Ovarian and endocrine responses in the cat after coitus*

V. M. Shille†¶, Coralie Munro†, Susan Walker Farmer‡, H. Papkoff‡§ and G. H. Stabenfeldt†

†Department of Reproduction, School of Veterinary Medicine, University of California, Davis, California 95616; ‡Hormone Research Laboratory, and §Reproductive Endocrinology Center, University of California, San Francisco, California 93141, U.S.A.

Summary. LH release leading to ovulation was induced in 17 of 29 oestrous periods. The time of ovulation after coitus was determined by histological examination or by observation at laparotomy of ovaries *in situ*. Histological methods revealed that ovulation was complete in most follicles (9 of 13) at 32 h *post coitum* and in all follicles that were involved in the ovulatory process by 36 h. When laparotomy was used, no signs of preovulatory change were noted at the first observation time, 22 h *post coitum*, but in 4 cycles in which the entire process of ovulation was observed, the ovulatory process occurred between 23 and 28 h (3 follicles), 23 and 27 h (2 follicles), 25 and 28 h (3 follicles), and 25 and 29 h (3 follicles) *post coitum*. The first ovulatory process noted was complete at 25 h *post coitum*.

In cats, LH release continued over a 16-h period before returning to baseline (long surge), values being 616 ± 180 ng/ml at $\frac{1}{2}$ h and 941 ± 154 ng/ml at 2 h *post coitum*. In 6 cats the LH release pattern was limited to a 4-h period (short surge), values being 537 ± 218 ng/ml at $\frac{1}{2}$ h and 353 ± 245 ng/ml plasma at 2 h and basal (49 ± 18 ng/ml) by 4 h *post coitum*. Decreased secretion of oestrogen by follicles in animals undergoing ovulation was first observed at 16 h *post coitum*.

It is concluded that coitus induces LH release within minutes in the cat and that ovulation begins about 24 h later and finishes by about 32 h *post coitum*. Only one coital input can cause LH release for as long as 16–20 h although shorter periods of LH release (4 h or less) can result in ovulation.

Introduction

Mating or a comparable stimulus is needed to initiate the neural and endocrine sequences which result in ovulation in the domestic cat (*Felis catus*) (Longley, 1911), the jaguar (*Panthera onca*) (Wildt, Platz, Chakraborty & Seager, 1979) and the puma (*F. concolor*) (Bonney, Moore & Jones, 1981). The time and sequence of preovulatory events in the domestic cat have been described from observations of the ovary *post mortem* (Longley, 1910; Foster & Hisaw, 1935; Dawson & Friedgood, 1940) or from laparoscopy (Wildt & Seager, 1978) at timed intervals after coitus. Friedgood & Dawson (1940) described a degranulation of carmine cells in the anterior hypophysis of cats after coitus, and several authors have followed the changes of luteinizing hormone (LH) in plasma after coitus (Concannon, Hodgson & Lein, 1980; Shille & Stabenfeldt, 1980; Wildt, Seager & Chakraborty, 1980; Johnson & Gay, 1981b; Wildt, Chan, Seager & Chakraborty, 1981).

* Reprint requests to Professor G. H. Stabenfeldt, Department of Reproduction, School of Veterinary Medicine, University of California, Davis, California 95616, U.S.A.

¶ Present address: Department of Reproduction, College of Veterinary Medicine, University of Florida, Gainesville, Florida 32610, U.S.A.

This report presents a study designed to determine (1) the interval from coitus to ovulation in the cat and (2) the endocrine events that are associated with ovulation. Periovarial follicular changes are also described. Some of the data have been previously presented (Shille & Stabenfeldt, 1980).

Materials and Methods

Animals

Mature cats of mixed breeds were maintained under controlled light (14-h light : 10-h dark) and temperature (20–23°C). They were fed a standard commercial ration (Purina) and water *ad libitum*. The criteria used to determine changes in behaviour, including oestrus, and in the exfoliate vaginal epithelium have been previously described (Shille, Lundström & Stabenfeldt, 1979). Animals were allowed to undergo at least one oestrous cycle before the ovulatory stimulus on Day 3 or 4 of oestrus. The ovulatory stimulus consisted of coitus with vasectomized males which was limited to $\frac{1}{2}$ h; 1–4 coital inputs occurred within this time. Ovarian and endocrine responses to coitus in cats were as variable within animals as between animals, and therefore each oestrous period for animals used twice was reported as an independent observation. Overall hormonal responses were tested by one-way analysis of variance using Scheffé's (1959) method of multiple comparisons to detect significant differences amongst groups.

