FSH AND LH LEVELS IN THE INTACT AND UNILATERALLY OVARIECTOMIZED CYCLING RAT

By

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ABSTRACT

Intact 5-day cycling rats were killed between 8–10 a.m. on each day of the oestrous cycle; experimental rats were unilaterally ovariectomized (ULO) at 9 a.m. on day 1 (oestrus) and killed between 8 and 10 a.m. on days 2, 3, 4 or 1 of the subsequent cycle. Pituitary and plasma concentrations of FSH and LH were measured in both groups of rats. Pituitary FSH concentration was measured by the Steelman-Pohley method with slight modification; plasma FSH by the Igarashi-McCann assay and pituitary and plasma LH concentration by the OAAD method.

In intact rats, pituitary FSH values remained constant for the first three days of the cycle, increased on day 4 and reverted to early cycle values by day 5. Plasma FSH increased between days 2 and 3 and days 5 and 1. Pituitary LH concentration remained the same for days 1 and 2; increased two-fold on days 3 and 4, and increased further by day 5. Plasma LH increased between days 2 and 3; other differences between successive cycle days were not apparent.

Following ULO on day 1, pituitary FSH increased steadily, but not significantly, for the remaining cycle. Plasma FSH did not change from day 2 through day 1 of the subsequent cycle. Pituitary LH remained low on day 2, increased sharply by day 3 and decreased (50%) by day 4. Plasma LH also increased between days 2 and 3. Other differences between successive days following unilateral ovariectomy on day 1 were not apparent.

Correlation of gonadotrophin activity with follicular development suggests that the mechanism of compensatory ovulation in the rat may be one of an increase in time of exposure to a constant gonadotrophic level for the duration of the oestrous cycle rather than to increased levels of the gonadotrophin.
The mechanism of compensatory ovulation following unilateral ovariectomy (ULO) involves the interaction between the hypothalamus, anterior hypophysis and the ovary. Several theories have been proposed to account for compensatory ovulation: increased synthesis and/or release of gonadotrophins from the pituitary; increased uptake of an unchanged level of circulating gonadotrophins by the remaining ovary and hence increased utilization; a more sustained peripheral level of gonadotrophins throughout the oestrous cycle. In the cycling rat, compensatory ovulation occurs within the immediate oestrous cycle if ovariectomy is performed before the follicles destined to ovulate are determined (Peppier & Greenwald 1970b). However, many investigators have ignored this fact that compensatory ovulation is related to the events encompassing the oestrous cycle in measuring pituitary and plasma FSH and LH.

The purpose of the following experiments was (a) to measure FSH and LH in the anterior pituitary and plasma in intact 5 day cycling rats, (b) to measure FSH and LH in the anterior pituitary and plasma in rats ULO on the morning of day 1 (oestrus), (c) to establish the mechanism of compensatory ovulation following ULO in the rat, and (d) to correlate the detected gonadotrophin patterns with previously reported ovarian changes (Peppier & Greenwald 1970a,b).

MATERIALS AND METHODS

A total of 504 Holtzman, female virgin rats were obtained at 51–63 days of age (180–200 g). Animals were maintained in groups of 6 with water and laboratory chow provided ad libitum. The lighting schedule was regulated for 14 hours of daylight (5 a.m. – 7 p.m.) and 10 hours of darkness. Day 1 of the cycle refers to oestrus, characterized by newly ovulated tubal eggs and by a vaginal smear of cornified cells devoid of leucocytes. Daily vaginal smears were taken between 8 and 10 a.m. and after three consecutive 5 day cycles, rats were divided into control and experimental groups. Controls were killed between 8 and 10 a.m. on each day of the 5 day oestrous cycle; experimental rats were ULO at 9 a.m. on day 1 and killed between 8 and 10 a.m. on days 2, 3, 4 or 1 of the subsequent cycle. ULO at 9 a.m. on day 1 in rats with 5 day cycles shortens the immediate cycle by one day (Peppier & Greenwald 1970a). The rats were anaesthetized with ether and decapitated. Blood was collected in an ice-packed beaker containing 0.5 ml heparin and 0.5 ml saline per animal. Plasma was separated by centrifugation in a cold room, removed, pooled (respective days) and frozen until assayed. Anterior pituitaries were removed and stored in a desiccator at room temperature for assay.

This study consisted of four series of experiments.

