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# Luteal Activity in the Pseudopregnant Cat

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## ABSTRACT

Ovulation was induced in 5 cats by copulation with a vasectomized male and corpus luteum (CL) activity was studied by the determination of peripheral plasma progestins. Luteal activity averaged 36.5 ± 1.1 days (m ± SEM, n = 12) in duration. Progestins rose to peak levels of 25.8 ng/ml plasma at 16 to 17 days postovulation (Day 0 = estimated day of ovulation). Regression of the CL began on about Day 21 postovulatuon. Sexual receptivity (estrus) averaged 7.1 ± 0.8 days (m ± SEM, n = 12). The interestrual intervals averaged 40.7 ± 4.1 days (m ± SEM, n = 10). Sexual receptivity continued for several days despite the presence of significant levels of peripheral plasma progestins. One cat was not allowed to copulate during 2 periods of sexual receptivity. The lack of a rise in progestins in this cat supports the concept that ovulation did not occur and that the cat is an induced ovulator. Interestrual intervals were considerably shorter (13 and 18 days) as compared to interestrual intervals during the pseudopregnancy cycles (40.7 days).

## INTRODUCTION

The domestic cat is considered to be an induced ovulator similar to the ferret and mink (Dawson and Friedgood, 1940) with sexual activity confined to the early spring and summer of the year in moderate latitudes (Fabian and Preuss, 1966; Klug, 1969). During the breeding season, several estrous cycles may occur, with their outcome dependent primarily on the individual animal's contact with a male. Three possibilities have been suggested: First, an anovulatory cycle may occur in which there is no coital contact with the male during estrus. Second, a pseudopregnancy cycle may occur as the result of a failure of fertilization following coitus where the ovulatory stimulus is adequate. Corpora lutea develop, and a progestational phase continues for 40–50 days. Lastly, pregnancy can occur with the delivery of full term fetuses in 58–65 days.

The purpose of this study was to determine the duration of luteal function in the pseudopregnant cat.

## MATERIALS AND METHODS

### *General*

Five mature mixed-breed female cats were used. All were housed in standard individual cages and were fed Purina Cat Chow with water available *ad libitum*. They were allowed to exercise together in the room for about 2 h every 12 h. They were subjected to normal daylight except for additional light during the night exercise period. The temperature in the animal room was maintained at 21°C. Various procedures, such as observation of sexual behavior and copulatory attempts and blood collection were carried out during the 2 exercise periods. A Siamese male was vasectomized 1 month prior to start of the project.

### *Behavior*

Females were judged to be approaching estrus when they first exhibited lordosis and rolling tendencies. These postural responses intensified until the cat would not walk at all, preferring to creep with her elbows on the ground and her pelvis elevated. This behavior was recorded as fully-expressed estrus. Successful intromission was judged to have occurred by visual observation and when the female uttered a sharp cry during coitus. Such females characteristically would roll about spasmodically and groom their genitalia after the male dismounted. These behavioral criteria have been previously described (Michael, 1961).

### *Luteal Activity and Ovulation*

Ovulation was estimated to have occurred 48 h before the day on which plasma progestin levels first rose above 1 ng/ml plasma followed by a sustained increase. The first day of the luteal phase (Day 1) was thus the day before the progestin level rose above 1 ng/ml plasma. The end of luteal activity was defined as the first day that progestins were below 1 ng/ml plasma.

### *Progestin Assay*

Total progestins were assayed using a competitive protein-binding radioassay (CPBR) technique similar

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to that described by Bassett and Hinks (1969). The assay system has been previously described (Baldwin and Stabenfeldt, 1974). All samples were assayed against progesterone standards. The assay system allowed the differentiation of 0.075 ng progesterone from 0. There appeared to be no significant difference when samples were assayed by GLC with electron capture detection or by CPBR (Stabenfeldt et al., 1972). The average assay error measured as the coefficient of variation of 231 sample pairs in 20 assays was less than 7 percent in samples containing over 4 ng/ml progestins.

*Blood Sampling*

Jugular vein samples were collected by venipuncture daily between 2200 and 0100 h. Cats were restrained in a wooden holding box for the collection; they had been preconditioned to this procedure and did not appear stressed. Blood (2–3 ml) was withdrawn into a heparinized syringe and refrigerated (5°C) until centrifuged. The plasma was stored at −20°C until assayed. The complete series of samples for each animal was assayed at one time.