Determination of ovulation

In the first experiment, ovulation was determined in 14 cycles by gross observation and microscopic examination of ovulatory points on ovaries removed at predetermined intervals after coitus, i.e. at 28 h (3 cats), 32 h (4 cats), 36 h (4 cats) and 40 h (3 cats). Ovaries were prepared for histological examination by a modification of the method developed for the rabbit (Bjersing & Cajander, 1974) in which the ovary is freed with scissors without stretching or clamping the vessels in the ligamentum latum and immersed in 4% glutaraldehyde in 0.15 M-cacodylate. After 5 min, blocks of tissues containing ovulated follicles were sliced while the ovary was still in the fixative; slices stored in the fixative at 5°C were later processed and stained with haematoxylin–eosin. Criteria previously developed to determine the ovulatory endpoint in the rabbit (Markee & Hinsey, 1935) were adapted for the cat.

In a subsequent experiment the ovulatory process was observed *in situ*. Changes in follicle size, the number of ovulatory follicles and the time of ovulation were observed in 15 cycles with the aid of a $\times 3$ –60 zoom surgical microscope in laparotomized animals anaesthetized with ketamine HCl (Vetalar: Parke Davis). Ovaries were viewed at 30-min intervals starting at 22 h after the ovulatory stimulus and continuing until ovulation occurred or to 30 h if ovulation did not occur.

Determination of endocrine events

The pattern of endocrine change *post coitum* was determined by analysis of plasma for luteinizing hormone (LH), oestradiol-17 β and progesterone in all 29 cycles studied. Blood (3–6 ml) was taken from the jugular vein of unanaesthetized cats just before the ovulatory stimulus ($\frac{1}{2}$ h or less) which was designated the zero time and at intervals of $\frac{1}{2}$, 2, 4, 6, 8, 12, 16, 20 and 24 h after stimulation. The plasma was obtained immediately by centrifugation, split into two aliquots for protein and steroid hormone analysis, and frozen at –20°C until assayed. Animals had been conditioned to the bleeding restraint, which involved restraint in a canvas bag and box, for a period of several months before this study.

LH assay

Standard. LH was prepared from whole cat pituitaries removed within 1 h of death, quickly frozen at -70°C and stored at -20°C until extracted. Isolation and purification methods were similar to those previously employed for a variety of mammalian and non-mammalian LHs (Papkoff, Gospodarowicz, Candiotti & Li, 1965; Papkoff & Gann, 1970; Licht *et al.*, 1977). In brief, the pituitaries were homogenized in a Waring blender, the pH adjusted to 9.5 with CaO, and the homogenate extracted at 4°C for 3 h. The alkaline extract was separated from the insoluble residue by centrifugation, adjusted to 0.15 M-(NH_4) $_2\text{SO}_4$ and to pH 4 by the addition of freshly prepared HPO_3 . The resulting precipitate (containing GH) was removed by centrifugation and the glycoprotein hormones in the supernatant were heat treated (55°C , 2 min) to destroy proteolytic enzymes, and concentrated by extraction with ammonium acetate-ethanol. The dialysed and freeze-dried glycoprotein extract was then chromatographed on DEAE-cellulose equilibrated with 0.03 M- NH_4HCO_3 , pH 9. Of the 3 fractions obtained from this chromatographic procedure (see Licht *et al.*, 1977, for detail), LH activity was concentrated in the unabsorbed material. This fraction was freeze-dried and subsequently chromatographed on sulphoethyl Sephadex C-50 equilibrated with 0.03 M- NH_4HCO_3 . The LH was absorbed on this column and eluted with 1 M- NH_4HCO_3 . Final purification was achieved by gel filtration on Sephadex G-100 equilibrated with 0.05 M- NH_4HCO_3 . The LH eluted with a V_e/V_o of 1.6 and the yield was 1.6 mg (160 mg/kg).

