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Coital Stimuli Controlling Luteinizing Hormone Secretion and Ovulation in the Female Ferret[1]

R. S. CARROLL,[2] M. S. ERSKINE, P. C. DOHERTY, L. A. LUNDELL, and M. J. BAUM

Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

ABSTRACT

A series of experiments focused on the masculine coital behaviors controlling pituitary luteinizing hormone (LH) secretion and reflex ovulation in the estrous female ferret. An initial experiment investigated which coital stimuli from the male are required to induce ovulation. It was found that corpus luteum formation, which served as an index of ovulation, occurred in estrous female ferrets only if the male achieved a penile intromission. Neck gripping, mounting, and pelvic thrusting behavior without intromission by the male failed to induce ovulation. A second experiment investigated the timing and magnitude of the coitus-induced LH surge associated with ovulation. Blood was obtained via jugular catheters from estrous females in various mating situations. Plasma LH concentrations were measured by a heterologous radioimmunoassay that was validated for use in the ferret. A significant surge in plasma LH occurred only when an intromission was achieved by the stud male. Plasma LH was significantly elevated 2.0 h after the introduction of the male, peak values were reached 6.0 h later, and this elevation lasted on average 5.7 hours (5/5 females). No LH rise occurred in 2/2 female ferrets in which only neck gripping, mounting, and pelvic thrusting, but no intromission, were allowed to occur. The ferret mating pattern and the resultant LH response differ from those seen in three other induced ovulators (cat, vole, and rabbit) in which the male's intromission latency and duration are much shorter than in the ferret, and in which a distinctive peak in plasma LH often occurs within 1 h after mating.

INTRODUCTION

Much variation exists among reflex ovulating species in the pattern of mating behavior that precedes an ovulatory surge in luteinizing hormone (LH) and in the timing of this LH peak and ovulation after mating. Although in most of these species (vole, rabbit, cat, and ferret) it has been presumed that penile intromission is necessary to induce ovulation, there have been very few studies on the actual effects of the various components of masculine copulatory behavior on coitus-induced LH secretion in females. Studies characterizing the coitus-induced plasma LH surge have been carried out for the vole (Charlton et al., 1975), rabbit (Dufy-Barbe et al., 1973; Goodman and Neill, 1976), and cat (Wildt et al., 1980; Johnson and Gay, 1981). In general, a 20–40-fold increase in plasma LH concentrations occurred within 30–60 min after coitus, and these levels reached peak values approximately 4 h later. The duration of the LH rise was shorter in the vole (2 h) than in the rabbit (5–6 h) and the cat (10–12 h). The LH response to coitus in the female ferret has not been characterized until now.

In the ferret the neural control of the seasonal reproductive cycle and the tonic secretion of gonadotropins is subject to synchrony by the photoperiod. Estrus occurs in response to lengthening photoperiod, and may last for up to 5 mo if copulation does not occur (Marshall, 1904). Mating begins with the male gripping the female on the dorsal surface of her neck. While the male maintains a neck grip, he mounts the female and after several minutes begins to display intermittent periods of pelvic thrusting, which terminate when an intromission is achieved. In one study (Baum and

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[2] Correspondence: R. S. Carroll, Boston University, Dept. of Biology, 2 Cummington St., Boston, MA 02215.

Schretlen, 1975) single intromissions varied from 2.5 to 151.3 min in duration. This paper addresses two related questions. First, which coital stimuli from the male are needed to induce ovulation in the estrous female ferret? Second, is there a coitus-induced plasma LH surge in females and, if so, which aspects of the male's sexual behavior cause this surge?

MATERIALS AND METHODS

Animals

Adult female Fitch ferrets (*Mustela furo*) in postpartum estrus were purchased from Marshall Research Animals (North Rose, NY). The ferrets were housed individually in rabbit cages with water available ad libitum. Purina Cat Chow was provided in bowls each morning. The lights in the ferret colony were off between 2200 and 0600 h.

Materials

Catheters for blood collection were comprised of an extrajugular portion (i.d. 0.040 mm × o.d. 0.070 mm, polyvinyl; approximately 8″ in length) and an intrajugular portion (i.d. 0.030 mm × o.d. 0.048 mm, polyethylene PE-60; approximately 6 cm in length). The catheters were constructed by using a hemostat to enlarge the end of the polyvinyl tube, into which the small polyethylene tube was then inserted. O-rings, used to secure the catheter (cut from #5 French intragastric feeding tube; Argyle), were placed 5 mm apart at the junction of the two tubes. All junctions were sealed with dichloromethane. Catheters were left to air dry for 24 h and then were gas sterilized.