*Morphological Observations*

To confirm ovulation, gross and histological observations were made on the ovaries and uteri of cats 980, 1027, 1076, and 995 on Days 4, 7, 8 and 19, respectively, following first coitus. Cat 1079 was sacrificed at the beginning of the winter anestrus, approximately 40 days after the end of the last anovulatory cycle. The genital tracts were fixed in Carson's modified fixative with haematoxylin-eosin stain used on all sections. Surgical procedures were done under Ketamine HCl anesthesia (10 mg/lbs body weight I.M.) and animals were sacrificed by injecting Pentobarbital Sodium (390 mg/10 lbs body weight I.C.).

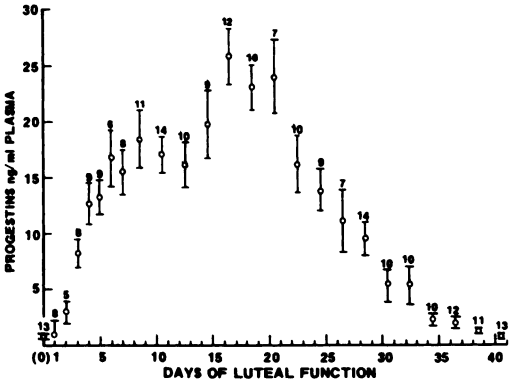


FIG. 1. Progestin concentrations in the peripheral plasma of 5 cats (total of 12 complete pseudopregnancy cycles) mated to a vasectomized male. The number above each point represents the number of observations. After Day 7, each point represents the combined data of 2 adjacent days. Day 0 is the estimated day of ovulation. Vertical bars represent SEMs.

## RESULTS

The concentration of progestins in the peripheral plasma of 5 cats during several pseudopregnancy cycles are shown in Fig. 1. Luteal activity increased rapidly following ovulation in that progestin concentrations were 8.3 ng/ml (mean) by the 3rd day of luteal function. Peak levels (25.8 ng/ml, mean) were achieved by Days 16 to 17. A progestin decline which began between Days 20 and 23, continued until about Day 40, although progestin levels were relatively low by Day 35 of luteal function. The mean length of luteal activity was found to be 36.5 ± 1.1 days (mean ± SEM) in 12 pseudopregnancy cycles.

Progestin levels, which increased rapidly up to Day 8 of luteal activity, appeared to plateau between Days 8 and 13 with further increase to a higher plateau between Days 16 and 20.

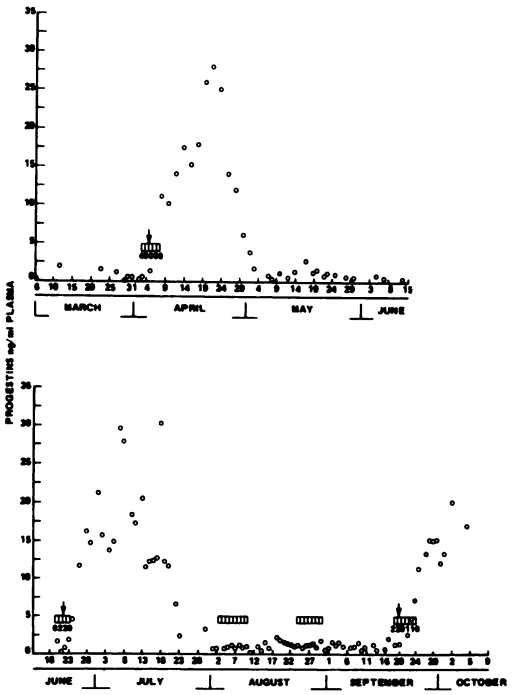


FIG. 2. Peripheral plasma levels of progestins in a cat (995) during ovulatory (nonfertile) and anovulatory cycles. No coital contact was allowed with a male during August. The horizontal boxes (connected) represent the days of sexual receptivity as determined by behavior of the female, regardless of whether she actually copulated. The number of copulations per day of estrus are indicated below the horizontal boxes. The estimated day of ovulation is indicated by an arrow.

