Follicular Development after the First Ovulation in Immature Rats Pretreated with PMS*

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Synopsis

A single injection of PMS induces ovulation in immature rats on the third day morning after the treatment. The second estrus occurred 9 days after the first ovulation. Follicles of the succeeding cycle developed the capacity to ovulate 3 days after the first ovulation in 3 out of 5 rats, but could not be maintained in an ovulable state during the next 24 hr.

When supplementary PMS was given just after the first LH surge, follicles developed the capacity to ovulate within 1.5 days after the first ovulation and the second ovulation occurred in 7 out of 10 on the fourth day morning after the first ovulation. Vaginal smears showed a 4-day-cyclic pattern in these animals after supplementary PMS administration.

From these experiments, it is concluded that immature rats pretreated with PMS were in an anestrous state during 8 days after the first ovulation, not due to the activation of corpora lutea, but due to the failure of follicular development. Plasma levels of gonadotropin responsible for follicular growth and maintenance after the first ovulation were discussed.

A single injection of PMS produces ovulation in immature rats on the third day morning after the treatment. Detailed studies have led to the view that ovulation itself depends upon the LH surge on the second day afternoon following PMS administration (McCormack and Meyer, 1962, 1963; Strauss and Meyer, 1962; Zarrow and Quinn, 1963; Quinn and Zarrow, 1964; Wagner and Brown-Grant, 1965; Klawon et al., 1971) and endogenous gonadotropin requirements for follicular development have been clarified (Sasamoto and Kennan, 1973). The second estrus, however, was observed about 9 days after the first ovulation in these rats (Hashimoto and Wiest, 1969; Taya et al., in press), and little is known about the follicular development after PMS-induced ovulation in immature rats.

The present experiments are concerned with the follicular development of the succeeding cycle after the first ovulation in immature rats pretreated with PMS.

Materials and Methods

The rats were of the Wistar strain bred in the laboratory and aged 25 days old (day 25) at the start of the experiments. The animals were housed in an air-conditioned room illuminated from 5.00 to 19.00 hr throughout the experiments.

Three IU of PMS was administered subcutaneously on day 25 between 9.00 to 10.00 hr. Vaginal smears were taken daily after vaginal opening.

Follicular responsiveness to HCG after the first ovulation

In order to examine the follicular development following the first ovulation in immature rats pretreated with 3 IU of PMS, 10 IU of HCG was singly
injected intravenously between 17.00 to 18.00 hr on day 30, 31, or 32, under light ether anesthesia and the animals were killed 20 to 24 hr after HCG to examine oviducts for ova with an adherent cumules.

**Follicular development after the first ovulation by supplementary PMS**

Immature rats pretreated with 3 IU of PMS on day 25 were given supplementary PMS (3 IU sc) at 18.00 hr on day 27, just after LH surge had occurred, in order to examine the ability of follicles to develop to the state capable of ovulation. To test the ability of follicles to ovulate, 10 IU of HCG was singly injected intravenously between 17.00 to 18.00 hr on day 28, 29, 30, or 31. The animals were killed 20 to 24 hr after HCG and oviducts were examined for the incidence of ovulation.

**Ovarian and uterine weight changes after supplementary PMS**

Ovarian development and estrogen secretion after supplementary PMS were compared with those of non-PMS-supplementary controls. Rats were given 3 IU of supplementary PMS subcutaneously at 18.00 hr on day 27 and 4 to 10 rats were killed every day (at 10.00 hr) from day 29 to day 32, 4 days after the first ovulation. At autopsy, ovaries and uteri were removed for weighing. Uteri were weighed after the uterine fluid was pressed out on blotting paper. Each hormone was given in 0.2 ml of 0.9% NaCl.

**Results**

**Effect on induction of ovulation of HCG given at varying time intervals after the first ovulation in immature rats pretreated with PMS**

When 25-day-old immature rats received a single injection of 3 IU of PMS, ovulation was regularly induced on the morning of day 28 with 6 to 14 ova (Table 1). No ovulation occurred from day 29 to 32 unless further treatment was made. When HCG was given between 17.00 to 18.00 hr on day 31, ovulation was induced on the next day morning in 3 out of 5 with 2 to 4 ova, whereas the HCG injection one day earlier or later produced no ovulation. Vaginal smears of the animals in these experiments continuously showed an anestrous state from day 29 to day 32, irrespective of HCG administration.

**Discussion**

Corpora lutea formed in these immature rats following PMS-induced ovulation were non-functional, unless cervical stimulation was made on the day of ovulation (Hashimoto and Wiest, 1969). The data in Table 1 indicate that only a small number of follicles developed the capacity to ovulate 3 days after the first ovulation. However, the estrogen required for vaginal cornification was not
Table 1. Responsiveness of follicles to HCG given at varying time intervals after the first ovulation in immature rats pretreated with PMS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Autopsy</th>
<th>Ovulation</th>
<th>No. of ova (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMS on day 25</td>
<td>day 28</td>
<td>5/5*</td>
<td>6-14</td>
</tr>
<tr>
<td>PMS on day 25</td>
<td>day 29</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>PMS on day 25</td>
<td>day 30</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td>PMS on day 25</td>
<td>day 31</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>PMS on day 25 + HCG on day 30</td>
<td>day 31</td>
<td>0/3</td>
<td></td>
</tr>
<tr>
<td>PMS on day 25</td>
<td>day 32</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td>PMS on day 25 + HCG on day 31</td>
<td>day 32</td>
<td>3/5</td>
<td>2-4</td>
</tr>
<tr>
<td>PMS on day 25 + HCG on day 32</td>
<td>day 33</td>
<td>0/4</td>
<td></td>
</tr>
</tbody>
</table>

PMS (3 IU) was given sc in 0.2 ml of 0.9% NaCl.
HCG (10 IU) was given iv in 0.2 ml of 0.9% NaCl.
* No. of rats ovulating/no. of rats examined.