Due to the small amount of LH obtained, limited characterization studies were performed. Examination of LH (F9B) by disc gel electrophoresis in 7.5% gel at pH 8.3 (Ornstein, 1964) revealed a single, but diffuse, band at an R_f of 0.1 which was similar to patterns obtained with the LH preparations from sheep, cows, pigs and horses. The absence of any other stained bands was taken to indicate a very low contamination with other pituitary hormones. The potency of F9B in terms of NIH-LH-S1 was 1.8 (1.3–2.6, 95% confidence interval) as assessed in the rat interstitial cell testosterone production assay (Dufau, Mendelson & Catt, 1974). This potency is comparable to those of a variety of other mammalian LHs tested in this same assay (Farmer, Suyama & Papkoff,

Table 1. Amino acid composition of sheep and cat LH

Amino acid	Sheep LH*	Cat LH†
Lysine	12	10.1
Histidine	6	2.9
Arginine	11	9.9
Aspartic acid	11	11.4
Threonine	16	13.4
Serine	14	13.1
Glutamic acid	14	15.2
Proline	27	28.0
Glycine	11	16.8
Alanine	15	20.9
Half-cystine	22	20.2
Valine	13	14.1
Methionine	7	4.7
Isoleucine	7	4.3
Leucine	14	15.0
Tyrosine	7	6.1
Phenylalanine	8	8.5

* Calculated from structural analysis, 215 residues (from Papkoff, Sairam, Farmer & Li, 1973).

† Amino acid analysis: 20 h hydrolysis, uncorrected for hydrolytic destruction, calculated on the basis of 215 residues per mol.

1977). An amino acid analysis was performed by the method of Spackman, Stein & Moore (1958) in an automatic amino acid analyser (Beckman Model 120B). The results are shown in Table 1 with the composition of ovine LH included for comparison. The only noteworthy difference between cat LH and the LHs of other animals was a reduced content of histidine and isoleucine in the cat LH.

Assay. LH was determined by a heterologous radioimmunoassay as described by Follett, Scanes & Cunningham (1972). Cat LH (F9B), stored as 3500 ng/200 μ l diluent, was used as the reference standard and iodinated ovine LH (LER-1056-C2) was used as the tracer because of the limited quantity of cat LH available. This was possible because displacement curves for the F9B standard were identical when obtained with 125 I-labelled cat LH or 125 I-labelled ovine LH as tracer (Text-fig. 1).

Text-fig. 1. Inhibition curves for cat LH (F9B), ovine LH (LER 1374A and NIH-LH-S18) and rat LH (RPI) using cat LH (F9B) or ovine LH (LER 1056-C2) for iodination and rabbit anti-ovine LH antiserum (GDN No. 15). The plasmas were obtained from cats after coitus-induced release of LH (Cat 5 = 147.5 ng/ml; Cat 26 = 88.8 ng/ml).

Highly purified ovine LH (LER-1056-C2, courtesy of Dr L. Reichert) and cat LH (F9B) were each dissolved in 0.5 M-phosphate buffer without saline, pH 7.5, at a concentration of 5 μ g in 20 μ l and iodinated with approximately 1 mCi 125 I (Amersham) in the presence of 20 μ g chloramine T in 10 μ l 0.5 M-phosphate buffer. Iodination was stopped after 30–60 sec by the addition of 30 μ g sodium metabisulphite in 25 μ l 0.5 M-phosphate buffer. Each mixture was immediately transferred to a previously prepared 10 ml Sephadex G75 column, and 0.5 ml fractions were eluted with 0.05 M-phosphate buffer, pH 7.5. The fraction containing iodinated LH was stored in 1.0 ml 3.0% bovine serum albumin and diluted to 10 000 c.p.m. in 50 μ l for use in the assay.

Rabbit anti-ovine-LH (No. 15, courtesy Dr G. D. Niswender) was diluted to 1:400 with distilled water for storage, and further diluted on the day of the assay to 1:40 000 with phosphate buffer (0.04 M, pH 7.4) containing 0.15 M-NaCl, 0.01 M-EDTA, 0.1% purified calfskin gelatin (Eastman Kodak) and 0.1% sodium azide (assay diluent). The antiserum (GDN No. 15) has been reported to be specific for LH (Niswender, Reichert, Midgley & Nalbandov, 1969) in a variety of species (Millar & Gehnelt, 1977).

