# On the Correlation Between Sexual Behavior and Ovarian Hormone Level During the Menstrual Cycle in Captive Japanese Monkeys

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ABSTRACT. The amount of estradiol and progesterone in the systemic blood plasma of six adult female Japanese monkeys was measured by radioimmunoassay. Data on heterosexual pairing tests was also collected and examined for correlation with these hormones. The relationship between ovarian hormones and frequency of ejaculation varied with each female. In nine normal menstrual cycles, the frequencies of invitation and approach by the female reached peaks on the day of the estradiol surge, after which they rapidly decreased. Frequencies of male behaviors including approach, leaving, invitation, and yawning significantly decreased during the luteal phase, as compared with those at the midcycle. Grooming by both sexes of the partner reached low points during the late follicular phase. The number of ejaculations per test increased significantly at the midcycle and reached a maximum a few days after the estradiol surge. The present results suggest that female proceptivity is highly correlated with the amount of estradiol, but her attractiveness is little correlated with estradiol and is lowered by progesterone.

#### INTRODUCTION

The sexual activity of both sexes clearly fluctuates during the menstrual cycle in the rhesus monkey (ROWELL, 1963; MICHAEL, HERBERT, & WELEGALLA, 1967; MICHAEL & ZUMPE, 1970; CZAJA & BIELERT, 1975), and in the pigtailed macaque (GOLDFOOT, 1971; BULLOCK, PARIS, & GOY, 1972). The fluctuation of sexual interaction during the menstrual cycle probably depends on changes in endocrine factors (MICHAEL & WELEGALLA, 1968). Many findings have accumulated concerning the hormonal regulation of sexual behavior after administration of various sex steroids to ovariectomized monkeys. For example, in the rhesus monkey (JOHNSON & PHOENIX, 1976; WALLEN & GOY, 1977), estrogens heighten female attractivity (i.e., the female stimulus value in evoking sexual responses by the male), female receptivity (i.e., female willingness to accept male mounting), and proceptivity (i.e., invitations by female). Progesterone, in combination with estradiol, enhances female proceptivity (BAUM, KEVERNE, EVERITT, HERBERT, & DE GREEF, 1977).

There have, however, been only a few works concerning the correlation of sexual interactions with ovarian hormones during the menstrual cycle. Although EATON and RESKO (1974) examined this problem in the pigtailed macaque, studies on other simian species have not been performed. Nevertheless, this problem must be considered with reference to the phylogenic and sociological position of the monkey species. The present work, therefore, aims to make clear the relationship of ovarian hormones and sexual interaction between the sexes of the Japanese monkey during the female menstrual cycle.

# MATERIALS AND METHODS

### SUBJECTS

Six adult intact female Japanese monkeys, weighing 6.2–9.2 kg, and two adult males weighing 10.5 and 11 kg, were used. All animals were individually housed in an air-conditioned room (25°C), with artificial lighting between 08:00 and 20:00. They were fed monkey chow (CMK-1, Japan CLEA, Tokyo) supplemented with sweet potatoes. Females regularly showed menstrual bleeding from August to January the following year, and the mean cycle was  $28.1 \pm 4.2$  (S.D.) days (N = 35), ranging from 21 to 40 days. Tests were performed during this period.

## **TESTING PROCEDURES**

Females were introduced into a pairing cage (1.5 m height, 2.2 m width and 1 m depth) and paired alternately with one of the two males either daily or at intervals of a few days. The behavior of the pair was observed and recorded at 15-sec intervals for 30 min, but if copulation continued even after 30 min had passed, the test was prolonged until the male finished ejaculating. In the latter case scores of the behavior were converted into values per 30 min. Behaviors recorded in the female were estrous vocalization, grooming, approach, and invitations consisting of presenting, beating-the-ground, head-ducking, and grasping. In the male the behaviors recorded were testing, grooming, approach, leaving, invitations consisting of hindquarters-display, turning, lip-smacking and muzzle-to-the-partner, yawning, ejaculation, and ejaculatory latency (time interval from the beginning of the test to the first ejaculation). These behaviors were scored according to the definitions of ENOMOTO (1974). Mounting rate (number of mountings per min) and thrust per mounting were also calculated.