Surgical Procedures

Animals were anesthetized using pentobarbital anesthesia (45 mg/kg), and sterile surgical procedures were used at all times. Females were ovariectomized via a single midline incision. Females were given jugular catheters using a procedure similar to that described by Florczyk and Schurig (1981). A small skin incision was made on the top of the head, between the ears, and the catheter was threaded subcutaneously to emerge at this site. The catheter was maintained patent by flushing it with 2 ml of heparinized saline (100 U/ml) every other day.

Luteinizing Hormone

A heterologous radioimmunoassay was used to measure ferret LH (Niswender et al., 1969; Donovan and terHaar, 1977; Ryan et al., 1983; Sisk and Desjardins, 1983). A rabbit antiserum (GDN 15) raised against ovine LH was used at a final concentration of 1:32,000. Highly purified ovine LH (LER-1056-C3) was radiolabeled with ^{125}I (New England Nuclear, Boston, MA) using a solid-phase oxidizing agent, 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (Iodogen, Pierce Chemical Co., Rockford, IL; Franker and Speck, 1978; Salacinski et al., 1981). Each assay tube contained 85 μl of 1% bovine serum albumin (BSA) in 0.05 M phosphate-buffered saline (PBS), 40 μl of plasma or standard (NIH-LH-S20) in PBS/1% BSA, and 50 μl of antiserum (1:32,000 in PBS/0.05 M EDTA with 1:500 normal rabbit serum; Antibodies Incorporated, Davis, CA). Tubes were incubated at 5°C for 24 h and then 25 μl of ^{125}I-labeled LH (diluted 1:50 in PBS/1% BSA) were added. Twenty-four hours later the precipitating antibody, 50 μl of sheep antirabbit gammaglobulin (Antibodies Incorporated; diluted 1:40 in PBS/1% BSA), was added.

After an additional 24 h at 5°C, the tubes were centrifuged (30 min at 4°C, 1006 × *g*), the unbound label in the supernatant was decanted, and the activity in the pellet was counted on a Micromedic gamma counter. A standard curve ranging from 0.02 to 10 ng LH (NIH-LH-S20) was used (Fig. 1). Increasing volumes of serum from ovariectomized ferrets and dilutions of homogenized ferret pituitaries yielded competition curves parallel to the ovine LH standard (Fig. 1). The pituitaries were first weighed and then homogenized in an equivalent volume of normal saline. Serial dilutions were taken from this stock. It was also found that a single intrajugular injection of 4 μg of gonadotropin-releasing hormone (GnRH; Beckman Instruments, Palo Alto, CA) caused a 3-fold increase in plasma LH, which peaked in about 15 min and returned to baseline after an additional 45 min (Fig. 2). The intraassay coefficient of variation was 8.8%. This value was based on 40 samples run in duplicate on two assays with LH values ranging from 0.5 to 4.5 ng/ml. All samples collected in this study were run in one of two LH assays, each of which included samples from females in the intromitted group and the nonintromitted group. The peak LH values for both groups were comparable in the two different assays. Concentrations of LH were calculated using the four-parameter logistic method (Rodbard and Hutt, 1974).

The levels of LH measured by RIA in the ferret were very low compared to those reported in other species. Therefore, increasing volumes of ferret plasma from mated estrous females (n=3) were run on a mouse Leydig cell bioassay (courtesy of Dr. Tony Plant) in order to determine whether the LH being measured using the ovine:antiovine RIA was physiologically active. Plasma samples containing basal levels of LH failed to stimulate testosterone secretion in the Leydig cell bioassay. However, increasing volumes of postmating samples, which contained peak levels of LH (approximately 4 ng/ml by RIA), caused dose-dependent increases in testosterone secretion that were parallel to those caused by the ovine LH standard.

Histology

Ovaries were removed from their capsules, immersed in Bouin's solution for 24 h and embedded in paraffin. Serial 30–μm sections were cut, mounted on gel-coated glass slides, and stained with hematoxylin-eosin prior to being examined for the presence of corpora lutea. The total number of corpora lutea per two ovaries is presented in the *Results*.