Plasma samples (50 μ l), or different amounts of the cat LH standard (F9B), were added to the tubes and made up to a volume of 200 μ l in the assay diluent; 50 μ l LH antiserum (1:40 000 in assay diluent) and 50 μ l 1:200 normal rabbit serum in assay diluent were also added. After 24 h, 125 I-labelled LH (LER-1056-C2) was added to all tubes at a concentration of 10 000 c.p.m. per 50 μ l assay diluent. This was allowed to incubate overnight before 50 μ l of a 1:25 dilution of sheep anti-rabbit gamma globulin (Antisera for Science, New Rochelle, New York) was added which was

followed by another 24 h incubation period. The total assay volume was 350 μ l. Each plasma sample was assayed in triplicate and each concentration of the standard curve was done in quadruplicate. Samples with concentrations higher than 10.0 ng on the dose-response curve were re-run with a smaller plasma volume (10 μ l).

The quantity of iodinated ovine LH bound by the antibody used in the assay (GDN No. 15, dilution 1:40 000) ranged from 30.6 to 38% while non-specific binding was 2.3%. Under the conditions of the assay, the useful range of the standard curve extended from 0.1 to 10.0 ng cat LH/tube and the minimum detectable concentration of LH in cat plasma was 2.0 ng/ml. Using ratios between replicate determinations, the mean intra-assay coefficient of variation was 9.5% ($n = 21$). The interassay coefficient of variation for plasma samples containing 5–100 ng/ml was 11.1% ($n = 14$) and 100–1000 ng/ml was 16.7% ($n = 14$).

Parallel standard dose-response curves were obtained from the sheep and cat standards with 125 I-labelled sheep and cat LH (Text-fig. 1). Curves parallel to these standards were also observed for the LH preparations NIH-S18 and NIH-RP1; relative to these, cat LH had a potency of 0.39 (0.31–0.49, 95% confidence interval) and 105 (93–119), respectively. Inhibition curves parallel to the cat LH were obtained using serial dilutions of 2 different pools of cat plasma obtained *post coitum* (Text-fig. 1).

LH levels were determined, by radioimmunoassay and the rat interstitial cell assay, in pools of plasma obtained *post coitum* from 11 cats with LH concentrations ranging from about 200 to 600 ng/ml. Means of the values determined by the two methods did not differ significantly ($P = 0.45$) when tested by Student's *t* test for paired samples; the correlation coefficient was $r = 0.997$.

Steroid assays

Concentrations of plasma oestradiol and progesterone were determined by radioimmunoassay methods described and validated previously (Shille *et al.*, 1979; Shille & Stabenfeldt, 1979). The practical detection limits for these steroids were established as the mean + 2 $\times$ s.d. of the concentrations measured in the plasma of ovariectomized cats, i.e. 6 pg oestradiol/ml and 80 pg progesterone/ml. A coefficient of variation of 20% was found for an oestradiol plasma pool which contained 30 pg/ml ($n = 26$). The variation between duplicate samples containing < 16 or 20–35 pg oestradiol/ml in plasma was 13 and 9%, respectively. The interassay coefficient of variation was 16% ($n = 17$) for progesterone as measured in pooled plasma from an ovariectomized cat with a mean of 101 pg progesterone/ml. The within-assay variability was 12% for replicates in the range from 0.5 to 1.0 ng/ml, and 7% for 1–20 ng/ml.

Results

Ovulation

Ovulation was induced by coitus in 17 of 29 cycles after one (6 cycles), 2 (5 cycles), 3 (3 cycles) and 4 (3 cycles) coital inputs. In the first experiment, ovaries were removed from 14 cats at 28, 32, 36 and 40 h *post coitum*; 10 animals had ovulated and there was a total of 26 ovulated follicles (corpora haemorrhagica) on one or both ovaries. Serial sections of ovulated follicles showed that ovulation had been completed and organization of the corpus haemorrhagicum was in progress as early as 28 h *post coitum* in 1/3, 1/1 and 2/2 follicles (3 of 3 cats), at 32 h in 2/6, 2/2 and 5/5 follicles (3 of 4 cats), at 36 h in 6/6 follicles (2 of 4 cats) and at 40 h in 7/7 follicles (2 of 3 cats). Ovulated follicles had ovulation craters that were bounded by extruded mural theca and granulosa cells which formed a loose reticulum. The surface epithelium was undermined by eosinophilic homogeneous material suggesting the presence of oedema. Unovulated, mature follicles (3–4 mm diameter) that were grey, flattened and spherical in appearance were seen on ovaries that showed ovulatory points as well as in some ovaries from animals that did not ovulate.