## HORMONE ASSAYS

Systemic blood (2 ml) was collected between 12:00 and 13:00. It was collected daily around the expected time of ovulation, and every two or three days during other times of the menstrual cycle. The blood was centrifuged at 4°C. The blood plasma was recovered and stored at  $-20^{\circ}$ C until analysis. The amount of estradiol-17 $\beta$  and progesterone in each sample was determined by radioimmunoassay in duplicate using the method of MATSUMOTO et al. (1976) with some modifications. To separate the bound steroid from the free one, dextran-coated charcoal (0.5% charcoal and 0.05% dextran) was used. For estradiol assay, 0.8 ml of plasma was taken. The range of the standard curve was 16–500 pg. Water blank value was 14.8+6.5 (S.D.) pg (N = 12). Intraassay accuracy, measured by the addition of 500 pg of estradiol to the distilled water, was 524.3  $\pm$  29.2 (S.D.) pg (N = 6). Two hundred and sixty different samples ranging from 37.4 to 580 pg resulted in a within assay variation of  $\pm 19.9$  (S.D.) pg. For progesterone determination, 0.1 ml of blood plasma was taken. Water blank value was  $7.0 \pm 1.9$  (S.D.) pg (N = 12). Intraassay accuracy was measured by adding 500 pg of progesterone to the distilled water. A mean accuracy of 469.7  $\pm$  58.9 (S.D.) pg (N = 12) was obtained. Intraassay precision was estimated by determination of normal plasma pool (each 0.05 ml) and the coefficient of variation was determined as 12.4%  $[100.7 \pm 12.5 \text{ (S.D.) pg}; N = 6].$ 

#### Sexual Behavior and Ovarian Hormones

#### STATISTICAL ANALYSIS

The difference between values was evaluated with the Student's t test.

## RESULTS

Three types of ovarian hormone pattern were observed in females. The first type showed estradiol and progesterone peaks at different stages of the menstrual cycle, ejaculation being frequently observed throughout the cycle (Fig. 1a), around the midcycle and during the luteal phase (Fig. not shown), or around the midcycle and during the follicular phase (Fig. not shown). In the second type, showing only an estradiol peak, ejaculation was limited to the days around the estradiol surge (Fig. 1b). In the last type, showing neither an estradiol nor a progesterone peak, ejaculation was observed at a fairly high frequency throughout the menstrual cycle (Fig. not shown). Thus the relationship between ovarian hormones and the frequency of ejaculation varies with each female.

In those female monkeys who underwent normal menstrual cycles with estradiol

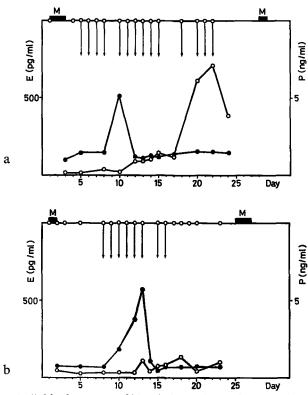


Fig. 1a & b. Two individual patterns of blood plasma level of ovarian hormone secretion and male ejaculation during the menstrual cycle. E and  $(\bigcirc - \bigcirc)$ : estradiol; P and  $(\bigcirc - \bigcirc)$ : progesterone. Open circles on the upper line show the day of test, and arrows mean ejaculation on the test day. Black bar M shows the menstrual bleeding.

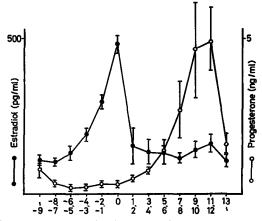


Fig. 2a. Representative pattern of ovarian hormones in the systemic blood plasma during the menstrual cycle. Each point represents the means  $\pm$ S.E.M. for five normal cycles.

and progesterone peaks, the secretion of both hormones fluctuated (Fig. 2a). The mean length of time from the first day of menstruation to the day of the estradiol surge and the mean length of time from the day of the estradiol surge to the day before the onset of next menstruation were  $13.1\pm2.6$  (S.D.) and  $17.4\pm2.1$  (S.D.) days, respectively. The mean length of time from the day of estradiol peak to the day of the maximum secretion of progesterone was  $10.5\pm2.2$  (S.D.) days.

