</td>
<td>2.0 (i)</td>
<td>4.4 ± 1.5 (i)</td>
</tr>
<tr>
<td>448-517</td>
<td>1.0 (i)</td>
<td>1.0 ± 0.3 (i)</td>
</tr>
<tr>
<td>516-570</td>
<td>1.0 (i)</td>
<td>5.0 ± 2.0 (i)</td>
</tr>
<tr>
<td>571+</td>
<td>1.0 (i)</td>
<td>4.0 ± 2.3 (i)</td>
</tr>
<tr>
<td>Another number of follicles</td>
<td></td>
<td>12.3 ± 4.3 (i)</td>
</tr>
</tbody>
</table>

1 Number in parentheses signifies number of animals in group with follicles of this size range.

2 Brackets figure indicates only one animal of group had follicles of this size range.

3 P<0.05 vs control group.

4 P<0.05 vs preceding group (i.e. 72 hrs. vs 48 hrs.).
prevalent. The injection of 20 IU HCG to intact rats on any day of the cycle indicates that follicles > 448 μm are capable of ovulating (Pepper and Greenwald, 1970b). At proestrus, both 4- and 5-day cycling rats have more than eight follicles of this size or larger, and this number is more than enough to account for the expected number of eggs shed late that evening from the one ovary. In the present study, one ovary in the intact, immature rat had only 2.3 follicles > 448 μm and a total of 15.1 follicles > 352 μm 48 hrs. after treatment with PMSG. Thus, follicles of a smaller size (< 352 μm) must be ovulated to account for the expected ovulation number of 35 eggs/ovary.

Following unilateral ovariectomy, the total number of follicles > 352 μm does not vary from day to day (Pepper and Greenwald, 1970b), and indicates the same progressive pattern of follicular development that is observed in cycling, intact rats occurring following ULO. However, the remaining ovary does contain more follicles 48 and 72 hrs. following ULO and this increase is the result of a greater number of follicles > 448 μm being present. Twice the number of large Graafian follicles (over 448 μm in diameter) found in one ovary of intact rats mature in the remaining ovary following ULO. Similarly, in the present study, the remaining ovary in ULO, immature rats had 5.6 follicles of this size and this was twice the number found in the ovary of the intact rat. However, this number was not enough to account for the number of eggs ovulated and follicles smaller than 448 μm had to be ovulated. Only so many smaller sized follicles can be recruited and thus, explains the lack of compensatory ovulation in the ULO, immature rat treated with 25 IU (Ying and Gove, 1973) or 30 IU (Zarrow, Sundaram and Stob, 1965) of PMSG and substantiates these investigators maximum stimulation hypothesis.

Johnson and Mallampati (1973) reported that ovarian weight doubles after 22 hrs. of exposure to PMSG, regardless of the dosage used. In the present study, ovarian weight increased two-fold after 24 hrs. and remained parallel between the two groups up to 48 hrs. post-injection. This weight increase was probably caused by the increased follicular development at this time. In contrast, the dramatic increase in ovarian weight between 48 and 72 hrs. was due to the formation of corpora lutea following ovulation.

Thus, the results of the present study demonstrate that smaller sized follicles are being ovulated in both the intact and ULO, immature PMSG-treated rat. Similarly, Osman (1965) reported that follicles 250 to 499 μm in size are being shed during the first spontaneous ovulation at puberty. Since smaller sized follicles are being shed in the PMSG-treated animal, there must be some affect on follicular development during the subsequent cycle. Immature, PMSG-treated rats remain in an anestrous state for eight days after the induced ovulation due to the failure of follicular development (Sawamoto and Sasamoto, 1973). In other words, the PMSG injection depletes the ovary of the follicles which would mature and ultimately be shed during the subsequent cycle, and thus, the reason for the period of anestrus. Whether these eggs that are shed are physiologically similar to those ovulated in the adult animal, remains to be determined. Nonetheless, the results of the present study demonstrate an important difference between these immature and adult rats that investigators should be aware of in designing experiments using these animal model systems.

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LITERATURE CITED