BIOLOGY OF REPRODUCTION 30, 945–951 (1984)

Serum Progesterone Profiles and Corpora Lutea of Pregnant, Postpartum, Barren and Isolated Females in a Laboratory Colony of Woodchucks (Marmota monax)

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ABSTRACT

Female woodchucks, >1 year old (n=10) or recently weaned locally in the wild at 42°N (n=10), were maintained indoors in standard laboratory animal facilities from one summer solstice to the next except for those killed after 11 months of study. Available ambient light was supplemented with a 12L:12D incandescent light schedule. Food and water were provided ad libitum, except for a 63-day period when feeding and supplemental lighting were discontinued, starting at the winter solstice. Temperatures fluctuated with outdoor temperatures >4°C. During the winter, females within each age class (adult vs. subadult) were housed with (n=5) or without (n=5) a male cagemate, and scored for hibernation at variable intervals. Blood samples for serum progesterone assay were collected at intervals of 1–4 weeks. Birth of litters occurred in 2 of 5 adults (March 19, April 2) and in 0 of 5 subadults. Neither the incidence nor frequency of hibernation were correlated to the occurrence of pregnancy or to the extent or timing of elevations in progesterone in barren or isolated females. Serum progesterone was elevated during the 31-day gestation and for 1–2 months postpartum in the 2 fertile colonized animals and in 7 others captured pregnant during the same breeding season and which gave birth April 12–24. Postpartum animals had progesterone levels equal or above those near midgestation and had 3.1 ± 0.2-mm diameter corpora lutea which numbered 2.5 ± 0.3 per ovary. Progesterone levels at 8–36 h postpartum were lower (2.5 ± 0.3 ng/ml) than those present prepartum (9.8 ± 2.3 ng/ml) in 5 of 8 animals, but were not reduced (>10 ng/ml) in 3 others.

Serum progesterone in most barren or isolated females was progressively elevated above 1 ng/ml starting March 25–April 26, and reached peaks of 5–60 ng/ml prior to the summer solstice. Their corpora lutea were more numerous (14.4 ± 1.3 per ovary) but smaller (1.1 ± 0.1 mm) than those of postpartum animals.

These results demonstrate that, in woodchucks: *1)* the peripartum decline in serum progesterone is either very transient and/or occurs inconsistently, *2)* the corpora lutea of pregnancy are maintained and/or rejuvenated for a considerable period postpartum, and *3)* barren or unmated females, including yearlings, spontaneously form multiple corpora lutea which secrete progesterone during the period when parturient females are lactating.

INTRODUCTION

Woodchucks (*Marmota monax:* Rodentia: Sciuridae) in the wild enter hibernation at or shortly after the autumnal equinox and have a single limited breeding season following emergence in late winter. Near the center of their geographical distribution (40–42°N, 75–77°W), and as recently reviewed (Concannon et al., 1983), the majority of adult females give birth to a single litter during the first 3 weeks of April following a 31- to 32-day gestation while subadult (<1 year old) females are infrequently fertile. Mean litter size is about 4, the young are weaned at 4–6 weeks of age, and no instances of more than 1 litter per year have been reported. Information has not been reported on the potential for multiple estrous cycles, on the duration of the estrous period(s), or on the extent to which ovulation and/or formation of functional corpora lutea occur spontaneously.

The corpora lutea of pregnancy are large and distinct and persist for 3–4 months postpartum (Snyder and Christian, 1960). These findings were recently confirmed and extended in a

Accepted February 10, 1984.
Received September 23, 1983.

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study in which progesterone concentrations were determined in single samples obtained at various times before or after parturition in individual woodchucks captured after breeding in the wild (Concannon et al., 1983). In that study, mean serum progesterone levels during the first, second and third months postpartum were 25 ± 6, 61 ± 19 and 23 ± 9 ng/ml, respectively, and were similar to or greater than those observed during the first, second or third trimesters of the 31-day pregnancy, which were 7 ± 2, 23 ± 4 and 17 ± 3 ng/ml, respectively. Mean corpus luteum diameters were larger postpartum than during pregnancy and their presence postpartum, along with that of elevated serum progesterone levels, occurred independently of suckling. The correlation between placental scars and ipsilateral corpora lutea confirmed that the postpartum corpora lutea were those of the recent pregnancy, since the woodchuck has a duplex cervix which precludes transcornual embryo migration (Concannon et al., 1983). The latter study, however, was restricted to single progesterone determinations within individual recently captured animals, did not evaluate the extent of any peripartum decline in serum progesterone levels, and failed to consider ovarian activity in subadult and/or nonbreeding animals. The present studies were conducted to consider such factors and to monitor reproductive success and ovarian activity in woodchucks chronically maintained indoors under laboratory colony conditions. They involved serial progesterone determinations in adults captured following breeding in the wild, and in adults and yearlings maintained under laboratory conditions and housed with or without a male during the expected breeding season. In addition, the ovaries of the majority of animals were subsequently removed to permit an evaluation of gross ovarian morphology in relation to prior changes in serum progesterone levels.

