COITAL BLOCK TO SUPEROVULATION IN THE HAMSTER

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Summary. Hamsters injected with 60 i.u. pregnant mares' serum (PMS) on the morning of metoestrus (Day 1 of oestrous cycle) will ovulate sixty to seventy ova if they are isolated from males. However, if similarly treated females are caged with males on Day 2 or 3, coitus takes place and the inactive corpora lutea of the oestrous cycle are transformed into functional corpora lutea of pseudopregnancy. Consequently, the animals do not ovulate. The corpora lutea block ovulation by producing progesterone, which inhibits the release of pituitary luteinizing hormone (LH). The corpus luteum of ovulation thus can be brought to full secretory activity as late as 54 hr after ovulation. Pregnant mares' serum-treated hamsters mating late on Day 3 do ovulate presumably because of regression of the corpora lutea. Hamsters placed with males on Day 4 mate at the normal time and ovulate as many ova as isolated controls. Animals injected concurrently with 60 i.u. PMS and 5 mg of progesterone on Day 1 and subsequently isolated from males fail to ovulate, thus duplicating the effects of coitus. This experiment also indicates that the ovulation of follicles matured by PMS is due to endogenous gonadotrophins rather than the inherent LH activity of the PMS preparation.

INTRODUCTION

Hamsters injected at metoestrus with 30 or 60 i.u. pregnant mares' serum (PMS) will ovulate seventy eggs at the next oestrus instead of the normal number of ten (Greenwald, 1962a). As an extension of this research, it was decided to determine how many of the superovulated ova could be fertilized and would subsequently implant. Accordingly, females primed with PMS at metoestrus (Day 1 of oestrous cycle) were caged with fertile males and killed on Day 2 of the next cycle. Preliminary experiments revealed an unexpected finding in that some females ovulated sixty to seventy eggs whereas others failed to ovulate. In checking the protocols of the PMS-treated animals, it was apparent that the stage of the oestrous cycle at which the female was introduced into the males' cage was the deciding factor in determining whether ovulation would occur.

The following experiments were therefore designed to answer two questions: firstly, at what stage in the oestrous cycle of PMS-treated hamsters will intro-
duction of the male prevent ovulation; secondly, what mechanism(s) normally leading to ovulation are blocked by the presence of the male?

MATERIALS AND METHODS

These experiments utilized forty-five female golden hamsters weighing 75 to 120 g. Vaginal smears were used to establish the stages of the oestrous cycle. Day 1, as used in this paper, corresponds to the morning of metoestrus, while Day 4 designates the morning before the next ovulation. Females were caged in groups of five. After at least two consecutive 4-day cycles had been recorded, the hamsters were divided into four groups and treated as follows:

Group 1
Hamsters were injected subcutaneously with 60 i.u. PMS on Day 1 between 9 and 10 a.m. and recaged with other females until killed at Day 2 of the next cycle.

Groups 2, 3 and 4
Females similarly injected with 60 i.u. PMS on Day 1, were placed with males at 9 a.m. on either Days 2, 3 or 4 (Groups 2, 3 and 4, respectively). Daily vaginal smears were continued after the injection of PMS. In each instance, one female was introduced into a cage containing two fertile males and the animals remained together until necropsy on Day 2 of the subsequent cycle. Ova, if present, were flushed from the oviducts with a 30-gauge needle attached to a 1 cc tuberculin syringe; granulosa cells surrounding recently ovulated eggs were dispersed with hyaluronidase. Representative ovaries of each group were sectioned serially at 10 µ and stained with haematoxylin and eosin. In addition, other animals of Group 2, in which PMS was administered, were killed on Days 3 or 4 of the cycle. Additional experiments are described in appropriate sections of the text.

RESULTS
Hamsters injected with 60 i.u. PMS and subsequently isolated from males ovulated an average of sixty-eight eggs (Table 1, Group 1); cycle length was prolonged to 5 days in five of the six animals. In contrast, six females, similarly treated, but caged with males on Day 2, had not ovulated when killed on Day 2 of the next cycle (Table 1, Group 2). When eight PMS-treated females were placed with males on Day 3, two distinct ovulatory responses were observed: four of them ovulated, with an average of thirty-six eggs (Table 1, Group 3a), whereas the other animals failed to ovulate (Table 1, Group 3b). All females introduced to the males' cage on Day 4 ovulated, with an average of sixty-one ova per animal (Table 1, Group 4).