Table 2. Effect of supplementary PMS on follicular responsiveness to HCG given at varying time intervals after the first ovulation in immature rats pretreated with PMS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Autopsy</th>
<th>Ovulation</th>
<th>No. of ova (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMS on day 25 + PMS on day 27</td>
<td>day 29</td>
<td>0/5*</td>
<td></td>
</tr>
<tr>
<td>PMS on day 25 + PMS on day 27 + HCG on day 28</td>
<td>day 29</td>
<td>1/5</td>
<td>1</td>
</tr>
<tr>
<td>PMS on day 25 + PMS on day 27</td>
<td>day 30</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>PMS on day 25 + PMS on day 27 + HCG on day 29</td>
<td>day 30</td>
<td>3/3</td>
<td>4-7</td>
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<tr>
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<td>day 31</td>
<td>0/5</td>
<td></td>
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<td>day 31</td>
<td>5/5</td>
<td>3-7</td>
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<td>day 32</td>
<td>7/10</td>
<td>4-9</td>
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<td>PMS on day 25 + PMS on day 27 + HCG on day 31</td>
<td>day 32</td>
<td>7/7</td>
<td>5-11</td>
</tr>
<tr>
<td>PMS on day 25 + PMS on day 27</td>
<td>day 33</td>
<td>0/3</td>
<td></td>
</tr>
</tbody>
</table>

Supplementary PMS (3 IU) as well as pretreatment PMS (3 IU) were given sc in 0.2 ml of 0.9% NaCl.
HCG (10 IU) was given iv in 0.2 ml of 0.9% NaCl.
* No. of rats ovulating/no. of rats examined.

Secreted from these follicles, which lost their ability to ovulate within next 24 hr probably due to degenerative changes. These are the striking contrast to the observations in adult cyclic rats in which ovulable follicles were maintained in an ovulable state when LH surge was inhibited on 2 successive days (Everett and Sawyer, 1950). Within 9 hr after the neutralization of circulating gonadotropin, ovulable follicles completely lost their ability to ovulate (Sasamoto and Kennan, 1972). Plasma levels of gonadotropin in these immature rats may be lower than the threshold level required for the maintenance of follicles.

Ovulation could be advanced one day earlier by HCG injection on the afternoon of diestrus (Eto and Imamichi, 1955), or by steroid treatment in adult cyclic rats (Everett, 1961). These results indicate that follicles of the succeeding estrous cycle in adult cyclic rats could develop the capacity to ovulate within 2.5 days after ovulation. Failure of ovulation by HCG at a 2.5 day-interval after the first ovulation (Table 1) indicates that no follicles developed to the stage where they are capable of ovulation at the time of HCG administration.

When supplementary PMS was given at 18.00 hr on day 27, just after LH surge, and followed by HCG between 17.00 to 18.00 hr on day 29 or later, all the animals ovulated on the next day morning after HCG (Table 2).
Follicles developed the capacity to ovulate within 1.5 days after the first ovulation (48 hr after supplementary PMS). Without HCG administration, 7 out of 10 ovulated on the morning of day 32, 4 days after the first ovulation, indicating LH surge on day 31. With supplementary PMS administration patterns of follicular development of the succeeding cycle were similar with those observed in adult cyclic rats (Nakamura et al. 1968). Large amounts of gonadotropin secretion during the periovulatory period have been assumed to be responsible for development of one set of follicles of the succeeding cycle in adult cyclic...
rats (Schwartz, 1969; Welschen, 1973; Schwartz et al., 1973). In immature rats pretreated with PMS, failure of follicular development after the first ovulation may be due to the difference in the patterns of gonadotropin surge during the periovulatory period as compared with those of adult cyclic rats.

When immature rats pretreated with PMS were given supplementary PMS, uterine weights increased significantly from day 30 to day 31 (Fig. 1), the day when LH surge occurred. These patterns of uterine weight changes were similar with those observed in adult rats (Hori et al. 1968). Hori et al. demonstrated that the time course of the uterine weight changes closely paralleled that of ovarian venous estrogen concentration with a delay of 3 hr. Patterns of estrogen secretion in these immature rats may be similar with those in adult cyclic animals and play a role in LH surge on day 31.

From these experiments, it is concluded that immature rats pretreated with PMS were in an anestrous state during 8 days after the first ovulation, not due to the activation of corpora lutea, but due to the failure of follicular development.

Acknowledgements

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References


Quinn, L. and M. X. Zarrow (1964). Endocrinology 74, 309.