MATERIALS AND METHODS

Colony-Maintained Animals

Colony-housed females maintained for reproductive studies in the 1982 breeding season included 4 groups of 5 animals each. These were adult females each pair-housed with an adult male; adult females housed in isolation from males; yearling females each pair-housed with a male; and yearlings housed in isolation from males. Each group of adults included 2 intact and 1 hemiovariectomized female retained from 1981 studies and captured in 1980 as either an adult or yearling; one of the 2 retained intact females in each group gave birth to a litter the year before, the other did not. The remainder of each group of adults was comprised of 2 adult females captured during the summer of 1981. The groups of yearlings were comprised of females captured as recently weaned juveniles in the late spring of 1981. The 5 juveniles paired with males had either an adult (n=1) or yearling (n=4) male introduced to their cage or pen on December 20 or earlier; 3 of the 4 initially paired with yearling males had adult males substituted between March 18 and March 31. Housing of adult females with males occurred from December 20 (n=2) or February 25 (n=3) until the females were palpated as pregnant (n=2) or were killed (n=3).

The animals were housed indoors in stainless steel cages or in pens constructed of galvanized steel; the enclosures provided 0.8–1.7 square m per animal or pair of animals and contained a nest box with 0.1-square m bottom. All animals were provided water ad libitum. Commercial rabbit chow was provided ad libitum until December 21, 1981, at which time food was withdrawn from all animals until again provided on February 22, 1982 and thereafter. Lighting was provided by ambient sunlight. Additional lighting was provided by incandescent light (12L:12D) before and after the 63-day period of withdrawal of food. Room temperature was maintained above 4°C by thermostatically controlled heaters and fluctuated in relation to outside temperatures. The mean daily room temperature (°C) was 7.3 ± 0.2, 6.3 ± 0.8 and 6.9 ± 0.8 in the months of December, January and February, respectively.

All female woodchucks were checked for hibernation status once before, once after, and at intervals of 5 to 10 days during the 9-week period of food deprivation. A woodchuck was determined to be hibernating if it was cool to the touch, its eyes were closed, it was tightly curled, and it did not respond to physical stimulation other than by slight, slow movement.

Blood Collections and Progesterone Assays

Blood samples were collected from adult females at approximately monthly intervals from December 11 to February 17, and at approximately weekly intervals therafter, until the animals were euthanized in May. Samples were collected from 8 yearling females intermittently prior to March 15, and at approximately 2-week intervals thereafter for 12–16 weeks; only a limited series of samples were obtained for the other 2 yearlings. Between March 10 and April 25, abdominal palpations were performed at the times of blood collection to determine the presence or absence of palpable fetuses. The samples were collected by femoral venipuncture and aspiration into evacuated tubes 5–10 min after administration of an anesthetic dose of a ketamine-xylazine mixture (Concannon et al., 1983). Blood was allowed to clot 8–18 h at 5°C; serum aliquots were harvested following centrifugation and stored frozen until assayed.

Serum progesterone content was assayed using procedures previously described in detail (Concannon et al., 1983). The method involved duplicate extraction of 0.5-ml aliquots of serum with petroleum ether, and assay of each extract utilizing a progesterone antiserum for which cross-reactivities have been

reported (Reimers et al., 1983). The coefficients of variation within and between assays were 10% and 14%, respectively. All samples from an individual animal were included within a single assay.

Captured Pregnant Animals

In order to supplement findings in colony-maintained animals, 7 females captured between March 26 and April 15 of the same year and determined pregnant by palpation were monitored daily for the birth of litters. They were housed indoors in Horsfal-type, air-filtered isolation units and were provided feed and water ad libitum. Bedding material was comprised of straw and wood shavings, and a 0.4-m^3 nest box was constructed of wood. Fluorescent lighting was on a schedule of 12L:12D. Blood samples collected from each animal 1 day after capture, on the day the litter was first observed, and at variable times pre- and postpartum were assayed for progesterone content. At 13–23 days postpartum, 3 animals were killed for examination of the reproductive tract immediately after collection of a final blood sample; 2 of them had lost their litters within 5 days of parturition, the other was suckling a single pup. The remaining 4 animals were bled at 1 and 2 months postpartum.