OVARIAN HISTOLOGY
The ovaries of hamsters placed with males on Day 2 (Group 2) contained numerous healthy vesicular follicles when examined on Days 3 or 4 of the
cycle in which PMS was administered. However, by Day 2 of the next cycle, all vesicular follicles were atretic (Pl. 1, Figs. 1 and 2). While the granulosa cells of most follicles were normal, all ova were degenerating as evidenced by the presence of pseudomaturatation figures or complete disappearance of the nuclear contents. It was of considerable interest that the ovaries also contained an average of five or six well-developed corpora lutea (Pl. 1, Figs. 1 and 2) which were identified as the persisting corpora lutea of the previous cycle.

As previously mentioned, females caged with males on Day 3 either super-ovulated (but ovulated fewer ova than isolated females) or failed to ovulate. The ovaries of the former animals on Day 2 of the next cycle had numerous atretic follicles, but newly formed and well-vascularized corpora lutea were also abundant (Pl. 1, Fig. 3). The corpora lutea of the previous cycle were involuting (Pl. 1, Fig. 3). The anovulatory ovaries were identical in histological appearance to those of Group 2 (vide supra).

**Table 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>N</th>
<th>Mean No. ova ovulated ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated from males</td>
<td>1</td>
<td>6</td>
<td>68.0 ± 3.54</td>
</tr>
<tr>
<td>Placed with males on Day 2</td>
<td>2</td>
<td>6</td>
<td>No ovulation</td>
</tr>
<tr>
<td>Placed with males on Day 3</td>
<td>3a</td>
<td>4</td>
<td>No ovulation</td>
</tr>
<tr>
<td></td>
<td>3b</td>
<td>4</td>
<td>35.5 ± 6.3</td>
</tr>
<tr>
<td>Placed with males on Day 4</td>
<td>4</td>
<td>6</td>
<td>61.8 ± 5.05</td>
</tr>
</tbody>
</table>

*All females injected with 60 i.u. PMS on Day 1.

Ovaries of animals placed with males on Day 4 (Group 4) were similar to those of isolated females in that numerous new corpora lutea were present by Day 2 of the next cycle. This was consistent with the recovery of numerous newly ovulated eggs from the oviduct (Table 1, Group 4). In addition, remnants of the vacuolated regressing corpora lutea of the previous cycle were still present.

**Behavioural oestrus in PMS-treated hamsters**

Twenty-four hours after females were caged with males on Day 2, vaginal smears revealed a consistent but unexpected pattern. Spermatozoa were recovered from the vaginas of all animals although the hostility of the females usually restricts mating to late Day 4 or early Day 1.

After the investigator noted spermatozoa in the vaginal smear of the first animal examined, the remaining animals of Group 2 were first tested for behavioural oestrus before being placed with males. An excellent lordosis response was elicited from all females by pudendal stimulation, but coitus was not observed over a period of several hours after females were introduced into the males’ cage. However, the next morning (Day 3), spermatozoa were present in every vaginal smear. Vaginal smears on Day 4 varied from animal
to animal with some containing abundant spermatozoa, whereas spermatozoa were lacking or sparsely distributed in others. This suggests that some PMS-treated animals copulated on more than 1 day. When females were examined on Day 1 of the next cycle (i.e. the day that spermatozoa are normally present), the vaginal smears contained variable numbers of spermatozoa, as on Day 1.

On Day 3, sexual receptivity was consistently shown by the PMS-treated hamsters before they were caged with males. Four females copulated repeatedly at 9 a.m. and abundant spermatozoa were recovered from vaginal smears taken a few minutes after coitus. These females constituted the group that failed to ovulate (Table 1, Group 3a). On the other hand, four other females placed with males on Day 3 did not copulate immediately and there were no spermatozoa in vaginal smears taken as late as 4 p.m. of Day 3. By the next day, however, spermatozoa were present in every smear. These females went on to ovulate variable numbers of ova at the next cycle (Table 1, Group 3b).

When PMS-treated hamsters were placed with males on Day 4, the females aggressively resisted all mating attempts, but by the next morning spermatozoa were recovered from the vaginas of all animals. All animals had superovulated when killed on Day 2 of the new cycle (Table 1, Group 4).

