Quantitative Aspects of Follicular Development in the Untreated and PMS-treated Cyclic Hamster

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ABSTRACT Hamsters injected at 0900 on day 1 of the cycle (metestrus) with either 0, 5 or 15 IU pregnant mare’s serum (PMS) were killed at 1500 of days 1 to 4 of the cycle and the ovaries prepared for light microscopy and for a quantitative evaluation of follicular development. In the untreated cyclic hamster, the maximal number of preantral follicles with eight or more layers of granulosa cells occurred between the afternoon of day 4 (proestrus) and day 1, coinciding with the highest blood levels of FSH and LH. It is concluded that the elevated preovulatory levels of gonadotropins not only induce the ovulation of the mature antral follicles but at the same time recruit the next set of follicles for development during the new cycle.

By the afternoon of day 1, treatment with either 5 or 15 IU PMS recruited more follicles into large preantral and incipient antral stages than in the untreated hamsters. However, by day 2 the pattern of follicular distribution was similar between the 5 IU PMS and untreated group whereas considerably more antral follicles had differentiated in the animals given 15 IU PMS. The ability of 15 IU PMS to elicit superovulation therefore depends on the levels being initially high enough to mature more follicles at critical stages of their development; the prolonged biological half life of PMS then sustains these follicles throughout the cycle.

Studies of follicular development in the untreated or pregnant mare’s serum (PMS) treated hamster during the estrous cycle are limited to quantitative changes in the number of antral follicles (Greenwald, '61, '62). The present study was designed to extend these observations to include much earlier stages of the follicular population and to use as an endpoint the number of granulosa cells surrounding the ovary rather than the diameter of the follicle.

MATERIAL AND METHODS
Golden hamsters (Mesocricetus auratus) weighing 75 to 100 gms were followed for at least three consecutive estrous cycles before being used in the experiments. The vaginal discharge occurring on the morning of ovulation was used to designate day 1 of the cycle; day 4 therefore corresponds to proestrus. The hamsters were given a single subcutaneous injection at 0900 of day 1 of the cycle of either 0.1 ml saline, 5 IU PMS or 15 IU PMS (Ayerst); the PMS was dissolved in 0.1 ml saline. Subsequently, 3 animals for each group were killed at 1500 hours on days 1, 2, 3 and 4 of the cycle. One ovary was removed from each animal and prepared for light microscopy by standard procedures. The ovaries were sectioned serially at 10 μ and stained with hematoxylin and eosin.

Follicles were counted in every fourth section at a magnification of 100 × and classified according to their stage of development. Only follicles in which the nucleolus of the oocyte was present were included in the counts. The one exception is that all antral follicles were counted whether the nucleolus was present or absent in the sections that were scanned. Follicular development was divided into six stages:

Stage I. Preantral follicle consisting of two or three layers of granulosa cells.
Stage II. Preantral follicle with four or five layers of granulosa cells.

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Stage III. Preantral follicle with six or seven layers of granulosa cells.

Stage IV. Follicle has eight or more layers of granulosa cells. These are the largest preantral follicles encountered.

Stage V. Incipient antral formation as evidenced by a series of isolated lacunae that are the forerunners of the antral cavity. The follicle has about 12 layers of granulosa cells.

Stage VI. Antral follicles characterized by a single coalesced cavity. This stage includes follicles varying in diameter from 400 μ to 570 μ, depending on the stage of the cycle.

Only follicles that were histologically normal were counted. The results were expressed as the mean number of follicles ± SE for each stage per ovary and the averages were based on three ovaries from three different animals for each subgroup.

To determine the number of ovulations resulting from PMS treatment, another series of hamsters were injected subcutaneously at 0900 of day 1 with either 0, 5, or 15 IU PMS. The animals were killed on the morning of the next day 1 and ova flushed from the oviducts. The newly ovulated eggs were freed from cumulus cells with hyaluronidase and then counted.

