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The Effect of Mating on the Afternoon of Proestrus on Serum LH (and FSH) and Ovulation in the Rat (38300)

CHARLES H. RODGERS AND NEENA B. SCHWARTZ

Biological Laboratories, Department of Psychiatry, University of Illinois School of Medicine, and Department of Physiology, Northwestern University Medical School, Chicago, Illinois 60612

Ovulation in spontaneous ovulators, by definition, usually occurs in the absence of coitus. However, mating has been shown to influence the number of ova ovulated, and the timing or the occurrence of ovulation in many spontaneously ovulating species (1–5). In view of such observations, recent attempts have been made to determine whether mating on the afternoon of proestrus increased serum LH (6, 7). Rodgers and Schwartz (6) were unable to demonstrate coitus-induced increases in serum LH (blood taken by cardiac puncture, CP) 30, 60, or 90 min post-coitus, whereas Moss and Cooper (7) reported increases. While the LH data may differ because of strain variability and/or procedural differences, it was clear that Rodgers and Schwartz (6) also failed to confirm earlier work showing reflexive increases in the number of ova ovulated in the cycling rat (3, 8). Moss and Cooper (7) did not investigate possible effects of coitus on ovulation.

The purpose of the present study was to further investigate possible relationships between coitus, serum gonadotrophin levels, and ovulation, controlling for method of blood sampling. The latter point was raised because of our recent demonstration that CP on the afternoon of proestrus in the pentobarbitalized rat could partially overcome the blockade of ovulation (9).

Materials and Methods. Females used in this experiment were Charles River CD Sprague–Dawley derived rats, exposed to 14L:10D (midnight is the mid-point of the dark period). Food and water were available *ad libitum*. Prior to use in the experiment the animals were required to show at least two consecutive 4-day estrous cycles.

One group of females was mated between 1700 and 1730 hr, another group between 1800 and 1830. One-half of each mated group was subjected to withdrawal of 1.3 ml of blood by cardiac puncture (CP) 30 min after cessation of mating. The remaining mated rats did not receive CP. An additional group of females was not mated, and one-half of these received CP at times equivalent to those in the mated groups.

Stimulus males were preselected for mating vigor. Coitus occurred in semi-circular observation arenas with Plexiglas fronts, each lighted by a 6-W bulb. The time the female was placed with the male was noted and behavior allowed to continue until five intromissions occurred. The female was then removed and the time again noted. Thirty min from the time she was removed the sample of whole blood was obtained under light ether anesthesia from one-half of the rats. Females not mated were placed in the observation arenas for 15 min without a male; the time of removal was noted, and CP carried out 30 min later.

Serum LH was measured in the samples taken by CP. Autopsy occurred on the morning of estrous between 0930 and 1030. The oviducts were removed and counts made of tubal ova as in previous studies (3, 6, 10). FSH and LH were measured in all animals from terminal blood samples. RIA was performed on serum LH (100λ samples) by using ovine:ovine assay with the NIH-LH-S16 standard. RIA of FSH was carried out using the NIAMDD kit (200λ samples) with the FSH-RPI standard.

Student's t tests were used for statistical comparisons ($P < 0.05$) of all variables.

Results. Animals mated at 1700–1730 receiving no CP showed significantly more ova than those mated at the same time, but receiving CP ($P < 0.05$), and those in the no CP–no mate group ($P < 0.05$) (Table I). No statistical dif-

TABLE I. Relationship between Mating and Cardiac Puncture (CP) on Serum Gonadotrophin Levels, and Number of Tubal ova on the Morning of Estrus.

			CP serum sample[a] (proestrus)	Terminal serum sample[b] (1000 hr estrus)	
Groups	*N*	Ova	LH (ng/ml)	LH (ng/ml)	FSH (ng/ml)
Nonmated					
No CP	9	12.8 ± 0.47[c]	—	3.4 ± 0.5[c]	235.6 ± 42.1[c]
CP	8	12.4 ± 0.50	58.6 ± 3.1[c]	2.1 ± 0.3	222.3 ± 22.2
Mated. 1700—1730					
No CP	6	15.2 ± 1.40	—	4.7 ± 1.3	267.3 ± 43.2
CP	6	12.3 ± 0.44	65.5 ± 5.1	2.6 ± 0.5	236.0 ± 40.1
Mated. 1800—1830					
No CP	6	13.3 ± 1.20	—	2.9 ± 0.4	231.4 ± 12.3
CP	6	13.0 ± 0.26	66.8 ± 4.4	2.6 ± 0.4	288.4 ± 42.7

[a] Sample taken by cardiac puncture – 30 min after removal from mating arena.
[b] Sample taken at autopsy.
[c] Mean ± standard error.

ferences were found between non-CP females mated at 1700–1730 and 1800–1830, owing to the large variance in each group, nor were differences demonstrated between the 1800–1830 mated group and the no mate groups (Table I).