Toward the end of the experiments, the males of one cage failed to mate consistently, and on three occasions PMS-treated females placed with these animals on Day 2 did not have spermatozoa in the vaginal smear until Day 1 of the next cycle. When killed on Day 2 of the new cycle, the females had ovulated twenty-seven, forty-three and fifty-two eggs, respectively.

Two salient facts were revealed by the above experiments: firstly, behavioural oestrus was induced much earlier in the PMS-primed hamsters than in normal untreated animals. Secondly, the life span of the corpora lutea of the oestrous cycle was lengthened in animals mating on Day 2 or early on Day 3. Taken in conjunction, these results suggested that the relatively inactive corpora lutea of the oestrous cycle were transformed by coitus into functional corpora lutea and enough progesterone was consequently produced to inhibit ovulation on Day 4.

In order to test for the functional activity of the corpora lutea of animals mating on Days 2 or 3, the uterus was traumatized to see whether a decidual reaction could be elicited. Three PMS-treated animals placed with males on Day 2, were laparotomized on Day 4 after spermatozoa were noted in the vaginal smears. The cornua were opened along their antimesometrial borders with scissors. When the animals were killed 2 days later, massive deciduo-mata had been induced in all cornua. In all instances, the ovaries contained persisting corpora lutea of the previous cycle and numerous large atretic follicles.

The above experiment demonstrated that the corpora lutea were producing enough progesterone to induce a decidual response. The inhibition of ovulation as a result of early coitus could be attributed to high progesterone levels which suppressed the release of luteinizing hormone (LH) from the pituitary. If this explanation was correct, administration of exogenous LH should induce ovulation in PMS-treated animals mating by Day 3. Accordingly, four females were given 60 i.u. PMS on Day 1 and placed with males on Day 2. The next morning, spermatozoa were noted in every vaginal smear. On Day 4, three of the ham-
Fig. 1. Ovary of a PMS-treated animal that was placed with males on Day 2. Spermatozoa were found in the vaginal smear on the morning of Day 3. The ovary on Day 2 of the next cycle contains persisting corpora lutea of the oestrous cycle (A) and numerous atretic follicles (B). x25 (original magnification).

Fig. 2. Enlarged detail of Fig. 1. x100.

Fig. 3. Ovary of a PMS-treated animal that was caged with males on Day 3. Spermatozoa were found in the vaginal smear by Day 4. The ovary on Day 2 of the next cycle shows an involuting corpus luteum of the oestrous cycle (A), a newly formed corpus luteum (B) and an atretic vesicular follicle (C). x50.

Fig. 4. Ovary of an animal injected with PMS and 5 mg progesterone on Day 1 and then replaced with females. The ovary on Day 2 of the next cycle has numerous healthy vesicular follicles; ovulation has not occurred. Contrast the follicles with those of Figs. 1 and 2. x50.

(Facing p. 220)
Coital block to superovulation

sters were injected with 20 i.u. human chorionic gonadotrophin (HCG) with the remaining animal left untreated as a control. When killed the next day, the three animals injected with HCG had superovulated but the untreated animal had not ovulated.

It was of interest to determine whether progesterone administered to isolated PMS-treated hamsters could duplicate the effects of coital stimulus. Four females were therefore injected on Day 1 with 60 i.u. PMS and 5 mg progesterone. One of the animals was injected at 4 p.m. on Day 4 with 20 i.u. HCG while the others received no further treatment. When killed the next day, the animal injected with HCG had ovulated seventy-two eggs. In contrast, ovulation had not occurred by Day 1 in one of the remaining animals nor by Day 2 in the other two hamsters. A striking difference was apparent on Day 2 of the next cycle between the anovulatory ovaries of PMS-treated animals either receiving progesterone or following coitus. In the former group, large healthy follicles were maintained (Pl. 1, Fig. 4) whereas follicles were all undergoing atresia in the animals that had mated.

DISCUSSION

These experiments demonstrate that the injection of PMS induces oestrous behaviour in the hamster 2 or 3 days before its normal occurrence. When such females are isolated from males, sixty to seventy ova are ovulated on Day 4 (Greenwald, 1962a). However, similarly treated females caged with males on Day 2 permit copulation to take place, and ovulation is suppressed.