Figure 2b shows the frequency of ejaculation per test during the menstrual cycle. The frequency of ejaculation rapidly increased during the follicular phase, reached a maximum one to two days after the estradiol peak, and then rapidly decreased until the fifth day after the estradiol peak.

Frequencies of several types of male sexual behaviors are shown in Figure 3a. Dur-

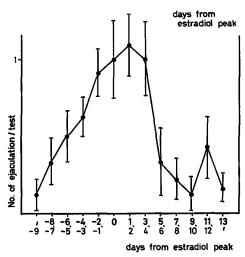


Fig. 2b. Frequency of ejaculation per test during the menstrual cycle. Each point represents the means  $\pm$ S.E.M. for nine normal cycles.

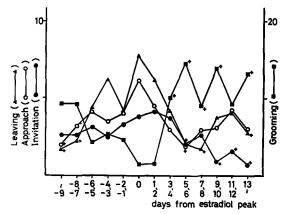


Fig. 3a. Frequencies of male behaviors including leaving, approach, invitation, and grooming during the menstrual cycle. Each point represents the means for nine normal cycles. +: p < 0.05 compared with value on day 0.

ing the menstrual cycle grooming by the male was most infrequently observed around the estradiol surge at the midcycle. In contrast to grooming, frequencies of approach and leaving increased during the follicular phase, reached dull peaks coincident with the estradiol peak, and then decreased during the luteal phase. The male intensively invites the female to initiate sexual interaction during the follicular phase and the midst of the cycle, followed by a significant decline of the behavior during the luteal phase.

Female sexual behaviors during the menstrual cycle are shown in Figure 3b. Grooming by the female reached a low point on the day of the estradiol surge. Approach and invitation towards the male were more often recorded on the day of the estradiol peak than during the other stages of the cycle. Vocalization was evoked during the luteal phase and around the midcycle rather than during the follicular phase.

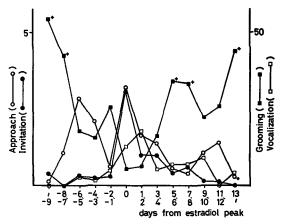


Fig. 3b. Frequencies of female behaviors including approach, invitation, grooming, and vocalization during the menstrual cycle. Each point represents the means for nine normal cycles. +: p < 0.05 compared with value on day 0.

	N-tests stage4)	E-tests stage	P-tests stage
Female behavior			
Grooming <sup>2)</sup>	37.2±6.85)	$18.9 \pm 2.2$	$22.2 \pm 7.1$
Approach <sup>1)</sup>	$3.0\pm$ .9	$3.7 \pm 1.1$	$1.3\pm$ .3
Invitation	$.3\pm$ .1	$1.0\pm$ .5	$.3\pm$ .2
Vocalization <sup>2)</sup>	$1.2\pm$ .7	$6.0 \pm 2.2$	$6.3 \pm 3.1$
Male behavior			
Testing	$1.0\pm.2$	.7±.0	.6± .2
Grooming <sup>1)</sup>	$7.3 \pm 2.1$	$4.2\pm$ .3	$11.5 \pm 3.3$
Approach <sup>1)2)</sup>	$3.2\pm$ .4	$4.3 \pm .1$	$3.0\pm$ .6
Leaving <sup>1)2)</sup>	$2.9\pm.4$	5.9±:.2	$3.1\pm$ .6
Invitation <sup>1)</sup>	3.0± .6	$3.8\pm$ .2	$1.8\pm$ .4
Yawning <sup>1)3)</sup>	$.8\pm$ .2	.8± .0	1.8± .4
ET1)2)	$.3\pm$ .1	$.8\pm$ .1	$.3\pm$ .1
MR	$1.7\pm$ .2	$1.8\pm$ .1	$1.8 \pm .2$
TM	$1.7\pm$ .3	$1.8\pm$ .1	$1.8\pm$ .1
EL	$18.9 \pm 3.4$	$24.0 \pm 2.4$	25.1±3.0

Table 1. Frequencies of sexual behaviors in each tests stage of the menstrual cycle

1) p < 0.05 between E-tests stage and P-tests stage; 2) p < 0.05 between N-tests stage and E-tests stage; 3) p < 0.05 between N-tests stage and P-tests stage. ET: number of ejaculation per test; MR: mounting rate; TM: number of pelvic thrust per mounting; EL: ejaculatory latency in minutes. 4) Details of classification of each tests stage are in the text. 5) Means  $\pm$ S.E.M. for nine normal cycles.