General Procedures

Experiment I. Fourteen estrous females were put into a test cage with a sexually experienced male. For one group of females (n=6), the male was allowed to neck grip, mount, and pelvic thrust. However, he was removed from the cage before penile intromission

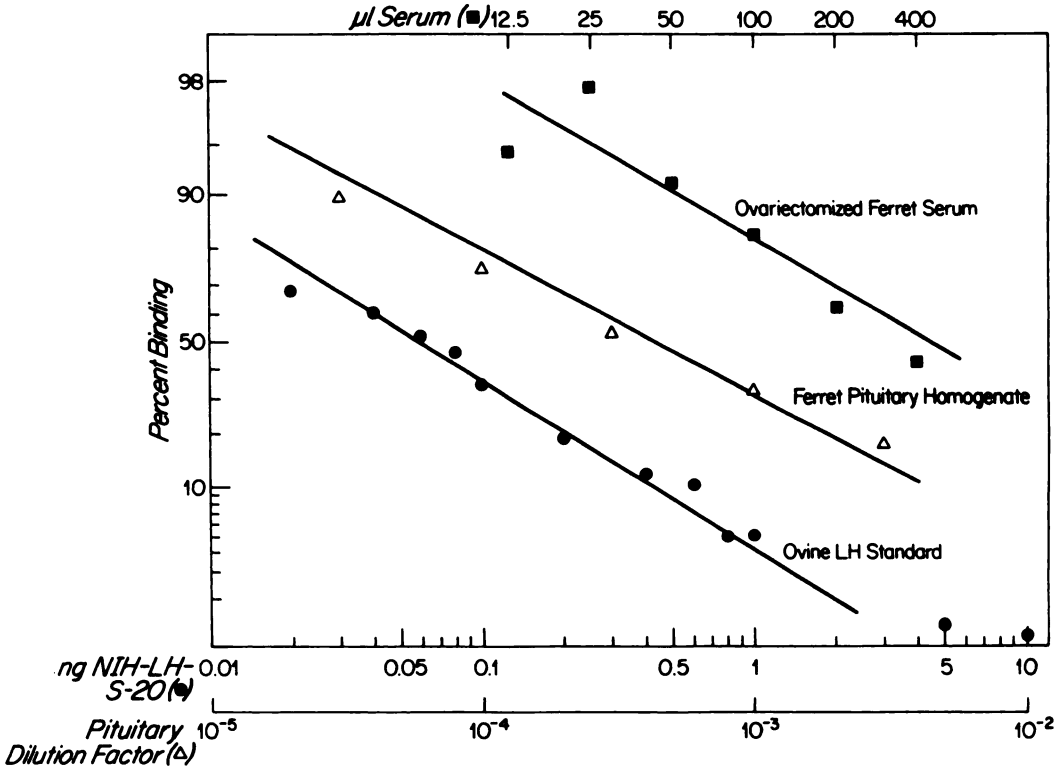


FIG. 1. Luteinizing hormone inhibition curves for ovine LH standard (m=−2.03), ferret pituitary homogenate (m=−1.98), and ovariectomized ferret serum (m=−2.47). (m=slope)

