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FOLLICULAR DEVELOPMENT IN THE PMSG-TREATED, IMMATURE RAT

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ABSTRACT

The induction of ovulation in immature rats by pregnant 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 ovariectomized (ULO), immature rat) with that reported for the intact and ULO, adult rat (Peppler and Greenwald, 1970b).

Holtzman, female, immature rats were rendered ULO and/or injected s.c. with 30 IU of PMSG between 2 and 4 PM at 28 days of age and killed 24, 48 or 72 hrs. later. Ovaries were processed histologically and follicular development assessed. PMSG injection resulted in more follicles $>$ 352 μm being present in the ovary after 24, 48 and 72 hrs. Similar increases occurred in the PMSG-treated, ULO, immature rat. Although the total number of follicles $>$ 352 μm did not differ between the two experimental groups at any of the time periods, twice the number of large Graafian follicles ($>$ 448 μm) found in one ovary of intact rats matured in the remaining ovary following ULO. However, more eggs than this number reflects are shed following PMSG treatment.

In the adult, intact or ULO rat, follicles $>$ 448 μm are the ones normally shed at ovulation. In contrast, in the PMSG-treated intact or ULO, immature rat, follicles $<$ 352 μm have to be shed to account for the expected ovulation number. Thus, investigators should be aware of this difference in follicular kinetics in designing experiments using these animal models.

INTRODUCTION

In 1936, Cole first demonstrated that a single injection of the gonadotropin found in the blood serum of the pregnant mare (PMSG) induced ovulation in the immature rat. Since then, this regime has been utilized repeatedly by many investigators as a model system for studying mechanisms involved in ovulation. The use of this model is preferred because it allows large numbers of synchronized animals to be obtained without having to contend with the variability in estrous cycle length common to adult rats.

Another animal model that has been used extensively to study the pituitary-ovarian axis is the unilaterally ovariectomized (ULO), adult rat. Following ULO, the remaining ovary compensates for this tissue loss by an increase in both weight and number of large follicles that develop, and by ovulating the same number of eggs originally shed by two ovaries (Peppler and Greenwald, 1970a & b). In contrast, in immature rats treated with 25 IU (Ying and Gove, 1973) or 30 IU (Zarrow, Sundaram and Stob, 1965) of PMSG, unilateral ovariectomy does not result in a doubling of the ova shed because the remaining ovary is presumably ovulating the maximum number possible at these dose levels. If this hypothesis is correct, then the remaining ovary in the PMSG-treated, ULO, immature rat should contain the same number of large follicles ($>$ 352 μm) as one ovary in the PMSG-treated, intact, immature rat.

Thus, the purpose of the present study was two-fold. First, to our knowledge, an analysis of follicular development for several days following the injection of PMSG in the intact or ULO, immature rat has not been reported. Second, because it is imperative that there be good agreement between reproductive processes occurring in a model system and the adult animal, follicular development in these immature models needs to be compared with that in the non-treated, intact or ULO, adult rat during the various days of the estrous cycle.

MATERIALS AND METHODS

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

The surgical procedure (ULO) and injection with pregnant mare's 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 received a single 30 IU s.c. injection of PMSG (Sigma, St. Louis, Mo.) dissolved in 0.3 ml of 0.9% saline solution and were killed between 2 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 31.

At autopsy, body weight was recorded and ovaries were removed, trimmed and weighed. Ovaries from three animals in each group were fixed in Bouin's solution, embedded in paraffin, sectioned serially at 10 μm and stained with hematoxylin and eosin. Each section was examined with the identity of individual ovaries remaining unknown until all were studied. To avoid counting the same follicle twice, only the section containing the nucleolus of the oocyte was measured. 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 447 μm; 448 to 517 μm; 518 to 570 μm; $>$571 μm.

Treatments were assigned, performed and data were analyzed according to a completely randomized design. Statistical probabilities were determined by analysis of variance or Student's *t* test.

RESULTS

The pattern of follicular development in the immature rat treated with PMSG is shown in Table 1. Since the ovary of a non-treated animal (control) contained only a few (2.0 $\pm$ 1.0) follicles $>$ 352 μm at day 31, it is assumed that a similar pattern, or less, existed at day 28. A significant ($p < 0.05$) 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 follicles 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 $\pm$ 1.1; Table II than one ovary (2.0 $\pm$ 1.0; Table I of intact controls and thus, one can assume that at the 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 injection, a significant increase ($p<0.05$) in 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 period, the remaining ovary of all animals contained corpora lutea and had follicles in all size groups with the exception that only 67% of the animals had follicles in the 571+ μm group.

TABLE I: *Pattern of follicular development in the immature rat treated with PMSG.*

Size of follicles (μm)	Control (Day 31)		Mean number of follicles per ovary $\pm$ SEM in rats injected with 30 IU PMSG at day 28 of age and killed post-injection at:		
			24 hrs.	48 hrs.	72 hrs.
352-394	1.0	(2)[1]	7.3$\pm$3.5(3)	7.6$\pm$1.5(3)	12.3$\pm$2.3(3)
395-447	2.0	(2)	4.4$\pm$1.5(3)	5.0$\pm$1.8(3)	11.3$\pm$2.4(3)
448-517			{1.0}[2]	1.3$\pm$0.3(3)	10.0$\pm$2.9(3)
518-570				{1.0}	5.3$\pm$2.0(3)
571+			{1.0}	{2.0}	4.0$\pm$2.5(3)
Mean number of follicles $>$ 352μm $\pm$ SEM	2.0$\pm$1.0(3)		12.3$\pm$4.3(3)[a]	15.1$\pm$1.8(3)[a]	43.0$\pm$9.4(3)[a,b]

[1] Number in parentheses signifies number of animals in group with follicles of this size range.
[2] Bracketed figure indicates only one animal of group had follicles of this size range.
[a] $P<0.05$ vs control group.
[b] $P<0.05$ 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 (Peppler 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 (Peppler and Greenwald, 1970b), and indicates the same progressive pattern of follicular development that is observed in cycling, intact rats occurs 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 anestrous. 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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