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Failure of Copulation to Affect Serum Prolactin, LH, and Estrogen Levels in Female Rhesus Monkeys[1] (39783)

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Introduction. Recently, coitus-induced hormonal release in spontaneously ovulating species has become the subject of renewed interest. In estrous rats postcoitum elevations in blood levels of LH, testosterone, and prolactin have been reported (1-4). Mating also resulted in serum LH and prolactin increases and induced ovulation in rats exposed to constant light (5). Some investigators suggested that the mere anticipation of mating could induce increases in plasma levels of LH, prolactin, and testosterone in male rats (4). Other reports, however, indicated no change in blood levels of prolactin, LH, and FSH after mating in males (6, 7). Conflicting reports have also appeared on mating-induced release of hormones in bulls (8, 9).

The effects of coitus on hormonal release in primates are also debatable. Some reports indicate no effect of coitus on serum levels of pituitary or gonadal hormones in men and women except for an inconsistent rise in serum prolactin in women (10-12), whereas other reports show significant elevations in testosterone levels in man during and after sexual intercourse (13) and some even suggest coitus-induced ovulations in women (14, 15). To our knowledge the effects of sexual stimulation on the release of pituitary hormones have not been investigated in rhesus monkeys, although one report indicates a two- to threefold increase in plasma testosterone levels in male rhesus monkeys during the season in which they frequently copulate (16).

The present studies were designed to determine the effects of copulation on serum levels of prolactin, LH, and estradiol-17β (E_2) in intact female rhesus monkeys on various days of the menstrual cycle. The effects of copulation were isolated from those of precopulatory behavior, anticipation of copulation, tactile stimuli, and the novel surroundings of the mating cage.

Materials and methods. animals. A total of 16 intact and 2 long-term ovariectomized rhesus females, weighing 5.7-8.3 kg each were used. Two 6-year-old intacts (described by symbols $\triangle$ and $\bigcirc$ in Figs. 1 and 2 and Table I) had no sexual experience. The remainder were feral born; they had at least one known sexual encounter during the past 2-6 years in captivity. All intact animals had recent menstrual cycles of 23-31 days each.

Experiment I. Eight intact females were used, each on three successive days. On Day 1 (Day 8-17 of the menstrual cycle) a female was transported from her home quarters to the copulation room down the hall and placed with a male in the mating cage. Number of mounts, intromissions, ejaculations, and behavioral events were recorded. Females refusing copulation with the first male were given a choice of one or two additional males. Each copulation session was terminated after a successful ejaculation (10-29.5 min) or after a maximum of 30 min, whichever occurred first. Blood samples (1.0 ml) were collected from the saphenous vein at the animals' home quarters, at 30 and 0 min before introduction into the cage, and at 10, 30, 60, 120, and 180 min postejaculation. On Day 2, the female spent 30 min alone in the mating cage and on Day 3, the female and the male that copulated on Day 1 were caged separately but within sight of each other for 30 min; the female occupied the mating cage. Bleeding protocols on Days 2 and 3 were the same as on Day 1.

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Experiment II. The remaining eight intact females were allowed copulation on Days 5, 12, and 22 of the menstrual cycle. Five of these copulated with the same male on each occasion but three preferred a different male on Days 12 and 22. The two ovariectomized females were injected with estradiol-17β (E_2) in oil (100 μg/day, ip) for 8 days, then permitted a single copulation on the ninth day. Blood samples were collected just before, and at 5, 15, 30, 45, and 60 min after, copulation.

General. Several precautions were taken in the experimental design to exclude intangibles. Cage cleaning and feeding routines were always in the early AM (17). To minimize the effects of diurnal fluctuations in hormone levels (17, 18), all experiments were performed between 10:00 AM and 12:30 PM each day. The control experiments (experiment I, Days 2 and 3) were conducted in the copulation room to prevent confounding of the effect of copulation with that of the novel surroundings and to determine the effect of the anticipation of sexual activity. Such a Pavlovian association was evident by the animals' searching behavior in the copulation room on Days 2 and 3. To minimize excitement in the copulation room, any male found unacceptable to a female was removed before violence ensued.