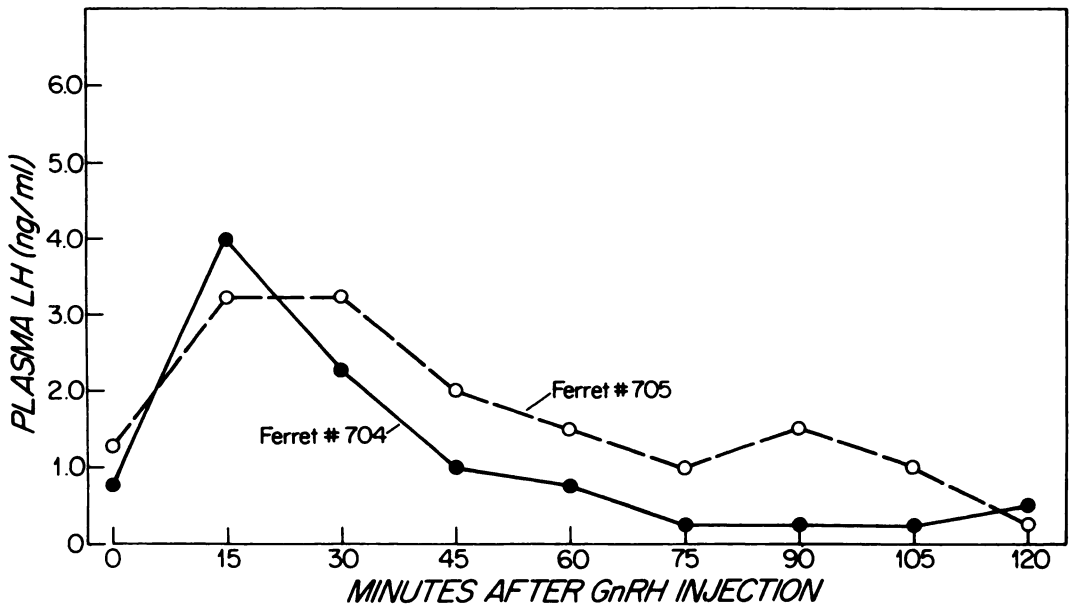


FIG. 2. Effect of intrajugular injection of 4 µg of GnRH on plasma LH levels in two ovariectomized female ferrets.

occurred. In the second group (n=8), all the male copulatory behaviors were allowed to occur, including an intromission. In some cases, the duration of the intromission received by various females was varied by removing the male from the test cage after different intervals. The durations of thrusting and intromission behavior were recorded during each test. One week after mating both ovaries were removed from all females and processed histologically.

Experiment II. Seven estrous females were removed from their home cages and an extension (2.5 ft) was attached to their intrajugular catheters to allow repeated blood sampling during testing. Each female was put into the test cage alone for 45 min, and baseline blood samples (0.7 ml) were taken every 15 min. A stud male was then put into the cage for two of three test situations and blood sampling continued every 15 min for 1 h. During this time an event recorder was used to record male and female sexual behaviors (duration of exposure to the male; neck gripping, mounting, pelvic thrusting, intromission; frequency of female bites; and duration of female receptive postures). Three different test situations were used: 1) the male was allowed to neck grip, mount, pelvic thrust, and intromit; 2) the male was allowed to neck grip, mount, and pelvic thrust, but no intromission was allowed; or 3) the female was placed in the test cage with no male present. At the end of 1 h (except if an intromission was still in progress), the male was removed from the test cage. Intromission was prevented in certain tests by using males that were known to have long intromission latencies.

For the subsequent 3 h samples were taken every half hour, and then for the next 12 h samples were taken every hour. After the samples were centrifuged and the plasma taken, the red blood cells were resuspended in heparinized normal saline and were returned to the female through the jugular catheter. One week after mating both ovaries were removed from all animals and were processed histologically.

Luteinizing hormone values for the five animals that received an intromission were analyzed by a one-way analysis of variance (ANOVA) for repeated measures. For each animal we computed mean LH values for pretest samples (n=4), preintromission samples (n=1–2), and samples (n=3–4) taken concurrently with intromission. These values were compared with the LH levels measured in individual samples taken 2.0–16 h after the introduction of the male. Post-hoc comparisons with preintromission values were made with Dunnett's test (Bruning and Kintz, 1977). For all females the area under the LH peak was calculated from the 60-min time point until the point at which the mean LH value was no longer significantly higher than the mean preintromission value (this occurred 12 h after introduction of the male).

RESULTS

Experiment I

Ovulation, as evidenced by the presence of corpora lutea in either ovary, only occurred when the male was allowed to intromit (Table 1). An intromission as short as 1 min was sufficient to induce ovulation. The other male behaviors that occurred prior to intromission were not by themselves sufficient to induce ovulation. Only large antral follicles were found in the ovaries of females that received no intromission. No evidence of luteinization was ever seen in these females. There was no correlation between intromission duration and the number of corpora lutea formed (r=−0.46; n.s.).

TABLE 1. Relationship between the occurrence of intromission and corpus luteum formation in estrous ferrets.