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These plateaus were not significantly different ($P = <0.01$), as tested by Scheffe's method of multiple comparisons (Scheffe, 1959). Thus one may conclude that there is a steady and continual increase in luteal activity which reaches its zenith by Days 16 to 20 postovulation.

The temporal relationship of estrous behavior, copulation and peripheral plasma progestins during 2 pseudopregnancy and 2 anovulatory cycles for one animal (995) are shown in Fig. 2. The progestin profiles are typical of those observed for other animals. The interestrual period between pseudopregnancies, however, was not typical in that this interval was 73 days, whereas the average interval for the 9 other intervals studied was 27.9 ± 1.8 days (m ± SEM). This animal was not allowed coital contact with the male during August (1973) at which time 2 anovulatory estrous periods occurred. The failure of ovulation to occur is supported by the fact that progestin levels averaged 1.0 ± 0.1 ng/ml plasma (m ± SEM, n = 69) during the estrous cycles (estrus and interestrual intervals) in a total of three cycles when coitus was not allowed. The 2 anovulatory interestrual periods were considerably shorter (13 and 18 days) than the average interval for pseudopregnancy cycles, 40.7 days, as shown in Table 1.

Data regarding the duration of sexual receptivity and the intervals between the ending of one estrous period and the beginning of the next in pseudopregnancy cycles are summarized in Table 1. In the pseudopregnancy cycles, the duration of sexual receptivity preceding the pseudopregnancy cycles ranged from 5–13 days, with a mean of 7.1 ± 0.8 days (mean ± SEM) in 12 cycles recorded for 5 cats. Average interestrous intervals were 40.7 ± 4.1 days (mean ± SEM) in 10 intervals for the same 5 cats, with a range of 30 to 73 days.

The length of estrus may be modified if ovulation is induced, although the data are very limited in this respect. Cat 995 (Fig. 2) had 3 estrous periods of 4, 5 and 6 days when ovulation was induced, whereas the estrous lengths were 7 and 8 days when ovulation was not induced. Cat 1079 (Table 1) had estrous periods of 8, 6 and 5 days associated with ovulatory cycles, whereas estrus was 9 days in the one anovulatory cycle recorded.

The relationship of sexual receptivity and circulating progestin levels following ovulation

TABLE 1. Estrous cycle data from pseudopregnant cats.

| Animal no. | Estrus no. | Estrus duration (days) | Copulation frequency/day of estrus | Interval between estrus (days)[2] |
|---|---|---|---|---|
| 980 | 1 | 5 | 1,0,4,0,0 | . . . |
| | 2 | 6 | 1,0,1,4,0,0 | 44 |
| | 3 | . . .[1] | 2,0,0,0,. . . | 36 |
| 995 | 1 | 5 | 4,0,0,0,0 | . . . |
| | 2 | 4 | 0,2,0,1,1,0 | 73 |
| | 3 | 6 | 2,2,0,1,1,0 | 40 |
| 1027 | 1 | 9 | 2,1,4,1,2,4,2,1,3 | . . . |
| | 2 | 8 | 4,2,1,2,0,4,4,0 | 37 |
| | 3 | . . .[1] | 1,1,2,1,7,4, . . . | 36 |
| 1076 | 1 | 10 | 1,1,0,1,0,5,1,0,0,0 | . . . |
| | 2 | 13 | 0,5,1,2,0,0,1,0,2,1,0,0,0 | 30 |
| | 3 | . . .[1] | 1,0,2,1,1,3,1,0, . . . | 31 |
| 1079 | 1 | 8 | 1,0,0,0,0,2,1,0 | . . . |
| | 2 | 6 | 3,1,1,1,4,0 | 40 |
| | 3 | 5 | 2,0,0,3,1 | 40 |
| | | $\bar{x}$ = 7.1 (n, 12) | | 40.7 (n, 10) |
| | | SD = 2.6 | | 12.3 |
| | | SEM = 0.8 | | 4.1 |

[1] Ovaries removed before end of estrus.
[2] Interval from last day of estrus to first day of the subsequent estrus.