Blood samples were stored overnight at 5°; the following morning they were centrifuged at 1000*g* and the sera were stored at −20° until they were assayed for prolactin, LH, progesterone (P_4), and E_2 by radioimmunoassays (17, 19, 20). Two-way analysis of variance, the *t* test, and Duncan's multiple range test were used to determine differences in hormone concentrations between sampling times (21).

Results. Experiment I. Details of each copulation session are shown in Table I. Five of the eight females copulated. The number of total mounts (3 to 13), intromissions (1 to 5), and thrusts (6 to 17) preceding ejaculation varied during each of the five copulation sessions, which lasted 10–29.5 min each. Behavior patterns of the females also varied; some exhibited intense sexual interest and copulated with the first male introduced, whereas others were more selective and accepted copulation only after males had been changed once or twice. Three females refused copulation despite a total of three to five mounts by two or three persistent males.

None of the females that copulated showed an increase in serum prolactin (Fig. 1A). Individual and mean serum prolactin profiles on the day of copulation (Day 1) were similar to those on the following 2 days when the females were left in the copulation

TABLE I. SEXUAL BEHAVIOR OF RHESUS MONKEYS DURING COPULATION SESSIONS.

Female symbol[a]	Day of menstrual cycle	Total number of Males[b]	Mounts	Intromissions + thrusts	Ejaculations	Total duration of session (min)	Behavior pattern
△	17	1	13	3 + 6	1	29.5	Female extremely interested and inviting. Male slow to respond.
●	13	3	12	5 + 9	1	27.0	First male unresponsive; second male ejaculated outside; third male was successful.
×	8	2	3	1 + 8	1	11.0	Female and first male marginally interested. Successful copulation by second male in 1 min.
○	8	1	5	1 + 10	1	10.0	Female slow to respond initially.
■	9	2	6	1 + 17	1	28.0	Female unreceptive to first male.
▼	16	3	3	–	–	30.0	Female unreceptive and extremely frightened of the third male.
+	15	2	5	–	–	30.0	Female unreceptive.
◇	16	3	3	–	–	30.0	Female unreceptive.

[a] Each symbol in Table I and Figs. 1 and 2 represents the same female.
[b] Total number of males.

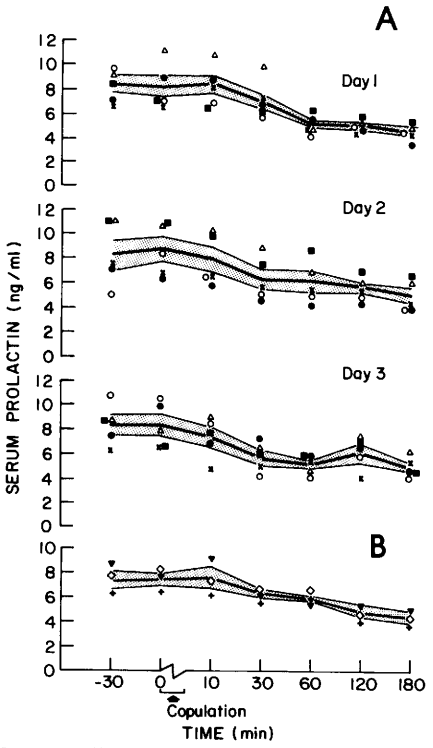

FIG. 1. Effects of copulation on serum prolactin in intact female rhesus monkeys. (A) Day 1, females were introduced into a mating cage at 0 min and removed after copulation (1–30 min); Days 2 and 3, females were allowed to spend 30 min in the mating cage, alone (Day 2) or in the presence of the male that copulated with her on Day 1 in a separate cage (Day 3). (B) Females that refused copulation with one to three males during a 30-min sojourn. Solid lines = means; shaded areas = SE of means; symbols = individual animals. Each symbol represents the same animal on each of 3 days in (A).

room alone (Day 2) or in the presence of a male in a separate cage (Day 3). On each of the 3 days mean serum prolactin levels showed a gradual decline which was maximum (≈38%, $P < 0.05$) at 180 min after copulation. A similar prolactin profile was observed in the females that refused copulation (Fig. 1B).