(1) Pituitary and plasma FSH concentration were determined during the normal 5 day oestrous cycle. – 269 females were killed on either day 1, 2, 3, 4 or 5 of the oestrous cycle.

(2) Pituitary and plasma FSH concentration were measured following ULO. – 178 rats were ULO on day 1 and killed on day 2, 3, 4 or day 1 of subsequent cycle.

(3) Pituitary and plasma LH concentration were determined throughout the normal
5 day oestrous cycle. - 31 rats were killed on either day 1, 2, 3, 4 or 5 of the oestrous cycle.

(4) Pituitary and plasma LH concentration were measured after ULO. - 26 females were ULO on day 1 and killed on day 2, 3, 4 or day 1 of subsequent cycle.

**Pituitary FSH**

Pituitary FSH concentration was determined by the Steelman-Pohley method with slight modifications (Steelman & Pohley 1953). Anterior pituitaries (dry) were weighed, pooled and homogenized in saline. Each assay animal received the equivalent of two pituitaries. Human chorionic gonadotrophin (HCG) (50 IU per assay animal) was added to the homogenate and the test solution injected over a 3-day period into 26 day old Holtzman female rats. Assay animals received 5 subcutaneous injections (0.5 ml per injection); twice on the first two days, and once on the third day. Animals were killed 24 hours after the last injection. Four standards were used in each of the 5 assays. HCG (50 IU) suspended in normal saline represented the control; reference standards consisted of 50, 100 and 150 µg of ovine FSH (NIH-S4 or S5) in combination with 50 IU HCG. Results were expressed as µg NIH-FSH-S1/mg. Student's t test was used to determine differences in FSH concentration. The 5 assays performed were averaged together in the final analysis.

**Plasma FSH**

A modification of the Igarashi-McCann assay was used to determine the plasma concentration of FSH in intact and unilaterally ovariectomized rats (Igarashi & McCann 1964). Frozen plasma was thawed, filtered and combined with HCG (5 IU per assay animal). Swiss-Webster, female mice (24 days old) received 2 subcutaneous injections daily for 3 days (animals received 1.0 ml on first day and 0.6 ml on other two days for a total volume of 2.2 ml) and were killed the day following the last injection. Four standards were used for each of the assays. As a control standard, 5 IU HCG was suspended in normal saline and reference standards consisted of ovine FSH (NIH-S5) in doses 1, 5 and 10 µg combined with 5 IU HCG. The results were expressed as µg NIH-FSH S1/100 ml. Two separate assays were performed and averaged together.

**Pituitary LH**

Pituitary and plasma LH concentrations were determined by the ovarian ascorbic acid method (Parlow 1958). Dry anterior pituitaries were weighed, pooled and homogenized in normal saline. Holtzman rats, previously primed with 50 IU PMS and 100 IU HCG, were anaesthetized with ether and injected intravenously via a tail vein with the standard or unknown solutions (1.0 ml per 100 g body weight). One ovary was removed 4 hours after the injection. The recipient rats received the test solutions in two different concentrations: 1/4 or 1/16 pituitary per 1.0 ml. In the four assays, ovine LH (NIH-S8 or S13) was used as reference standards in doses of 0.1, 0.4 and 1.6 µg. Results were expressed in terms of LH-S1 (NIH) on the basis that NIH-S8 has a relative potency of 0.73 × NIH-LH-S1/mg and NIH-S13, 0.93 NIH-LH-S1 units/mg. A total of 4 separate assays were performed. The data were analyzed by a fortran program designed for LH assay determination on a GE-625 computer.

**Plasma LH**

Plasma was thawed and filtered to remove fibrin clots. Each assay animal received
3.0 ml of the plasma and both ovaries were removed 4 hours after the injection. In the two assays, one saline standard (zero per cent depletion) and three reference standards of ovine LH (NIH-S13) were used (0.1 µg = 6.8%; 0.4 µg = 16.4%; 1.6 µg = 30.5%). The results are expressed as per cent depletion of ascorbic acid by 3.0 ml of plasma.

RESULTS

Pituitary and plasma FSH concentration in intact rats
(Table 1 and Figs. 1 and 2)

Pituitary FSH values remained relatively constant for the first three days of the cycle, increased on day 4 and reverted to early cycle values by day 5. Both the increase between days 3 and 4, and the decrease from days 4 to 5 were significant (P < 0.025). FSH concentration in the plasma increased (P < 0.025) between days 2 and 3, and days 5 and 1. Plasma FSH decreased (P < 0.025) on day 5 when compared with the value for preceding day.