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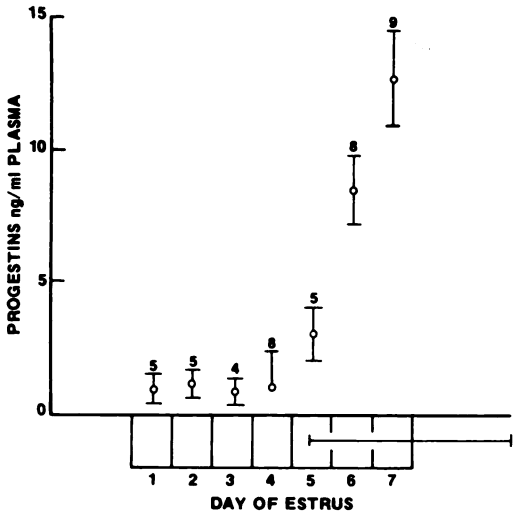


FIG. 3. Progestin concentrations in the plasma of 5 cats during the period of sexual receptivity. The vertical bars represent SEMs. The number above each point represents the number of observations. The horizontal line represents 1 SD around the average length of sexual receptivity (7 days).

are shown in Fig. 3. The most striking aspect of this relationship is the fact that these cats remained sexually receptive (allowed coitus) for several days in the presence of rapidly increasing progestin levels. The copulation frequency/day of estrus is shown in Table 1.

An attempt was made to estimate the time of ovulation based on the first rise in peripheral plasma progestins. Ovulation was estimated to occur 48 h before the first sustained rise in progestins was observed. We have no strong basis for selecting this time interval except that there is good evidence that significant progestin production can occur in other animals (mare) by 1 to 2 days postovulation (Stabenfeldt et al., 1975). Using this criterion for ovulation, the interval from the day of first copulation to the day of ovulation was calculated. The average interval for 15 observations was 1.9 days with a range of 1 to 5 days. Considerable variation occurred between cats in that the average interval from first coitus to the estimated time of ovulation was 1, 1.67, 2, 2 and 3 days, respectively, for the 5 cats.

Ovulation was confirmed in 4 cats through ovariectomy. Ovaries were obtained 2, 3, 5.5 and 17.5 days after the estimated time of ovulation (48 h before the beginning of a sustained rise in progestins) at which time plasma progestin levels were 4, 5, 9.8, 16.8 and 17.0 ng/ml, respectively, at the time of collection. All had corpora lutea that were in various stages of development commensurate with their maturity, as determined by histological examination, and non-fertilized ova were found by serially sectioning the oviducts. Cat 1079 was sacrificed 43 days after completion of an anovulatory estrus. She had several secondary follicles and no corpora lutea in her ovaries.

## DISCUSSION

Several carnivores such as the dog (Smith and McDonald, 1974), blue fox (Møller, 1973a), mink (Møller, 1973b), and ferret (Heap and Hammond, 1974) have luteal phases that are similar in length (within a series) without regard for the presence or absence of pregnancy. The cat, rabbit (Horrell et al., 1972) and rat (Pepe and Rothchild, 1974) appear to differ in that the luteal phase of the nonpregnant animal is only about one-half of the duration of the normal gestation period. The cat thus appears to have a reproductive advantage, compared to other carnivores, in that the shorter luteal phase allows for a more rapid return to a potentially fertile state. The cat also has a reproductive advantage over the dog in that the reestablishment of ovarian activity can begin within 7 to 10 days following pseudopregnancy, whereas it is not uncommon in the dog for at least 5 months to elapse after corpus luteum regression before significant ovarian activity begins. The maximal number of pseudopregnancies a cat can undergo in one season was not fully tested, but it appears that 4, or even 5, may be possible.

Plasma progesterone concentration indicate that corpora lutea of the pseudopregnant cat begin to regress about 3 weeks after their formation. A similar time of regression was seen in an earlier histological study by Foster and Hisaw (1935). One might speculate that the gradual decline in progestins which begins at about Day 21 postcoitum suggests that corpus luteum function in the pseudopregnant cat is not terminated by the acute application of lytic factors but may be simply due to a lifespan predetermined at ovulation (in the absence of gestational luteotropic influences).

One important physiological effect of progesterone in some species is the suppression of estrus. In some domestic species such as the sow, ewe and mare, sexual receptivity usually wanes within a few hours after ovulation,

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presumably due to progesterone production by the newly-formed corpora lutea (Stabenfeldt, 1974). The cat, in contrast, seems to be less sensitive to progestins as concerns diminution of sexual receptivity in that, on the average, sexual receptiveness continued to be manifested (last 3 days of estrus) despite significant increases in circulating progestins. The cat, in this respect, is similar to the dog (Smith and McDonald, 1974) and fox (Møller, 1973a).