Necropsy Examinations

Examinations of the reproductive tracts in addition to those performed on 3 of the recently captured females were performed on all adult and 3 yearling colony-maintained females. The examinations were performed between May 6 and May 27. Each animal was examined via a mid-ventral laparotomy following administration of an anesthetic dose of a ketamine-xylazine mixture. The numbers and diameters (to the nearest 0.2 mm) of corpora lutea on each ovary were recorded and the mean corpus luteum diameter for each ovary was calculated. Each ovary was removed attached to the ipsilateral uterine horn. In postpartum animals, the uterine horns were slit open and the number of placental sites recorded. Examinations were terminated by administration of a lethal dose of anesthetic.

All means are reported as mean ± standard error of the mean. Differences between means were determined by Student's *t* tests.

RESULTS

Fertility

Two of the chronically maintained adult females gave birth to litters. The first gave birth to 3 pups on March 19 and maintained 2 until she was killed on May 7 at 48 days postpartum. The other gave birth to 5 pups on April 2, lost all of them within 7 days, and was killed on May 25 at 53 days postpartum. Fetal vesicles of approximately 25 mm and 10 mm diameter were palpated in these females at 18 and 22 days prepartum, respectively. No other females among the colony-housed animals were observed to give birth or to have fetuses when palpated. Each of the 7 recently captured pregnant females gave birth to litters between April 12 and April 24. Litter sizes ranged from 3 to 6 and averaged 4.3 ± 0.4 pups.

Hibernation

The incidence of hibernation in chronically maintained animals before, during, and following the period of food withdrawal are shown in Fig. 1. No consistent differences among groups were noted in the incidence of hibernation within groups at the various times studied. The extent of hiberation within individual hibernating females varied within, as well as among, groups. The percentages of times hibernating were 38–100% in 3 of 5 adults paired with males, 50–75% in 3 of 5 adults isolated from males, 25–68% in 5 paired yearlings, and 25–50% in 3 of 5 isolated yearlings. Hibernation was not observed in the other 6 females. Of the 2 females which gave birth to litters, the first was never observed to hibernate and the second was hibernating 3 of the 8 times studied during food deprivation.

Progesterone

Serum progesterone levels in individual chronically maintained animals are shown in Fig. 2. Prior to March 9, progesterone levels

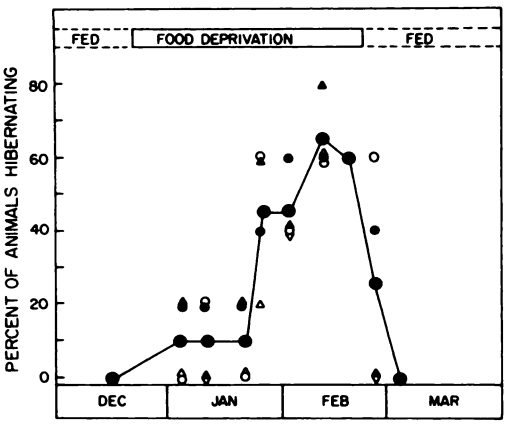

FIG. 1. Incidence of hibernation before, during and after a 63-day period during which feeding and supplemental lighting (12L:12D) were discontinued in female woodchucks maintained indoors under available ambient light and with temperatures ≥4°C allowed to fluctuate in relation to outside temperature. No consistent differences were noted among male-paired adults (●), isolated adults (○), male-paired yearlings (▲), and isolated yearlings (△).