<table>
<thead>
<tr>
<th>Day of oestrous cycle</th>
<th>Pituitary FSH concentration µg NIH-FSH S1/mg ± se</th>
<th>Plasma FSH concentration µg NIH-FSH S1/mg ± se</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Hematicrate*</td>
</tr>
<tr>
<td></td>
<td><strong>Intact</strong></td>
<td><strong>Hematicrate</strong></td>
</tr>
<tr>
<td>1</td>
<td>4.2 ± 0.5 (29)**</td>
<td>46.7 ± 14.9 (10)</td>
</tr>
<tr>
<td>2</td>
<td>5.1 ± 0.7 (32)</td>
<td>23.8 ± 10.0 (9)</td>
</tr>
<tr>
<td>3</td>
<td>5.8 ± 0.5 (27)</td>
<td>81.5 ± 25.2 (11)</td>
</tr>
<tr>
<td>4</td>
<td>7.2 ± 0.9 (24)</td>
<td>39.5 ± 12.9 (9)</td>
</tr>
<tr>
<td>5</td>
<td>5.2 ± 0.5 (26)</td>
<td>13.7 ± 2.9 (11)</td>
</tr>
<tr>
<td>1***</td>
<td>6.5 ± 0.8 (20)</td>
<td>37.7 ± 11.6 (9)</td>
</tr>
</tbody>
</table>

* Unilaterally ovariectomized on day 1 of oestrous cycle.
** Number in brackets indicates number of assay animals.
*** For hematicrate, day 1 means day 1 of next cycle.

Pituitary FSH concentration
Intact
Day 3 vs. Day 4 P < 0.025
Day 4 vs. Day 5 P < 0.025
Intact vs. hematicrate
Day 1 vs. Day 1*** P < 0.01

Plasma FSH concentration
Intact
Day 2 vs. Day 3 P < 0.025
Day 4 vs. Day 5 P < 0.025
Day 5 vs. Day 1 P < 0.025
Intact vs. hematicrate
Day 5 vs. Day 4 P < 0.025

270
Pituitary and plasma FSH concentration following ULO (Table 1 and Figs. 1 and 2)

ULO of 5 day cyclic rats on day 1 consistently shortens (82.2 %) the oestrous cycle by one day (Peppier & Greenwald 1970a). Following semispaying on day 1, pituitary FSH increased steadily but not significantly for the remainder of the cycle. Differences were not detectable within the immediate cycle when
pituitary FSH values of intact and hemicastrated rats were compared on respective days (i.e. day 2 intact vs. day 2 hemicastrate, etc.). However, by day 1 of the next cycle, the pituitary of the hemicastrate animal had 6.5 ± 0.8 µg FSH compared to only 4.2 ± 0.5 mg in day 1 intact rats ($P < 0.01$). Following ULO on day 1, plasma FSH did not change from day 2 through day 1 of the subsequent cycle. Comparison of plasma values on respective numbered days with intact rats revealed no differences. (i.e. day 2 intact vs. day 2 hemicastrated, etc.). However, when comparing pro-oestrous values (day 5 intact vs. day 4 hemicastrate), the hemicastrate had twice as much plasma FSH as the intact rat ($P < 0.025$).

*Pituitary and plasma LH concentration in intact rats*

*(Tables 2 and 4 and Figs. 3 and 4)*

Pituitary LH concentration remained the same for days 1 and 2; increased two-fold on day 3; remained at this level through day 4 and increased further by day 5. LH decreased sharply (greater than 60%o) between days 5 (pro-oestrum) and 1 (oestrus). Differences in values of per cent depletion of ovarian ascorbic acid produced by 3.0 ml of plasma from successive days in the oestrous cycle were not apparent except between days 2 and 3 ($P < 0.025$). This parallels the increase of pituitary LH. The percentage of animals responding to the 3.0 ml of plasma for respective days indicated the presence of more LH in the late stages of the cycle (days 3, 4 and 5). Only 39%o of the assay animals responded to day 1 plasma; 33%o to day 2; 88%o to day 3; 90%o to day 4 and 93%o to day 5.