The effects of copulation on serum levels of E_2 and LH are shown in Fig. 2A. No noticeable change in E_2 was found in any of the five monkeys. Likewise, serum P_4 concentrations in various monkeys (0.5–4.0 ng/ml) remained unaffected by copulation (not illustrated in the figure). Two of three monkeys in which LH was measured showed an increase ($P < 0.05$) in serum LH levels at 10 and 30 min postcopulation when compared with precopulation levels. However, one of these two monkeys showed a similar increase in LH on Day 2 when she was left alone in the copulation room. The animals which refused copulation showed no change ($P > 0.05$) in serum levels of E_2 or LH (Fig. 2B).

Experiment II. Inconsistent LH responses were observed after copulation on Days 5, 12, or 22 of the menstrual cycle in eight females (Fig. 3). In one of them (No. 6) serum LH increased at various time intervals after copulation on each of the 3 days, but remained unchanged or showed insignificant ($P > 0.05$) fluctuations in the remaining animals. Copulation had no effect on serum LH concentrations in ovariectomized E_2-treated females (Fig. 3, lower graph).

Discussion. These results show that serum prolactin, E_2, and P_4 concentrations in individually caged female rhesus monkeys are not influenced by copulation. There was considerable variation in the preejaculatory activities: number of total mounts, intromissions, and thrusts; number of males needed for successful ejaculation; and the duration of copulation sessions. Yet none of these stimuli, whether or not followed by ejaculation, influenced serum levels of these hormones. Furthermore, the anticipation of copulation and sexual stimulation on Days 2 and 3 when the females were in the copulation room alone or in the presence of a male in a separate cage failed to have any effect on prolactin or E_2 and P_4 levels. These results are in agreement with studies in human subjects in which all the men and a majority of the women showed no effect of coitus on the release of prolactin (10, 12) or ovarian steroids (10). However, there are reports in both men and monkeys indicating high testosterone levels in males during periods of sexual activity (13, 16, 22). The postcoitum rise in serum prolactin seen in some women was perhaps due to breast stimulation which has been shown to be a strong prolactin-releasing stimulus (23). In the present studies no breast stimulation occurred, and effects of this stimulus on prolactin release in the monkey have not been reported.

These studies failed to provide convincing

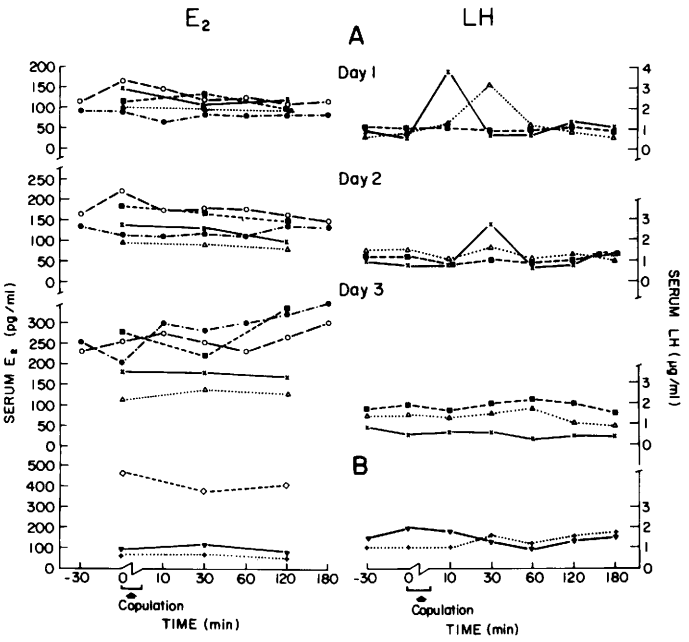

FIG. 2. Effects of copulation on serum estradiol-17β (E_2) and LH concentrations in intact female rhesus monkeys. See Fig. 1 for experimental protocols.