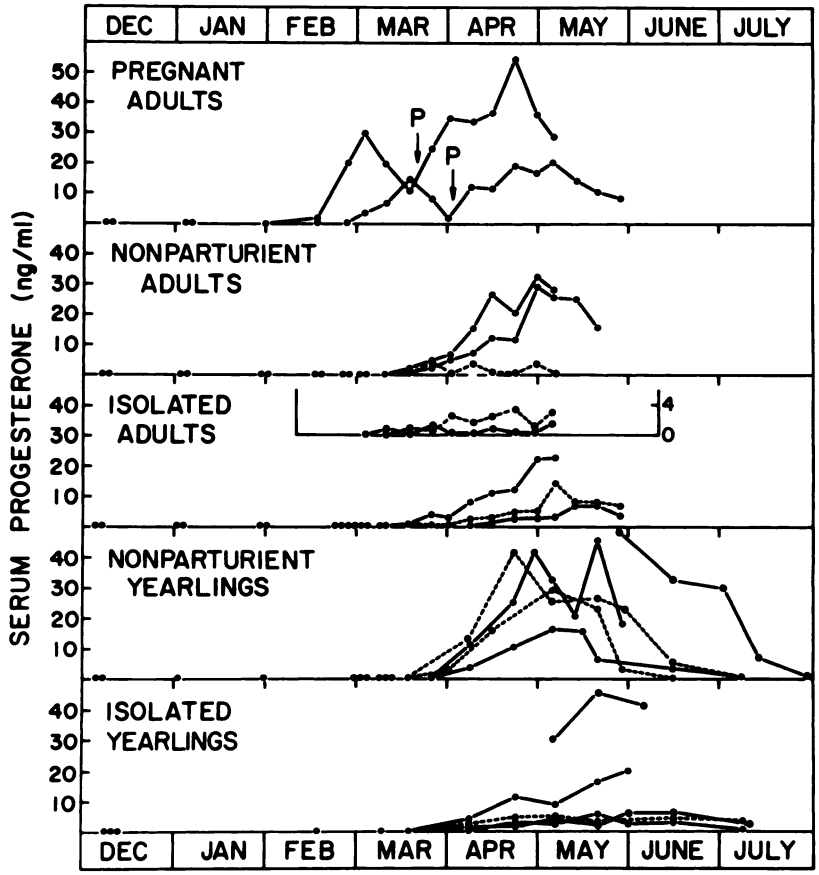

FIG. 2. Serum progesterone concentrations in individual adult (>1.5 yr) and subadult/yearling (<1.4 yr) female woodchucks either housed with males (panels 1, 2 and 4) or in isolation from males (panels 3 and 5). The times of parturition *(P)* are indicated for 2 females which gave birth to litters.

were <0.3 ng/ml in all animals except for the first observed increases in the 2 pregnant animals on February 17 and March 4. These increases to 2.1 and 3.0 ng/ml were at 31 and 29 days prior to parturition, respectively. In the first pregnancy, a peak level of 29 ng/ml at 16 days prepartum was followed by a decline to 11.2 ng/ml at 2 days prepartum. In the second pregnancy, a peak level of 14.0 ng/ml was followed in more frequently collected samples by a decline to lower levels of 7.9, 3.9 and 1.8 ng/ml at 8, 2, and 1 days prepartum, and by a subsequent increase to 3.4 ng/ml in a sample obtained 12–24 h after parturition. In both females, progesterone levels postpartum increased to levels higher than those observed during pregnancy; postpartum peak levels of 56 ng/ml in the female suckling a litter and of 21 ng/ml in the female that lost her litter occurred at 33 and 35 days postpartum, respectively. Levels remained elevated until the animals were killed for necropsy at 48–53 days postpartum, when progesterone was 29 and 8.6 ng/ml in the suckling and nonsuckling female, respectively.

In the remaining animals, serum progesterone levels >1.0 ng/ml were not observed until March 18 or later. In 16 of 18 females that did not have litters, protracted increases in serum progesterone levels above 2 ng/ml were observed for 1 mo or longer (Fig. 2). These included peak levels of 31–33 ng/ml in 2 of 3 nonwhelping adults paired with males, 12.5 ± 4.1 ng/ml in 4 of 5 adults housed separately from males, 43.0 ± 9.7 ng/ml in the 5 yearling females that failed to become pregnant, and 18.1 ± 10.0 ng/ml in the 5 yearling females housed separately from males. Peak progesterone levels in nonparous females occurred between April 23 and June 17. Initial elevations in

serum progesterone above 1 ng/ml first occurred between March 18 and April 23 (March 31 ± 6 days) in 6 nonparous adults, and between April 8 and May 6 (April 13 ± 4 days) in 8 of the yearlings.

Two adult animals failed to show protracted increases in progesterone prior to necropsy on May 6. One was an intact female which was housed in isolation from males and which had only minor elevations in progesterone (1–2 ng/ml) on 2 occasions. The other had been unilaterally ovariectomized during the previous year and had 3 elevations in serum progesterone to 2–5 ng/ml at intervals of 2–3 wk. The extent to which serum progesterone levels increased could not be related to either the occurrence or extent of hibernation previously observed within individual animals.