*Pituitary and plasma LH concentration in hemicastrated rats*

*(Tables 3 and 4 and Figs. 3 and 4)*

Following ULO on day 1, pituitary LH remained low on day 2 and increased sharply by day 3 as in the intact rat. However, instead of staying at this high level until the end of the cycle, the concentration decreased by day 4 (50%o reduction). Day 4 in rats ULO on day 1 corresponds to pro-oestrum *(Peppler & Greenwald 1970a)*. When pro-oestrous values of hemicastrates (day 4) and intact rats (day 5) were compared three times as much pituitary LH is found in intact rats.

Paralleling the increase of pituitary LH between days 2 and 3 there was an increase in the per cent depletion of ascorbic acid ($P < 0.025$). Other differences between successive days following ULO on day 1 were not apparent. In comparing the per cent depletion values with intact rats for respective days, no differences were detected. Percentage of assay animals responding for days 2, 3, 4 and 1 of subsequent cycle were 42, 81, 81 and 67 respectively. A greater number of assay animals responded to the plasma of day 1 of the next cycle (67%o) than to the day 1 plasma from intact rats (39%o).
Table 2.
Pituitary LH concentration (μg/mg) in hematicastrated rats1.

<table>
<thead>
<tr>
<th>Day of cycle</th>
<th>Assay number</th>
<th>Number of unknowns</th>
<th>Number of assay animals</th>
<th>μg LH Sl/mg</th>
<th>95% C. L. on relative potency</th>
<th>λ</th>
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<td>0.24</td>
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<td>0.23-0.58</td>
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<td>8</td>
<td>0.46</td>
<td>0.32-0.64</td>
<td>0.14</td>
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<tr>
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<td>0.20-0.56</td>
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</table>

1 Unilateral ovariectomy performed on day 1 of the oestrous cycle.
* Average of means.
** Day 1 of next cycle.

DISCUSSION

Pituitary FSH

Previous investigations have reported a decrease in pituitary FSH between the days of pro-oestrus and oestrus in intact rats (McClintock & Schwartz 1968; Goldman & Mahesh 1968). In the present study, pituitary FSH concentration decreased \((P < 0.025)\) between late dioestrus (day 4) and oestrus (day 1). Benson et al. (1969) reported a decrease in pituitary FSH 24 h following ULO. In contrast, others have detected no difference (Mandl & Zuckerman 1956; Edgren et al. 1965). The present experiments demonstrated that pituitary FSH increased steadily following ULO and did not decrease
Fig. 3.
Pituitary concentration of LH (μg/mg) in intact and unilaterally ovariectomized rats.
Intact: ●●●; Hemicastrated: ×—×.

at the end of the cycle as in the intact animal. This steady increase in pituitary concentration was consistently but not statistically below that measured in the intact rat on the respective days of the oestrous cycle. A significant increase in pituitary FSH occurred by day 1 of the next cycle. This was the only day where a difference in pituitary FSH existed between intact and hemicastrated rats.

Fig. 4.
Per cent depletion of ovarian ascorbic acid + se by 3.0 ml of plasma from intact and hemicastrated rats.
Intact: ●●●; Hemicastrated: ×—×.
Plasma FSH

Soliman & Nasr (1962) detected an increase of FSH during oestrus and early dioestrus, while others (Goldman & Mahesh 1968; Benson 1968) have measured more FSH in the blood at pro-oestrus than oestrus. In the present study, plasma FSH in intact rats increased between days 5 and 1 (pro-oestrus and oestrus). The level of FSH dropped on day 2, but increased markedly on

Table 3.

Pituitary LH concentration (µg/mg) in intact rats.

<table>
<thead>
<tr>
<th>Day of cycle</th>
<th>Assay number</th>
<th>Number of unknowns</th>
<th>Number of assay animals</th>
<th>S1/mg µg LH potency</th>
<th>95% C. L. on relative</th>
<th>( \lambda )</th>
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<td>1.56</td>
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</table>

C. L. refers to confidence limits.
* Average of means.
day 3. Between days 4 and 5, a significant decrease occurred. Following semi-
sparing, an increase of plasma FSH was detected 3–4 days later (Benson et al.
1969). In contrast, Edgren et al. (1965) could not detect this change. In the
present study, rats hemicastrated on day 1, showed a slight increase \((P < 0.05)\)
in plasma FSH by the following morning and the values remained relatively
constant through day 1 of the next cycle. There was no difference when the
plasma values for respective days of intact and ULO rats were compared. FSH
did not decrease at pro-oestrus in the ULO rat (day 5 intact vs. day 4 semi-
spayed). Instead, the hemicastrated rat had twice the amount of plasma FSH
as the intact rat (29.3 vs. 13.7).