It has been clearly established in several species that oestrous behaviour results from a rise in progesterone levels after an initial priming period of oestrogenic activity (rat: Boling & Blandau, 1939; guinea-pig: Dempsey, Hertz & Young, 1936; mouse: Ring, 1944). In the ovariectomized hamster, a similar sequence of oestrogen–progesterone treatment is necessary to produce psychic oestrus (Frank & Fraps, 1945).

It is likely that precocious secretion of progesterone is responsible for the early onset of oestrus in the PMS-treated hamsters. There are at least three possible sources of progesterone in these animals: (1) the corpora lutea; (2) the combined effect of the normally developing follicles plus the ‘reserve’ follicles brought to maturity by PMS, or (3) the developing follicles alone. The latter possibility is suggested by the fact that the injection of PMS accelerates the development of the follicles so that they are the same size as follicles in untreated animals that are 1 day further along in the oestrous cycle (Greenwald, 1962a). The earlier enlargement of the developing follicles might conceivably be paralleled by the earlier synthesis of progesterone.

As a result of the premature onset of oestrus, the PMS-treated animals mated on Days 2 or 3 and the corpora lutea that ordinarily would have involuted instead persisted for a life span of at least 6 days. It thus appears that pseudo-pregnancy, resulting from early coitus, was responsible for the inhibition of ovulation. Two lines of evidence suggested that the corpora lutea were functional after coitus: (1) the ovaries of animals killed on Day 2 of the next cycle contained only nine or ten well-vascularized solid corpora lutea as well as
n numerous atretic follicles; (2) decidual ma were produced in hamsters whose cornua were traumatized on Day 4 after mating earlier in the cycle.

While all females placed with males on Day 2 mated and became pseudo-pregnant, only half the animals introduced on Day 3 were similarly affected. Evidently, Day 3 represents a transitional stage beyond which pseudopregnancy can no longer be induced. It is noteworthy that animals copulating at 9 a.m. on Day 3 did not ovulate in contrast to those mating between 4 p.m. on Day 3 and the next morning. This suggests that the corpus luteum of oestrus can still be activated on the morning of Day 3, but thereafter degenerative changes prevent it from becoming functional. Histological observations indicate that the corpus luteum at 9 a.m. on Day 3 is normal, but by 10 p.m. of the same day marked luteolysis has occurred (Greenwald, unpublished findings).

The regression of the corpus luteum on Day 3 coincides with a wave of follicular atresia that destroys approximately half of the vesicular follicles that have developed during the oestrous cycle (Greenwald, 1962b). It is possible that the two events are causally related.

The hamster ovulates spontaneously between 2 and 3 a.m. on Day 1 (Strauss, 1956). That pseudopregnancy results from mating as late as 9 a.m. on Day 3 indicates that 54 hr after ovulation the corpora lutea can still be transformed into a functional state. The corpora lutea of the rat also retain the capacity for full secretory function over a similarly prolonged period. Thus, after transplanting the rat pituitary to the kidney as late as the 3rd day of dioestrus, a pronounced decidual response can be produced in the uterus (Nikitovitch-Winer & Everett, 1958).

It has been well documented that the functional corpus luteum prevents ovulation by its secretion of progesterone, which in turn inhibits the release of LH from the pituitary (Shipley, 1962). The current study indicates that early coitus blocks ovulation in the PMS-treated hamsters by this mechanism. Ovulation was also suppressed in PMS-treated hamsters given a single dose of progesterone and then isolated from males. However, when HCG—predominantly a LH preparation—was injected on Day 4, ovulation ensued. The latter finding confirms a previous suggestion (Greenwald, 1962a) that a single injection of PMS brings numerous reserve follicles to maturity, but subsequent ovulation depends on endogenous gonadotrophins.

In recent years, numerous investigators have shown that pregnancy in the recently mated mouse can be disrupted by the presence of an ‘alien’ male or even by his odour (see survey by Parkes & Bruce, 1961). However, in the PMS-treated hamster, coitus must occur in order to block ovulation. Females that did not mate while exposed to males superovulated, but ovulated fewer ova than the optimal number of sixty to seventy eggs.

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REFERENCES