No significant differences were noted in serum LH taken in the CP samples in the early evening of proestrus, whether mated or not mated, and all groups showed expected high proestrous values (6, 11). Terminal LH levels taken on the morning of estrus were significantly elevated in the no CP–no mate females in comparison to the CP–no mate animals ($P < 0.05$, two-tailed test).

Discussion. There are several findings from this investigation. (a) Coitus in the early evening of proestrus (1700–1730) caused more ova to be ovulated at estrus than in unmated animals, but the coitus effect occurred 1 hr earlier than noted in previous investigations (1800–1830) (3, 10). (b) Cardiac puncture in the late afternoon of proestrus decreased values of terminal serum LH in the non-mated groups. (c) Cardiac puncture inhibited the increase in ova ovulated in females mated at 1700–1730. (d) Mating in the late afternoon of proestrus failed to elevate significantly serum LH 30 min later, or terminal LH and FSH serum levels 15–16 hr later.

The coitus-induced increase in ova ovulated in rats mated at 1700–1730 confirms data from previous work using the Charles River Sprague–Dawley rats, although the time of induction shifted to an earlier time (3, 8, 10). The shift suggests an inherent variability in the substrain of rat used in coitus-induced ovulation studies.

In a recent study in which CP was used to obtain blood for LH measurements, mating between 1400 and 1900 hr failed to induce an increase in the number of ova ovulated (6). Data from the present study indicate that CP interfered with the coitus-induced increase in ova ovulated. However, in the presence of pentobarbital blockade, CP can cause ovulation by eventual LH and FSH release (9); the LH release is not seen at 30 or 60 min following CP, and was also not present 4 or 5 hr later (9, 11). In the absence of pentobarbital, CP does not reduce or enhance spontaneous ovulation (6; present study), nor does it alter serum LH levels within 30 or 60 min of collection during the proestrous surge (11). The noted differential effects of CP on reflexive ovulation, and on pentobarbital blockade of ovulation, is unexplained.

The present study confirms our previous findings that coitus during the time of normal mating does not increase serum levels within 30 min of mating, nor does it alter serum LH and FSH levels in the AM of estrus (6; Table I). Coitus in the human female has also not been found to increase gonadotrophin or steroid levels (12). These data in rat and human contradict those in the female rat found by Moss and Cooper (7) who noted significant elevations in serum LH within 30 min of mating. Obvious differences between the present study and the other (7) are in

the strains of rat used and the behavioral paradigms. Females in the Moss and Cooper (7) study were allowed to show lordosis in response to mounts by males for a 15 min period. If receptive, the females would be expected to have received a high number of intromissions providing the males were vigorous maters. In view of the increased genital-sensory area in estrogen-primed female rats (13), a high number of intromissions or mounts without intromission could increase serum LH. We have, however, measured serum LH in animals receiving between five and 21 intromissions without finding significantly elevated levels (6). Lastly, the serum LH values in both mated and unmated animals in the Moss–Cooper (7) study were lower (maximum in unmated rats about 12 ng/ml) than those usually observed on the afternoon of proestrus by other investigators. Thus, the potential for increases in LH subsequent to coitus may have been greater in their (7) study.

Finally, it has been shown that mating early on the morning of estrus, after ovulation has taken place, causes small but significant increases in serum LH, FSH, and prolactin (14–16). While these later hormone releases presumably have no influence on ovulation, they could be important for corpus luteum function in early pregnancy.

Summary. Coitus between 1700 and 1730 caused more ova to be ovulated than in unmated rats. It is clear that cardiac puncture (CP) 0.5 hr after mating inhibited the increases in the number of ova ovulated as a result of mating. Cardiac puncture also lowered terminal LH levels in the no mate group in comparison to the levels in the no mate–no CP group. Serum LH in the early evening of proestrus and terminal LH and FSH serum levels were not increased by mating. In earlier studies coitus between 1800 and 1830 increased the number of ova ovulated, while in the present study coitus was effective between 1700 and 1730. Cardiac puncture in combination with mating affects ovulation and LH levels differently than does CP subsequent to pentobarbital blockade of ovulation.

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