**Pituitary LH**

In the intact rat, pituitary LH is lowest during oestrus. LH concentration
remained low on day 2, but increased sharply by day 3 with a further in¬
crease by day 5 (pro-oestrus). A large decrease \((68\%\)\) in pituitary LH occurred
between the days of pro-oestrus and oestrus. As in the intact rat, pituitary
LH in the hemicastrated rat remained low on day 2 and increased sharply by
day 3. However, contrary to the finding in the intact rat, the content decreased
by day 4. Comparison of pro-oestrous values from intact and hemicastrated
rats showed three times as much LH in the intact rat’s pituitary.

**Plasma LH**

In the present study, an increase in the per cent depletion of ascorbic acid

<table>
<thead>
<tr>
<th>Day of oestrous cycle</th>
<th>Per cent depletion(^1) ± se</th>
<th>Intact rats</th>
<th>Hemastraeted rats(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.1 ± 2.2 (7/18)(^3)</td>
<td>6.0 ± 1.3 (5/12)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4.7 ± 0.4 (5/15)</td>
<td>10.3 ± 1.3 (13/16)</td>
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<tr>
<td>3</td>
<td>11.5 ± 3.0 (14/16)</td>
<td>7.4 ± 2.0 (17/21)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9.9 ± 1.4 (20/22)</td>
<td>6.8 ± 1.2 (14/15)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>9.0 ± 2.0 (12/18)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) LH-S13 depletions: 0.1 µg = 6.8 %; 0.4 µg = 16.4 %; 1.6 µg = 30.5 %; Saline = 0 %.
\(^2\) Unilateral ovariectomy performed on day 1 of the oestrous cycle.
\(^3\) Ratio represents number of assay animals responding from which mean % depletion
was calculated.
\(^*\) Day 1 of next cycle.
occurred between days 2 and 3 in both intact and ULO rats \((P < 0.025)\). This parallels the increase of pituitary LH detected at the same time. Differences in plasma LH on respective days between the two groups were not apparent. However, a greater number of assay animals responded to the plasma of ULO animals at oestrus of the next cycle (oestrus verified by presence of tubal ova) than to day 1 plasma from intact rats. This may indicate that more LH is released at the end of the cycle as is the case with FSH in the hemicastrate. The decrease in pituitary LH detected on day 4 in hemicastrates agrees with this hypothesis. The possibility that the synthesis of LH is reduced at this time cannot be eliminated. Nonetheless, elevated plasma levels of LH over control values could not be demonstrated following ULO in this study.

If one considers the detected concentration levels as an indication of overall activity, then the FSH:LH ratios (Table 5) suggest a decrease in pituitary activity at the end of the oestrous cycle in intact rats; on the other hand, pituitary ratios for hemicastrated animals showed an increase through day 1 of the next cycle. Plasma activity paralleled that of the pituitary for the intact animal; whereas that of the ULO animal remained constant until day 1 of the next cycle. The greater activity of the pituitary in the hemicastrate together with the constant plasma activity suggests that the mechanism of compensatory ovulation may be one of exposure to a constant or minimal gonadotrophic level for the duration of the oestrous cycle rather than one of increased concentration.

The same hypothesis can be made after correlating pituitary and plasma gonadotrophic activity with follicular development in the intact and hemicastrated animal. During the oestrous cycle of the intact rat, there is a gradual increase in the number of follicles with a diameter of 518 to 571 µm and

<table>
<thead>
<tr>
<th>Day of cycle</th>
<th>Pituitary FSH:LH ratio</th>
<th>Plasma FSH:LH ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>hemicastrated</td>
</tr>
<tr>
<td>1</td>
<td>8.6</td>
<td>9.6</td>
</tr>
<tr>
<td>2</td>
<td>9.4</td>
<td>4.6</td>
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<tr>
<td>3</td>
<td>5.0</td>
<td>11.1</td>
</tr>
<tr>
<td>4</td>
<td>6.9</td>
<td>2.0</td>
</tr>
</tbody>
</table>
| 5            | 3.3        |              | * Day 1 of next cycle.