There is some disagreement about the temporal relationship of the coital stimulus to ovulation in the cat (Fabian and Preuss, 1966; Klug, 1969; Greulich, 1934). While the interval from first coitus to ovulation, as estimated by this study, may not be precise, the between-animal comparisons should be valid in that the same criteria were used for each animal. The variation among cats was striking. It may be that a certain intrinsic maturity of the follicle is a necessary prerequisite for an ovulatory stimulus to be effective. If this is true, one explanation for the apparent variability of the coitus to ovulation interval could be due to variations in the relationship of sexual responsiveness and follicle maturity. Some females could be sexually receptive before follicle maturation had proceeded sufficiently to allow response to the ovulatory stimulus.

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## REFERENCES

Baldwin, D. M., and Stabenfeldt, G. H. (1974). Plasma levels of progesterone, cortisol and corticosterone in the pregnant rabbit. Biol. Reprod. 10, 495–501.

Bassett, J. M., and Hinks, N. T. (1969). Microdetermination of corticosteroids in ovine peripheral plasma: effects of venipuncture, corticotrophin, insulin and glucose. J. Endocr. 44, 387–403.

Dawson, A. B., and Friedgood, H. B. (1940). The time and sequence of preovulatory changes in the cat ovary after mating or mechanical stimulation of the cervix uteri. Anat. Rec. 76, 411–429.

Fabian, G., and Preuss, G. (1966). Messungen am Uterus der Katze in den Zyklusphasen. Zbl. Vet. Med. (A) 13, 337–351.

Foster, M. A., and Hisaw, F. L. (1935). Experimental ovulation and the resulting pseudopregnancy in anestrous cats. Anat. Rec. 62, 75–93.

Greulich, W. W. (1934). Artificially induced ovulation in the cat *(Felis domestica)*. Anat. Rec. 58, 217–224.

Heap, R. B., and Hammond, J., Jr. (1974). Plasma progesterone levels in pregnant and pseudopregnant ferrets. J. Reprod. Fert. 39, 149–152.

Horrell, E., Major, P. W., Kilpatrick, R., and Smith, B. M. (1972). Progestational steroids during pseudopregnancy in the rabbit. J. Endocr. 55, 89–96.

Klug, E. (1969). Die Fortpflanzung der Hauskatze *(Felis domestica)* unter besonderer Berucksichtigung der instrumentellen Samen-ubertragung: eine Literaturstudie. Doctoral Dissertation, Tierarztliche Hochschule Hannover. p. 51.

Michael, R. P. (1961). Observations upon the sexual behavior of the domestic cat *(Felis catus L.)* under laboratory conditions. Behaviour 18, 1–24.

Møller, O. M. (1973a). Progesterone concentrations in the peripheral plasma of the blue fox *(Alopex lagopus)* during pregnancy and the oestrous cycle. J. Endocr. 59, 429–438.

Møller, O. M. (1973b). The progesterone concentions in the peripheral plasma of the mink *(Mustela vison)* during pregnancy. J. Endocr. 56, 121–132.

Pepe, G. J., and Rothchild, I. (1974). A comparative study of serum progesterone levels in pregnancy and various types of pseudopregnancy in the rat. Endocrinology 95, 275–279.

Scheffe, H. (1959). The Analysis of Variance, John Wiley and Sons, London, p. 68.

Smith, M. S., and McDonald, L. E. (1974). Serum levels of luteinizing hormone and progesterone during the estrous cycle, pseudopregnancy and pregnancy in the dog. Endocrinology 94, 404–412.

Stabenfeldt, G. H. (1974). Physiologic, pathologic and therapeutic roles of progestins in domestic animals. J.A.V.M.A. 164, 311–317.

Stabenfeldt, G. H., Drost, M., and Franti, C. E. (1972). Peripheral plasma progesterone levels in the ewe during pregnancy and parturition. Endocrinology 90, 144–150.

Stabenfeldt, G. H., Hughes, J. P., Evans, J. W., and Geschwind, I. I. (1975). Unique aspects of the reproductive cycle of the mare. J. Reprod. Fert. In Press.

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