To examine hormone control of the sexual behaviors, the tests were grouped together, separately for the male and the female (Table 1), and the results compared for three stages of the menstrual cycle. The stages were: N-tests stage, from the first day of menstruation until day -5, during which the amounts of estradiol and progesterone were at their basal levels; the E-tests stage, from days -4 to 0, during which the secretion of estradiol rapidly increased to a maximum while, in contrast, the amount of progesterone remained at its basal level; the P-tests stage, from days +8 to +12, during which the amount of estradiol decreased to its basal level while, conversely, the amount of progesterone reached its peak value. Among the female behaviors only approach decreased significantly in the P-tests stage, in comparison with the level in the E-tests stage. Grooming by females was at a high frequency while vocalization by females was at a low frequency in the N-tests stage, compared to the frequencies in the E-tests stage. Both approach and leaving by the male was recorded at higher frequencies in the E-tests stage than in the N- and P-tests stages. The male showed invitations at a significantly higher frequency in the E-tests stage than in the P-tests stage. The male groomed the female at a significantly higher frequency in the P-tests stage than in the E-tests stage. Among indices showing copulation, only the ejaculation number per test in the E-tests stage was significantly higher than that in the N- and Ptests stages. The other indices did not change between the stages.

The female always responded to hand-on-back by the male, it was one of the most common behaviors by the male to initiate a mounting series, and fluctuation in responsiveness to this behavior was not observed in the menstrual cycle.

## DISCUSSION

There were peaks of the frequency of invitation and approach by the female towards the male which were associated with the estradiol surge. This finding supports the results of administration of the hormone to ovariectomized female monkeys (EVERITT & HERBERT, 1971; JOHNSON & PHOENIX, 1976; WALLEN & GOY, 1977) in which it was found that estradiol enhances female proceptivity. In contrast, EATON and RESKO (1974) did not report significant fluctuations of the female sexual behaviors during the menstrual cycle of the pigtailed macaque, although the hormonal pattern resembled that in the present study. This difference might be considered with reference to the higher plasticity in the social structure of the pigtailed macaque, as compared with that in the Japanese monkey.

The present study revealed that the frequency of invitation by females during the early follicular phase did not differ from that around the progesterone surge, while the estradiol levels during both periods were roughly equal to each other. This result might be contradictory to that of BAUM et al. (1976) who reported the enhancement of sexual invitation after administration of progesterone to the ovariectomized, estrogen-treated female rhesus monkey. This might be due to differences in androgen levels between follicular and luteal phases of the cycle as described by BAUM, EVERITT, HERBERT, and KEVERNE (1977), and this problem should be clarified in the future.

The peak of ejaculation per test appeared a few days after the estradiol peak in the systemic plasma was observed. This result indicates that the ejaculation per test did not depend simply on the amount of estradiol. Because ovulation takes place 46–52 hr after the estradiol peak in the rhesus (WEICK et al., 1973), and the optimal mating period for the rhesus is of an approximately 24 hr duration appearing immediately after the estradiol surge may have an adaptive value for efficient fertilization. This delayed ejaculation peak may be due to female attractivity rather than female proceptivity, because proceptivity showed a fairly sharp peak coincident with the estradiol surge, whereas the frequency of male invitation reached a dull plateau around the midcycle which resembled the pattern of ejaculation number per tert.

Acknowledgements. Special thanks are given to Dr. KIYOSHI OSHIMA, Primate Research Institute, Kyoto University, for his helpful criticism. We would also like to thank the staffs of the Division of Experimental Animals, Interdepartment Laboratory, Tokai University School of Medicine, for their careful maintenance of the monkeys.

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----Received November 2, 1978; Accepted March 15, 1979

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