In the second experiment, ovaries were observed *in situ* in 15 cycles every 30 min from 22 to 30 h *post coitum*; ovulation occurred in 7 cycles. No ovulatory points were present at 22 h. The first signs of the ovulatory process were observed at 23 h *post coitum* with the first ovulation complete at 25 h. The interval from the first coital stimulus to the end of ovulation ranged from 27 to 29 h. The entire process of ovulation was observed in 4 cycles (mature follicle to corpus haemorrhagicum) during 23–28 h (3 follicles), 23–27 h (2 follicles), 25–28 h (3 follicles) and 25–29 h (3 follicles) *post coitum*. Ovulation of up to 3 follicles was completed within a 3–5 h period in the 4 animals that were observed during the entire process. By 29 h, 21 of 21 mature follicles (7 cycles) that had undergone distinct structural change had ovulated. The number of coital inputs did not affect the interval from coitus to ovulation. In 8 cycles no ovulations were observed during the 8 h observation period; ovulatory failure was confirmed by the subsequent determination of plasma progesterone concentrations.

At the earliest observation time of ovaries *in situ* (22 h *post coitum*), mature follicles measured 3–6 mm at the greatest diameter and were dark grey in colour in contrast to the ivory-yellow of the surrounding ovarian stroma. Follicles that later ovulated could not be distinguished at this time from non-ovulatory follicles. Dilated branched capillaries surrounding a translucent pale area were seen in ovulatory follicles 2–3 h before ovulation (11 follicles, 4 cycles), but not in 6 other follicles on the same ovaries that were 3 mm in diameter and which subsequently failed to complete the ovulatory process. The first sign of approaching ovulation was the development of a cone shaped protuberance in the translucent area which became diffusely reddened. This occurred in <½ h (5 follicles, 2 cycles) or ½–1 h (6 follicles, 2 cycles) before ovulation when the domed apex gradually collapsed around the edge leaving a small depression in the centre. Delicate strands of fibrin occasionally adhered to the remaining tip of the dome. Extrusion of the cumulus was not observed. Mature follicles that failed to ovulate in response to coitus (13 follicles, 8 cycles) did not change in size or appearance during the period of observation. Immature follicles (<2 mm diam.) were observed on most of the ovaries at all times.

LH secretion patterns

The mean $\pm$ s.d. plasma levels of LH determined before coital stimulation in the 29 cats were 19.9 ± 6.6 ng/ml ($n = 121$). The level of discrimination for LH was established as the mean $\pm$ s.d. or 30 ng/ml. Surges of LH were induced in 18 of 29 cycles after coitus; ovulation occurred in 17 of these animals. Two types of LH release occurred in the responding animals (Text-fig. 2). In the first type (long surge), mean plasma LH concentrations in 11 cycles rose to 616 ± 180 ng/ml at ½ h and to 941 ± 154 ng/ml at 2 h *post coitum*, with a return to baseline not occurring until 16–20 h *post coitum*. Six animals had a second type of LH release, i.e. short surge, with values of 537 ± 218 ng/ml at ½ h and 353 ± 245 ng/ml plasma at 2 h *post coitum*. At 4 h *post coitum* LH concentrations in all 6 cats declined to near baseline with an average of 49 ± 18 ng/ml. One cat which failed to ovulate showed sporadic peaks of LH that reached 224, 157 and 287 ng/ml at 4, 6 and 12 h *post coitum*, respectively.

Coitus failed to initiate an LH surge in 11 cycles. Plasma LH levels were not elevated above the 95% confidence interval calculated for baseline concentrations at any time during the observation periods. The average number of coital stimuli for cats with a long, short or no LH surge was 2.0, 2.25 and 2.5, respectively.

The LH secretion pattern of one cat is shown in Text-fig. 3(a). Cat 22 had a long LH surge after two coital inputs with LH concentrations reaching 290, 616, 424 and 367 ng/ml at ½, 2, 4 and 6 h after coitus, respectively, and remaining above 100 ng/ml at 8 and 12 h with a decline to 22 ng/ml plasma at 16 h *post coitum*. Text-figure 3(b) depicts a short LH surge observed in Cat 46 after one coital stimulus. LH rose to 1960 ng/ml at ½ h, declining to 200 ng/ml at 2 h and to 27 ng/ml by 4 h *post coitum*. An apparent biphasic release of LH occurred in Cat 40 (Text-fig. 3c) in which the first LH peak was at ½ h (630 ng/ml) and the second at 6 h (595 ng/ml) *post coitum*; the animal subsequently ovulated. An example of failure to release LH following coitus is shown in Text-fig. 3(d).