In the 7 recently captured pregnant females, serum progesterone levels at 1 or more times before and after parturition as well as during the 32-h period following parturition are shown in Fig. 3. Progesterone levels at 8–32 h postpartum were reduced from prepartum values and ranged from 1.8 to 3.1 ng/ml in 4 of the 7 animals, while those in the other 3 animals were elevated and ranged from 10.7 to 28.6 ng/ml. Progesterone levels were ≥10 ng/ml in 3 animals killed for necropsy at 13–23 days postpartum and at 1 and/or 2 mo postpartum in the remaining 4 animals. The pups of 2 animals killed for necropsy all died within 7 days of birth; the other 5 females maintained litters of 1–6 pups throughout the period of study.

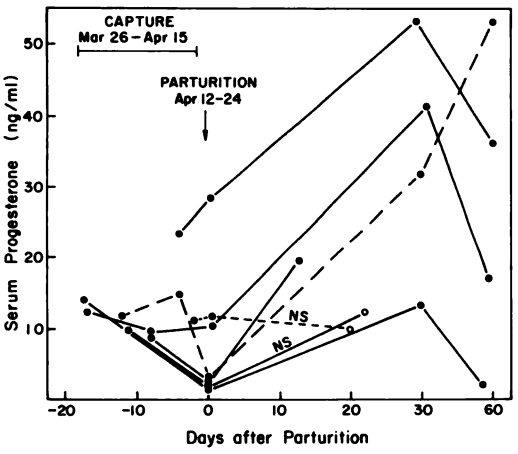

FIG. 3. Serum progesterone levels in 7 woodchucks captured following breeding in the wild shown aligned to a common time of parturition. Samples indicated as Day 0 from parturition were obtained 8–32 h postpartum. No pups in litters of nonsuckling *(NS)* animals survived beyond 5 days postpartum.

Corpora Lutea

Observations on the reproductive tracts and serum progesterone levels at the time of necropsy are summarized in Table 1. Mean serum progesterone levels in postpartum and nonparous animals at the time of necropsy were not different ($P>0.5$). Corpora lutea were smaller ($P<0.05$) and more numerous ($P<0.01$) in nonparous adults and yearlings than in the postpartum adults. In all postpartum animals, the number of placental scars in individual uterine horns was either equal to (n=8), or one less than (n=2), the number of corpora lutea on the ipsilateral ovary. In the 1 adult in which no elevations in serum progesterone were observed throughout the study, no grossly visible corpora lutea were present on the ovaries.

DISCUSSION

Only 2 of 5 adult females (4 intact and 1 hemiovariectomized) maintained indoors and paired with males were observed to give birth. Reduced fecundity in captive woodchucks has also been noted by Hamilton (1934), Grizzell (1955) and Young and Sims (1979). Estimates of reproductive success in feral adults vary depending on year and location but, in general, are high. Davis (1981) reported pregnancy rates in adults ranging from 64 to 100% (95 ± 4%) over 9 yr of study; Singles (1976) reported 93% in 1 yr. Hamilton (1934) reported a mean pregnancy rate of 98% in a 2-yr study. The pregnancy rate for yearlings in the wild is low and has been estimated at 17–26% (Hamilton, 1934; Grizzell, 1955). The absence of pregnancies in the yearlings of the present study may represent the reduced fertility of yearling woodchucks. However, all but 1 were cohoused only with a yearling male until the 3rd wk of March and after that time only 4 of the 5 were housed with adult males. If the fertility of yearling males is low compared to older males, the absence of fecundity in the yearling females may have been complicated by the age of the males and/or the time at which an adult male was introduced.

The reasons for poor reproductive performance by woodchucks in captivity is not known, mainly because of lack of information on the

TABLE 1. Reproductive parameters at the time of necrospy in postpartum adult, nonparturient adult, and nonparturient yearling woodchucks.