	Animal identity number	Time with male[a]	Duration of thrusting[a]	Duration of intromission[a]	Number of corpora lutea/2 ovaries
Ovulating females	1	29.4	7.9	15.0	11
	2	10.3	1.7	1.0	9
	3	49.0	4.3	39.7	6
	4	25.0	6.0	15.0	10
	5	94.0	4.8	86.8	8
	6	27.2	5.9	6.8	8
	7	44.3	8.5	2.5	10
	8	24.0	2.2	18.5	9
Nonovulating females	1A	5.0	1.3	–	0
	2A	21.0	1.6	–	0
	3A	7.0	0.3	–	0
	4A	20.6	2.4	–	0
	5A	25.0	0.4	–	0
	6A	25.0	0.2	–	0

[a]All times are given in minutes.

Experiment II

A surge in plasma LH was only observed in estrous females if the male achieved an intromission (compare Figs. 3 and 4). Fig. 3 shows the LH peaks (height and duration) for individual females that received an intromission; the occurrence of different male copulatory behaviors is also indicated. The mean (± SEM) pretest LH value was 0.66 ± 0.04 ng/ml; the preintromission value was 0.74 ± 0.08 ng/ml; and the LH concentration in samples taken concurrently with intromission was 1.4 ± 0.11 ng/ml. Plasma LH concentrations increased significantly over the course of testing ($F_{19,76}=5.21$, $P<0.01$). A significant ($P<0.05$) elevation over the preintromission value was first noted in samples collected 2.0 h after introduction of the male (2.35 ± 0.23 ng/ml), and peak LH values were found 6 h later (3.8 ± 0.80 ng/ml) (i.e., 8 h after introduction of the male). Plasma LH subsequently declined 10 h later (i.e., 12 h after introduction of the male) to a level (1.55 ± 0.21 ng/ml) that was not significantly different from the preintromission value.

As can be seen in Fig. 4, the behaviors that occurred prior to intromission were by themselves insufficient to induce an LH surge or even cause a significant increase in plasma LH levels. Fig. 4 also shows that frequent blood sampling with no male present had no effect on plasma LH. Table 2 shows the relative durations of different masculine copulatory behaviors, the number of corpora lutea formed in each female, and the area under each female's LH peak. There was no correlation between intromission duration and either the number of corpora lutea formed or the area under the LH peak ($r=-0.36$, n.s., and $r=-0.031$, n.s., respectively).