evidence of regularly induced changes in LH following copulation in caged female monkeys. The correlation between LH increase and copulation observed in two monkeys in experiment I became doubtful when one of these monkeys showed a similar rise in LH when she was left alone in the copulation room on Day 2. In experiment II, in which monkeys were mated repeatedly during various stages of the menstrual cycle, LH increased regularly in only one of eight monkeys indicating that the changing ratios of the sex steroids do not influence LH responses to coitus. This conclusion is borne out by the lack of an LH response to coitus in ovariectomized E_2-treated monkeys. Perhaps unknown factors surrounding coitus, other than the act per se, influence serum LH concentrations. Such a possibility has been discussed in an earlier report on men (24). These unknown stimuli may account for reports of ovulation following coitus in women (14, 15). Under normal circumstances, coitus does not affect blood levels of LH and FSH either in men or women (10, 11, 13) or in males and females of other species (6, 7, 9). In captive female rhesus monkeys also, prolactin, LH, and ovarian steroids are unresponsive to copulation, sexual stimulation, or anticipation of sexual activities.

Summary. Serum concentrations of prolactin, LH, and estradiol-17β (E_2) were measured on Day 1 (Day 8–17 of the menstrual cycle) at 30 and 0 min before and 10, 30, 60, 120, and 180 min after coitus in five intact female rhesus monkeys. Blood samples were also collected on a similar schedule on the following 2 days when the females were placed for 30 min in the mating cage alone (Day 2) or in the presence of a male in a separate cage (Day 3). None of the females showed an increase in serum prolactin or E_2 levels on any of the 3 days. Serum LH levels increased ($P < 0.05$) in two of the three monkeys in which they were monitored. However, of these two monkeys, one showed a similar LH increase when she was left alone in the mating cage. In three females which refused copulation, serum prolactin, LH, and E_2 levels remained unchanged.

The effect of copulation on serum LH was further investigated in eight additional females which were allowed to copulate on Days 5, 12, and 22 of the menstrual cycle

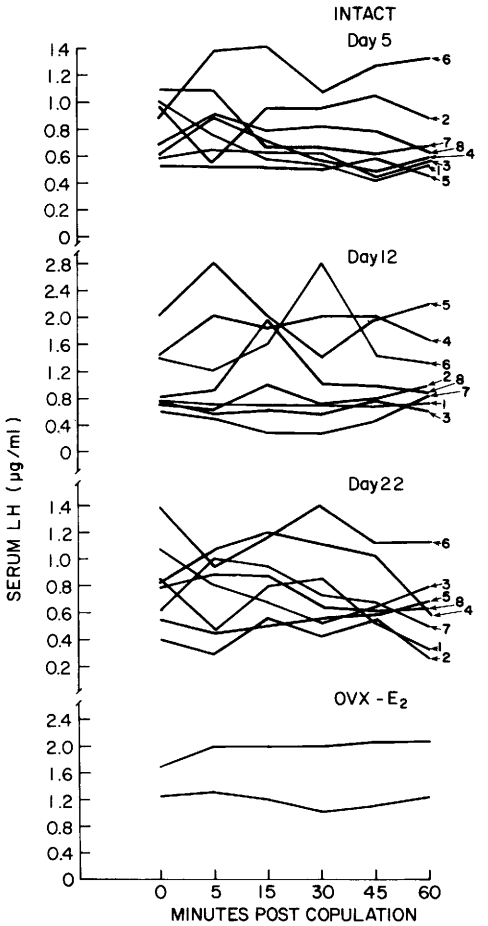

FIG. 3. Changes in serum LH concentration in eight intact rhesus females after copulation on Days 5, 12, and 22 of the menstrual cycle and in two ovariectomized females after an 8-day treatment with estradiol-17β (E_2). Each intact female is designated by the same Arabic numeral (1-8) on each of the 3 days.

and in two long-term ovariectomized E_2-treated females. Serum LH increased within 30 min after coitus in one female on each of the 3 days, but remained unchanged or showed inconsistent fluctuations in the remaining seven intact females and in the two ovariectomized E_2-treated females. We conclude that copulation per se does not influence serum prolactin, LH, or E_2 levels nor do changing ratios of circulating estrogen and progesterone before coitus account for occasional postcoital LH fluctuations in captive female rhesus monkeys after pairing with a sexually active male.

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