	Postpartum adults	Nonparturient adults	Nonparturient yearlings
No. of animals	5	7	3
No. of corpora lutea per ovary (Range)	2.5 ± 0.3 (1–4)	13.6 ± 1.5[a] (5–22)	15.3 ± 2.2[a] (11–23)
Corpus luteum diameter (mm) (Range)	3.1 ± 0.2 (2.4–4.4)	1.2 ± 0.1[a] (0.6–2.0)	1.1 ± 0.1[b] (0.8–1.4)
No. of placental scars per horn (Range)	2.3 ± 0.2 (1–3)	---	---
Corpora lutea in excess of ipsilateral placental scars (n) (Range)	0.2 ± 0.1 (0–1)	---	---
Serum progesterone (ng/ml) (Range)	16.0 ± 3.6 (9–29)	11.6 ± 4.0 (0.5–28)	31.3 ± 12.3 (18–56)

[a]Different from postpartum animals, $P<0.05$.

[b]Different from postpartum animals, $P<0.01$.

extent to which normal reproduction is dependent on environmental factors such as temperature, photoperiod, prior hibernation and diet, and on endogenous factors such as age, body condition, and metabolic changes associated with the annual cycle of changing food intake and hibernation. In the wild the period of hibernation lasts 4–5 mo, from October or November until February or March (Hamilton, 1934; Snyder and Christian, 1960; Davis, 1976; Young and Sims, 1979). It is possible that the shorter duration of the 2-mo period of food deprivation in the present study, coupled with the apparent failure of some animals to hibernate, may have contributed to the low fertility. However, the absence of prior hibernation in 1 of the 2 litter-bearing females demonstrates that hibernation per se is not a prerequisite for fertility. Furthermore, the latter observation was not a unique occurrence, since in a study of another colony performed concurrent with the present study, the pregnancy rate in adult females housed with males was higher in those in which the incidence of hibernation was curtailed by providing uninterrupted access to food, in comparison to those in which hibernation was induced by food deprivation (Baldwin and Concannon, unpublished data).

Pregnancy in the woodchuck lasts 30–32 days (Hoyt and Hoyt, 1950; Hoyt, 1952; Grizzell, 1955). Levels of serum progesterone observed during pregnancy and after parturition were within the range of those previously reported in single samples obtained from captured pregnant animals (Concannon et al., 1983). The finding of reduced serum progesterone concentrations near the time of parturition in several animals suggests that, in at least some woodchucks, there is a peripartum decline in progesterone, such as reported for most other species studied. However, the delince must be either extremely transient or inconsistent since progesterone levels 12–32 h postpartum in 3 captured females were elevated. Results to date suggest that retained corpora lutea of pregnancy are the major source of progesterone during the postpartum period. That the large corpora lutea present in both suckling and nonsuckling postpartum animals are the corpora lutea of the preceding pregnancy is supported by their correlation, in number, to the ipsilateral placental scars, determined in the present study (Table 1) and in previously studied animals (Concannon et al., 1983). A nonovarian source of significant amounts of progesterone in postpartum or nonpregnant animals seems unlikely since protracted elevations in progesterone were not observed in the female that did not have corpora lutea at the time of necropsy.

The formation of multiple, small corpora lutea and elevations in serum progesterone at the end of the natural breeding season in the nonparturient colony-housed females probably

represent events that also occur in natural populations. Snyder and Christian (1960) found nulliparous yearlings with large numbers of small corpora lutea which, like those in the present study (unpublished observations), could not be distinguished histologically from corpora lutea of pregnancy, except for their size. Whether they are postovulatory corpora lutea or nonovulatory luteinized follicles, or are formed simultaneously or sequentially, has not been determined. The present study demonstrates that such corpora lutea are functional and occur in nonparturient adults as well as yearlings. The adaptive significance of their formation at the end of the breeding season is not clear. It may serve to terminate the breeding season at a time beyond which fertile matings would result in births too late in the year for pups to develop sufficiently for successful hibernation.

The rise in progesterone in most females between late March and mid-April suggests that the annual reproductive cycle in these animals was not entirely disrupted by the conditions in which they were housed despite the small number of pregnancies. The mechanism of formation of the multiple corpora probably involves a change in secretion of gonadotropin(s), although the possibility of spontaneous luteinization of numerous, accumulated follicles without a change in gonadotropin secretion cannot be discounted. If the timing of this phenomenon is dependent on extant environmental factors, then the increase in hours of daylight following the vernal equinox could be involved.

ACKNOWLEDGMENTS

The authors thank B. Hodgson, J. Lawless and L. Potkewitz for technical assistance, Dr. W. Hornbuckle for clinical care of the animals, Mr. L. Eberhardt for animal maintenance, and Mrs. C. Drake for manuscript preparation. This research was supported by Contract NO1 AI 02651 from NIAID and NCI, National Institutes of Health.

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