* Table 5.

FSH:LH ratio of pituitary and plasma concentrations in intact and unilaterally ovariectomized rats.

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greater; however, the total number of follicles ranging in size from 352 to 571 μm and larger remained relatively constant (Peppler & Greenwald 1970b). ULO on the morning of days 1 to 3 resulted in doubling the number of ovulations in the remaining ovary by the next oestrus in all rats (Peppler & Greenwald 1970a). Five-day animals continued to double the number of ovulations when the operation was performed as late as 2 a.m. on day 4 (Peppler & Greenwald 1970b). Following ULO, the total number of follicles larger than 352 μm does not vary from day to day (Peppler & Greenwald 1970b). However, twice the number of large Graafian follicles (over 448 μm in diameter) found in one ovary of intact rats were maturing in the hemicastrate. Injection of 20 IU HCG to intact animals on any day of the cycle showed that follicles larger than 448 μm are capable of ovulating. Intact animals do not ovulate in response to 20 IU HCG when injected at 2 p.m. on day 1, 47% ovulated an average of 7.6 eggs when given HCG on day 2, and all animals responded to administration on the remaining days of the cycle (day 3, 4 and 5) ovulating an average of 7.5, 9.8 and 11.8 eggs respectively (Peppler & Greenwald 1970b). Five-day cycling rats ovulate 10.2 ± 0.2 ova (Peppler & Greenwald 1970a) and the normal ovulation rate via HCG treatment was not obtained until day 4. Both the ULO and HCG experiments demonstrated that follicles increased steadily in size throughout the cycle and follicles capable of ovulating in any one cycle are determined by 8 a.m. on day 4.

In this study, the observed increase in plasma FSH between pro-oestrum and oestrus in the intact animal is necessary for the early development of follicles destined to mature in any one cycle. Progressive follicular maturation occurs under a certain amount of FSH stimulation and development is completed as a result of the plasma FSH surge detected on day 3. By the morning of day 4, ovulatible follicles are differentiated. Hence, the minimal amounts of FSH through the remainder of the cycle are probably necessary to maintain the already matured follicles until ovulation. Thus, the increase in pituitary FSH concentration on day 4 in the intact animal is indicative entirely of a storage phenomenon.

Following ULO on day 1, the remaining ovary doubles the number of eggs shed. The method of compensatory ovulation involved a doubling of the number of large follicles maturing in that cycle and resulted from increased proliferation of smaller follicles in the course of the cycle (Peppler & Greenwald 1970b). ULO of pregnant rats on day 1, 10 or 15 also increased the number of antral follicles within 5 days and resulted in compensatory ovulation at the post-partum oestrus (Chatterjee & Greenwald 1971). It seems unlikely that an immediate surge of FSH in the plasma could account for this steady increase maturation, but rather that a constant minimal level could. In the present study, following hemicastration on day 1, plasma FSH values remained relatively constant through day 1 of the next cycle. Greenwald (1963)
reported that the reserve follicles shed during superovulation needed PMS throughout the cycle in the hemicastrated hamster and McLaren (1966), working with the mouse, suggested that an increase in the duration of PMS stimulation resulted in ovulatory compensation. This study has demonstrated that the level of FSH in the plasma is increased for a greater portion of the oestrous cycle following ULO on day 1.

Everett (1964) administered 2 and 4 µg of LH to 5-day cycling rats on the days of dioestrus and pro-oestrus. The injections at dioestrus resulted in 0 and 5.8 eggs ovulating while at pro-oestrus, 2.4 and 6.4 ova shed respectively. It appears that an increase of LH release should occur in the ULO rat. However, values of plasma LH did not show this increase in the present study. Hemicastration caused a decrease in pituitary LH concentration on day 4 and more assay animals responded to plasma from day 1 of the next cycle in the semispayed than that from the intact. However, relative values showed no difference.

Whether the mechanism would be the same if the animals were ULO later in the cycle (late day 3 and early day 4) can only be answered through further experiments. Many animals hemastraed at this time which showed compensatory ovulation at the next oestrus also displayed longer cycles (Peppler & Greenwald 1970b). This again suggests an increase in time of exposure to gonadotrophins rather than one of concentration as the factor responsible for compensatory ovulation.

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