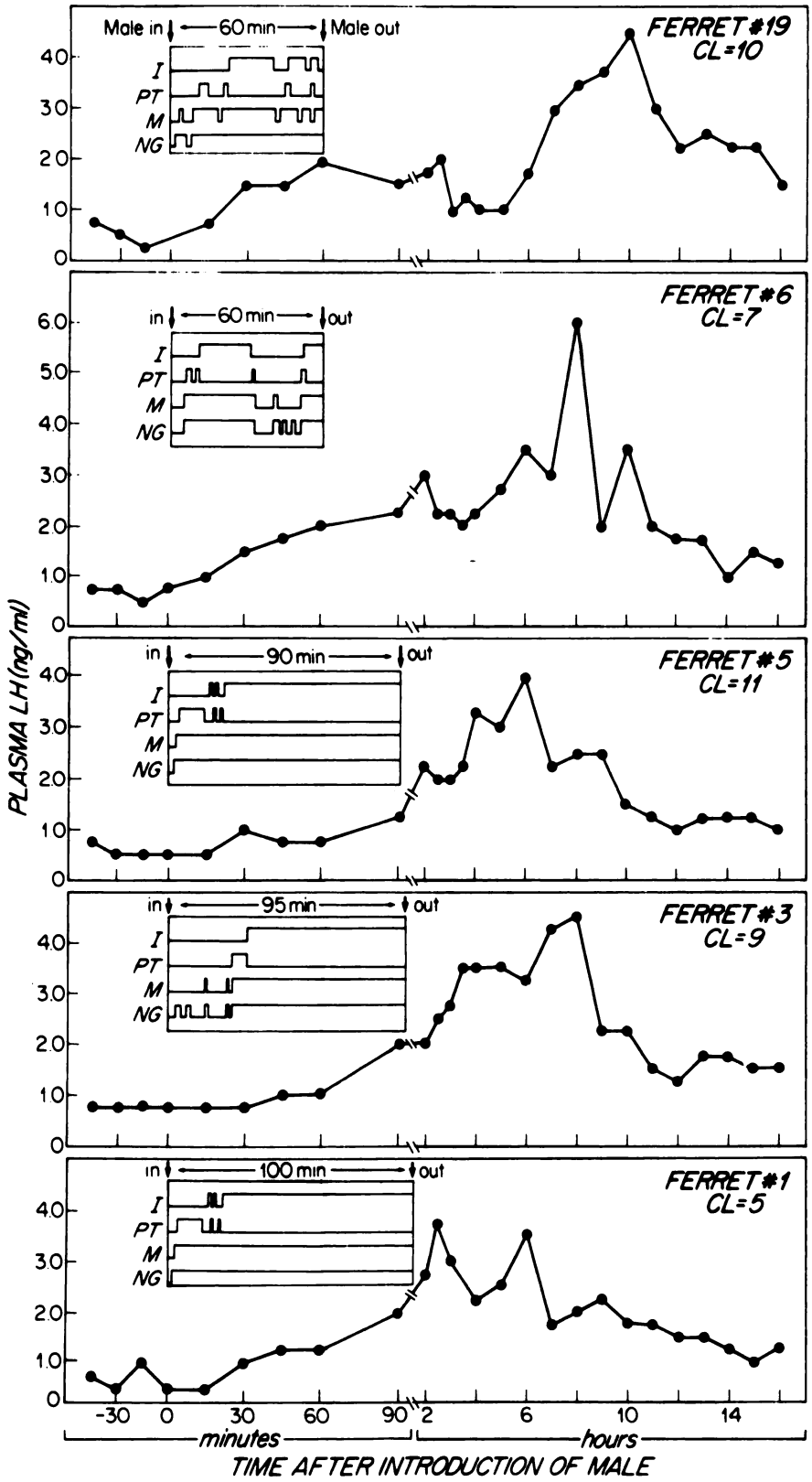
DISCUSSION

The present results show that the female ferret does exhibit a postcoital surge in plasma LH that results in ovulation, and that this occurs only after an intromission has been achieved by a male. The masculine coital behaviors that occurred prior to an intromission were by themselves not sufficient to cause a significant rise in plasma LH. The absence of an elevation in serum LH in one unmated estrous ferret and the minimal LH fluctuations in two other estrous ferrets that received neck grips, mounts, and pelvic thrusting without intromission suggest that the significant rise in LH measured in ferrets following intromission was the direct result of this stimulus, and not an artifact of the blood collection procedures used.

Ferrets differ from cats in that a single copulation, with an intromission, was sufficient to induce ovulation 100% of the time in both Experiments I and II. Ovaries from every ferret receiving an intromission had corpora lutea 1 wk after mating even though the intromission duration varied from 1 to 94 min. Corpora lutea were found in all ferrets that showed a plasma LH surge. We therefore infer that an LH rise is needed to induce ovulation. In the cat a single copulation induced ovulation in only about 50% of cases, whereas multiple copulations induced ovulation 100% of the time (Wildt et al., 1980). In cats, as with the ferrets of the present study, only those females that exhibited a definite rise in plasma LH were subsequently confirmed to have ovulated.

As in the cat (Wildt et al., 1980; Johnson and Gay, 1981), plasma LH concentrations in female ferrets increased following a single copulation. Neither the area nor the duration of the LH peak was related to the duration of the intromission or the number of corpora lutea formed. On the other hand, in the cat more prolonged coital stimulation from the male (multiple copulations), which induced an elevation in plasma LH lasting for 1 h or more, assured that ovulation would occur. In the ferret an intromission as short as 2.3 min was sufficient to induce a clear-cut rise in plasma LH that lasted for 6.0 h (Fig. 3).

The latency, duration, and magnitude of increases in plasma LH measured in ferrets postcoitally were different from those previously found in other reflex ovulators. In rabbits (Dufy-Barbe et al., 1973; Kanematsu et al., 1974), as in some queens (Concannon et al., 1980; Wildt et al., 1980; Johnson and Gay, 1981; Banks and Stabenfeldt, 1982; Schille et al., 1983), LH was elevated 20–40-fold within 10 min of copulation, reached a peak by 0.5–2 h, and was still elevated at 4 h. Johnson and Gay (1981) reported an even faster change in estrous cats following a single mating: plasma LH increased in 5 min, peaked in 20 min, and returned to baseline in 1 h. Only when their queens were allowed to remain with the male for 5–7 h or were permitted to mate at their own pace did LH surges of long duration occur. By contrast, in ferrets, a significant increase in plasma LH (5–9-fold) was not seen on the