Text-fig. 2. Mean $\pm$ s.e.m. plasma LH concentrations in cats that failed to release LH ($\circ$ --- $\circ$, $n = 5$), or released LH over a 4-h period ($\bullet$ — $\bullet$, $n = 6$) or a 16-h period ($\blacktriangle$ — $\blacktriangle$, $n = 11$). The zero time was the first coital input (subsequent coital inputs varied from 0–3 within a 30-min interval). The vertical bars represent s.e.m.

Text-fig. 3. Plasma hormone concentrations in cats after coitus (zero time represents the first coital input). (a) Example of a long-duration surge of LH after 2 intromissions; (b) example of a short-duration surge of LH after 1 intromission; (c) a biphasic LH response after 4 intromissions; and (d) no LH release in spite of 3 intromissions.

Ovarian steroids

Oestradiol-17 β levels in plasma of individual cats (Text-fig. 3) generally declined during the period of observation. Cats that ovulated after coitus showed a decline in oestrogen levels between the 6th and 8th h with the first significant decline ($P < 0.05$) observed at 16 h *post coitum* (Table 2). These changes occurred regardless of the duration of the LH surge. Significance of responses was tested by Sheffé's (1959) method of multiple comparisons in which oestrogen values were pooled for the first four sampling periods (coitus through 6th h) and compared with each subsequent post-coital sampling period.

Table 2. Oestradiol-17 β plasma concentrations in cats before and after coitus

Time	Oestradiol-17 β (pg/ml)
- 24 h	41.1 $\pm$ 3.9 (16)
Coitus ($\pm \frac{1}{2}$ h)	44.1 $\pm$ 6.2 (14)
+ 2 h	53.4 $\pm$ 6.2 (12)
+ 4 h	52.1 $\pm$ 6.8 (13)
+ 6 h	51.1 $\pm$ 4.9 (14)
+ 8 h	44.2 $\pm$ 5.0 (12)
+ 12 h	40.3 $\pm$ 3.7 (15)
+ 16 h	32.3 $\pm$ 2.6* (15)
+ 20 h	27.5 $\pm$ 2.5* (13)
+ 24 h	26.2 $\pm$ 1.9* (14)

Values are mean $\pm$ s.e.m. for the no. of samples indicated in parentheses.

* Significantly different from values obtained at coitus and through the first 6 h, $P < 0.05$ (see text for explanation of statistical method).

Plasma progesterone levels remained at baseline concentrations of 123 ± 62.4 pg/ml ($n = 24$) during the 24-h period after the ovulatory stimulus. The initial increase in progesterone was detected at 48 or 72 h following a successful ovulatory stimulus.

Coital behaviour

Coital inputs were limited to the number which could be accomplished by the males within $\frac{1}{2}$ h and ranged from 1 to 4, with one mating occurring in 10 cycles, 2 in 8 cycles, 3 in 6 cycles and 4 in 5 cycles.

Males mounted oestrous females for an average of 2 min before intromission (2.0 ± 1.5 , $n = 29$, range 0.5 to 7 min). Intervals between intromissions ranged from 1 to 60 sec with a mean of 12.3 ± 10.6 sec ($n = 29$).

Discussion

The interval between coital stimulation and ovulation has been reported to be variable in the cat, ranging from 25 to 50 h as determined by observations of ovaries removed from cats at intervals after coitus (Longley, 1911; Courier & Gros, 1933; Dawson & Friedgood, 1940). However, in many cases, the data presented do not preclude an earlier occurrence of ovulation. Wildt *et al.* (1981) used laparoscopy at 48-h intervals to determine the time of ovulation in response to coital stimuli (3 at 4-h intervals) which were repeated daily as long as the females were in oestrus; ovulation occurred in 5 of 12 cycles (6 cats) at the first observation period (48 h) with ovulation confirmed

in the other 7 cycles at the second observation period (at 96 h). It was concluded that ovulation occurs between 44 and 60 h following the first induced LH peak (48–64 h *post coitum*).

In the present study ovulation occurred as early as 25 h *post coitum* after coitus-induced LH release, with most follicles having ovulated by 32 h *post coitum*. We previously have found a variable response of LH release to coital stimulation with some cats not releasing an ovulatory amount of LH until the 2nd, 3rd or even 4th day of oestrus (Banks & Stabenfeldt, 1982). It is possible that in the study by Wildt *et al.* (1981) the ovulatory release of LH occurred after the first day of coital stimulation, although the composite LH results indicate that most animals had significant release of LH on the first day of oestrus. It is more likely that the time of observation of ovaries *post coitum* is the main cause for the difference reported for the coitus to ovulation interval.