RESULTS

Hamsters injected with 0, 5 or 15 IU PMS on day 1 ovulated the following number of ova (± SE): 9.2 ± 1.35 (n = 6); 14.0 ± 1.75 (n = 8) and 44.0 ± 1.58 (n = 6). The effects of these doses of PMS on follicular development are summarized in table 1 and figure 1. In the control group (i.e., saline-injected hamsters) the maximal number of preantral follicles was present on the afternoon of day 1. A few follicles had progressed as far as the early antral stage (stage V) but the majority of the largest follicles were multilayered ones without any trace of an antral cavity (stage IV). By the next afternoon, the number of stage IV follicles was reduced which was reflected in an increase in early antral and small antral stages (stage VI). By day 3, there was an average of six antral follicles per ovary accompanied by the depletion of all stage IV and V follicles. The number of follicles with six to seven granulosa cell layers (stage III) was signi-
Fig. 1 Follicular development at 1500 on days 1-4 of cycle after single injection of 0, 5 or 15 IU PMS at 0900 of day 1. Results are expressed as mean number of follicles/stage/ovary. For definition of stages see MATERIAL AND METHODS.
significantly reduced from the number present on the previous two days of the cycle. The number of antral follicles was unchanged on day 4 and preantral stages with eight to ten layers of granulosa cells reappeared in the follicular population. This was accompanied by an increase in the number of stage III follicles. Throughout the four days of the cycle, there was no statistical difference in the number of stage I and II follicles.

Following a single injection of 5 IU PMS the most obvious difference from the control group on day 1 was that all ovaries contained follicles on the verge of forming an antral cavity (stage V). However, by day 2 and thereafter the pattern of follicular development was essentially the same for both groups except that a few follicles still persisted at stage IV on day 3 of the cycle. Follicles with eight or more layers of granulosa cells (stage IV) again began to reappear on the afternoon of day 4, comparable to the situation in the control group.

The pattern of follicular development by the afternoon of day 1 was identical in animals treated with 5 or 15 IU of PMS. The major difference between the two groups was evident by day 2; for the 15 IU PMS group, 23 antral follicles had differentiated and this approximate number persisted throughout the remainder of the cycle. In addition, all stages of follicular development were represented continuously throughout the cycle except for stage V which was still absent on the afternoon of day 4. The large standard error on day 3 for the 15 IU PMS group was attributable to one animal with many more follicles in stages I–III than any other animal involved in the study.

**DISCUSSION**

In the normal cyclic hamster, a significant increase in the number of preantral follicles with eight or more layers of granulosa cells (stages IV and V) occurs between the afternoon of proestrus (day 4) and the next postovulatory day (day 1). The highest blood levels of FSH and LH are also found during this time span (Bast and Greenwald, '73). Therefore the heightened preovulatory levels of gonadotropins not only induce ovulation of the mature antral follicles but at the same time recruit the next set of follicles for development during the new cycle. Blood levels of FSH and LH have both begun to increase by 1500 of day 4 (Bast and Greenwald, '73). Therefore whether one or both gonadotropins are involved in the initial reappearance of follicles with eight or more layers of granulosa cells (stage IV) cannot be ascertained.

In hamsters hypophysectomized for one week, the largest follicles present have six to seven layers of granulosa cells (Moore and Greenwald, '73). This corresponds to stage III in the present study and it indicates that follicular development past this point is dependent on the pituitary. In the intact cycling hamster, there is no significant difference in the number of follicles in stages I–III for any day of the cycle contrary to the flux in the population of larger follicles (table 1, fig. 1). Follicles in stages IV and V on the afternoon of day 1 appear to be the ultimate source of the antral follicles ovulating at the end of the cycle as evidenced by the absence of the former stages on day 3 (fig. 1). Follicular atresia accounts for the discrepancy between the number of stage IV and V follicles on day 1 and the number of antral follicles on day 4. Pulse labeling with 3H-thymidine, similar to the studies carried out with the mouse (Pedersen, '70), offers the best means of verifying this hypothesis.

It is evident that on the afternoon of day 1, both 5 and 15 IU PMS have similar effects on follicular development (fig. 1); namely, to recruit more follicles into stages IV and V than in the untreated hamster. However, by day 2 the pattern of follicular distribution in the low dose PMS group is similar to the untreated hamsters whereas 23 antral follicles have developed in the 15 IU PMS group. The latter treatment therefore has enabled a greater number of stage IV and V follicles to undergo further differentiation thus accounting for the ultimate superovulatory response. The ability of high doses of PMS to elicit superovulation therefore depends not only on its prolonged biological half-life but also on the levels being initially high enough to recruit more follicles at critical stages of their development. Neither dose of PMS altered the number of follicles in stages I.
to III (fig. 1) indicating that these follicular compartments are not affected by gonadotropin levels.

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LITERATURE CITED