Male in
60 min
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FERRET #19
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I
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CL=11
95 min
FERRET #3
CL=9
100 min
FERRET #1
CL=5
PLASMA LH (ng/ml)
-30
0
30
60
90
2
6
10
14
minutes
hours
TIME AFTER INTRODUCTION OF MALE

FIG. 3. Plasma LH levels in five estrous female ferrets that received an intromission from a stud male. The inserts record the occurrence and duration of the masculine copulatory behaviors displayed by individual males in tests with each female (*NG*=neck grip, *M*=mount, *PT*=pelvic thrusting, *I*=intromission).

average until 2 h after the onset of mating (about 1.5 h after intromission was achieved), and plasma LH did not return to preintromission levels until 12 h after introduction of the male. The occurrence of this delayed LH peak would not have been predicted by previous work on mated ferrets (Hill and Parkes, 1932), in which hypophysectomy performed as early as 1 h and 50 min after the "commencement of copulation" failed to inhibit ovulation. Perhaps a 3–4-fold rise in plasma LH is sufficient to induce ovulation in estrous ferrets.

The prolonged LH release that occurs in estrous ferrets after mating is presumably due to the continuing and perhaps episodic release of GnRH, in light of the relatively brief burst of LH secretion seen after a single i.v. injection of GnRH (Fig. 2). Donovan and Gledhill (1984) found that the half-life of ferret LH can be as short as 19 min (anestrous females) or as long as 2 h (ovariectomized females) following a GnRH injection and acute hypophysectomy. These workers were unable to measure the half-life of LH in estrous ferrets because very