An interval to ovulation of 30–36 h after the onset of oestrus has been reported for a number of spontaneously ovulating species. Quirke, Hanrahan & Gosling (1981) observed ovulation in sheep as early as 22 h after onset of oestrus with 34/35 animals ovulating by 32 h. Also, Parsons, Hunter & Rayner (1967) reported average ovulation times of 26–30 h in sheep. Signoret, du Mesnil du Buisson & Mauléon (1972) observed ovulation between 34.1 and 35.0 h after the onset of oestrus in mated pigs. Marion, Smith, Wiley & Barrett (1950) reported the interval to ovulation in mated cattle from the onset of oestrus to be about 26 h. Because the onset of oestrus and the initiation of the ovulatory release of LH occur at about the same time in these species, the LH release to ovulation interval in these species is comparable to that which we have observed for the cat. In addition, an interval of 32 h from the LH rise to ovulation has been reported for women (W.H.O., 1980).

The mode of preovulatory LH release in spontaneously ovulating animals has been shown to be one of increased frequency of pulsatile release. Oestrogen, in increasing concentration over a period of time (hours), is required for this phenomenon through the sensitization of LH-RH neurones in the hypothalamus (Neill, Patton, Dailey, Tsou & Tinedall, 1977) and/or gonadotrophs of the anterior pituitary (Nakia, Plant, Hess, Keogh & Knobil, 1978). In contrast, LH release in the cat appears to be continuous after coitus (Johnson & Gay, 1981b), although pulsatile at other times of the oestrous cycle (Johnson & Gay, 1981a).

Johnson & Gay (1981b) observed a refractoriness of LH release which began about 90 min after the initial coital input regardless of the number of coital inputs. This agrees with our findings of peak concentrations of LH at 2 h although coital inputs were limited to a maximum of four which occurred within the first 30 min of contact. Wildt *et al.* (1981), however, found that LH release could be enhanced when coital inputs were repeated at 4-h intervals. This suggests that the refractory period may be relatively short in cats.

Coitus appears to influence the dynamics of LH release in spontaneously ovulating animals as well as in induced ovulators. Marion *et al.* (1950) found that ovulation occurred sooner in cows after the onset of oestrus if mating was allowed (28.8 h compared with 32.1 h in unmated controls). In sows mating decreased the interval from the onset of oestrus to the start and finish of ovulation (34.1 and 38.0 h, respectively) as compared to unmated controls (35.0 and 41.8 h, respectively) (Signoret *et al.*, 1972). These results suggest that coitus in the cow and sow enhances and/or accelerates the preovulatory release of LH. It has been shown in the rat that coitus does not affect the initial preovulatory surge of LH, but does extend the duration of LH release (Moss, Dudley & Schwartz, 1977).

The concentrations of LH reported in this study are similar to those reported by Johnson & Gay (1981a, b) who used the NIAMDD RP-1 rat standard whereas we used a cat LH reference standard. These concentrations are about 10-fold higher than those reported by Concannon *et al.* (1980) who used an ovine LH reference standard. When we have used ovine LH as both standard and tracer for cat LH determinations (Banks & Stabenfeldt, 1982), the LH values are about 10% of those reported in the present study. It is likely that LH values reported for some animals will change as species-specific LH becomes available for reference purposes. The use of an homologous standard as in the current study is desirable for the development of LH data for a particular species.

It is unclear why some cats in this experiment had relatively short LH surges after coital

stimulation (6/18). The duration of the LH surge was not affected by the number of preovulatory follicles present, the duration of mounting, the number of intromissions, or the use of a particular male. Some of the animals in our study released large amounts of LH in response to only 1 coital input. Thus one coital stimulus requiring only a few seconds can elicit a relatively sustained release of LH in the cat. It is also apparent that LH surges which lasted 2–4 h were sufficient to produce ovulation. The amount of LH required per unit of time to cause ovulation in the cat is about 6 times greater than the minimal ovulatory requirement (Banks & Stabenfeldt, 1982), and it is obvious that the cat does not require a prolonged release of LH as a prerequisite for ovulation.

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