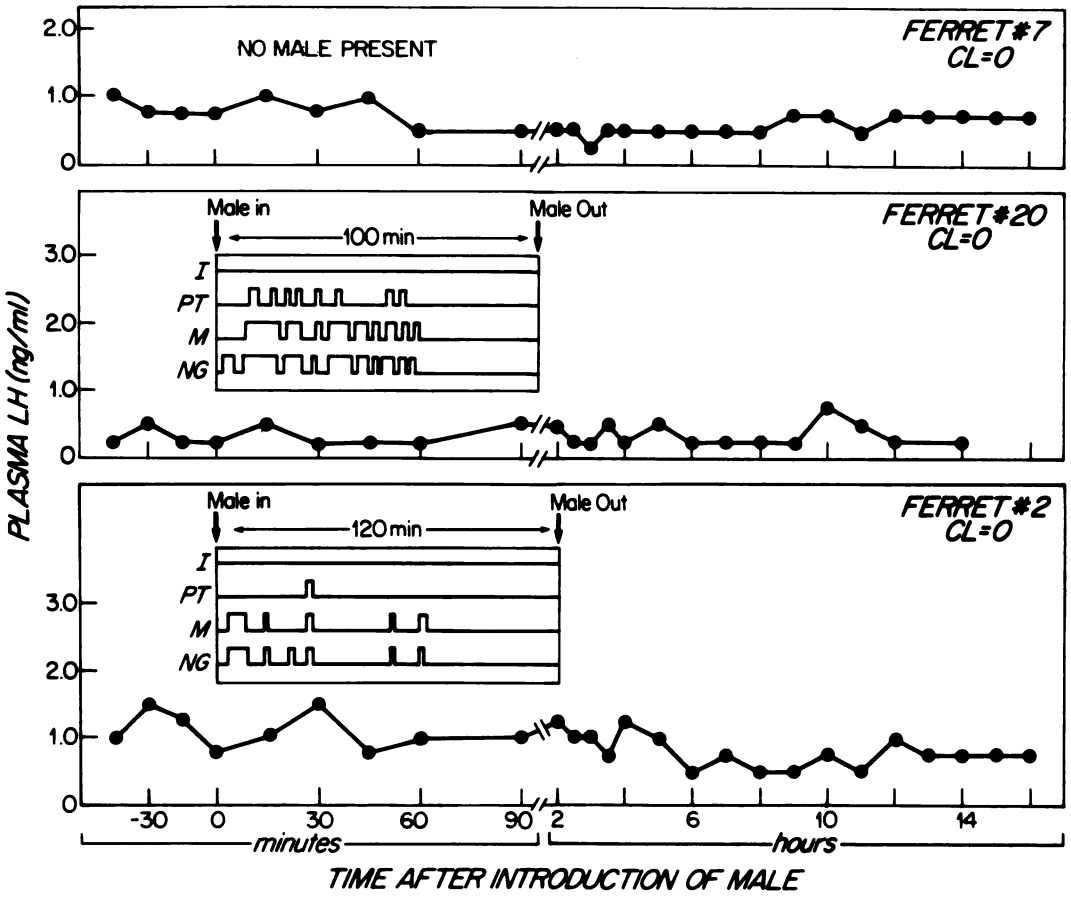

FIG. 4. Plasma LH levels in two estrous females that received neck gripping, mounting, and pelvic thrusting, but no intromission, from a stud male. Also shown are LH concentrations in plasma samples collected serially from one estrous female in the absence of a stud male.

TABLE 2. Relationship between the occurrence of different coital behaviors, an ovulatory surge of luteinizing hormone, and corpus luteum formation in estrous female ferrets.

Female number	Time with male[a]	Neck grip duration[a]	Mount duration[a]	Intromission latency[a]	Duration of thrusting[a]	Duration of intromission[a]	Number of corpora lutea/2 ovaries	Area under LH peak (mm^2)
1	100	72.4	71.2	15.8	7.56	46.2	5	910
3	95	89.1	88.3	31.8	3.23	81.4	9	1260
5	90	86.9	85.7	16.0	7.79	2.3	11	895
6	60	38.1	38.0	10.2	3.62	26.4	7	1245
19	60	58.3	53.0	11.0	4.7	33.4	10	973
2	120	71.6	40.9	—	0.79	—	0	0
20	60	28.0	20.5	—	7.5	—	0	0

[a]All times are given in minutes.

little LH was released after injection of GnRH. Therefore, even if circulating LH in estrous ferrets has a half-life as long as 2 h, one pulse of GnRH could not account for the long postcoital LH surges seen in the present study. In addition, GnRH infused continuously over 200 min, but not as a single injection, was able to induce ovulation in the estrous female ferrets studied by Donovan and terHaar (1977).

In the female cat, vole, and rabbit the potential exists for a rapid LH response to coital stimuli that was not seen in the ferret. This difference may be due in part to species differences in the estrogenic regulation of pituitary responsiveness to GnRH. In the cat there is evidence that estradiol primes the pituitary to respond to GnRH. A single injection of GnRH produced a much larger and more prolonged LH response in estrous than anestrous cats (Chakraborty et al., 1979). This is presumably due to the facilitatory effect of estradiol on LH release by the pituitary. In the rabbit estradiol benzoate priming alone has been shown to cause a significant increase in serum LH (Kanematsu et al., 1974). By contrast, in the vole Milligan (1978) found no apparent facilitatory effect of steroids on LH release, and in the ferret Donovan and terHaar (1977) found that GnRH injection caused a much larger release of LH and FSH in either anestrous or ovariectomized females than in estrous females. In that study the resting concentrations of LH were similar in anestrous and estrous females. Thus it seems that the sensitivity of the pituitary differs in these two endocrine states, presumably as a result of the direct action of estrogen on the pituitary gland.

Baum, Krey, and McEwen (unpublished results) obtained similar results in ovariectomized female ferrets given intrajugular injections of 4 μg of GnRH. Estradiol or a combination of estradiol and progesterone, but not progesterone alone, significantly decreased the ability of GnRH to induce LH secretion. The differential effect of estradiol on the pituitary responsiveness to GnRH existing in reflex ovulating species may account for the species differences in their LH responses to coital stimulation. Also, there is some evidence in the cat (Johnson and Gay, 1981) that GnRH itself increases the sensitivity of the pituitary to the subsequent action of GnRH (a self-priming effect). In the ferret the male's preintromission behaviors can last up to 30 min (Baum and Schretlen, 1975). By contrast, these behaviors

typically last only 5.5 min in the cat (Whalen, 1963), 6 min in the rabbit (Agmo and Kihlstrom, 1974), and 5 min in the vole (Gray and Dewsbury, 1974). Gonadotropin-releasing hormone or some other peptide, released into the hypophysial portal veins during the relatively prolonged preintromission courtship behaviors characteristic of the ferret, may be needed to prime the pituitary to respond to the pulses of GnRH that are presumably released following penile intromission in this species.

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