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Serum Hormone Concentrations and Their Relationships to Sexual Behavior at the First and Second Estrous Cycles of the Labrador Bitch

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ABSTRACT

Seven purebred Labrador bitches were monitored for sexual behavior at the first and second estrous cycles. Serum was analyzed for luteinizing hormone (LH), estrone (E_1), estradiol-17β (E_2) and progesterone (P) concentrations. All hormonal data were normalized to the day of preovulatory peak LH (Day 0) for both cycles. Mean E_1 concentration during the week preceding Day 0 was greater ($P<0.05$) in the second cycle compared with that at the first cycle (42.0 ± 3.0 vs 29.0 ± 2.0 pg/ml), but the mean peak E_1 levels were not different (52.0 ± 16.0 vs 34.0 + 6.3 pg/ml). Both the mean peak E_2 concentration (54.0 ± 8.0 vs 28.0 ± 7.0 pg/ml) and the mean level during the week before peak LH (47.0 ± 4.0 vs 22.0 ± 4.0 pg/ml) were greater ($P<0.05$) in the second cycle compared with the first cycle. Mean serum LH concentration during the week before Day 0 (4.8 ± 0.9 ng/ml) in the second cycle was higher ($P<0.05$) compared with that of the first cycle (2.5 ± 0.7 ng/ml). The peak preovulatory LH (Day 0) for the second cycle was also higher ($P<0.01$) than that of the first cycle (51.6 ± 6.9 vs 23.1 ± 4.2 ng/ml, respectively). The mean serum P concentration during the week before Day 0 was greater ($P<0.05$) in the second cycle (1.6 ± 0.1 vs 1.0 ± 0.3 ng/ml) but the mean P level during the second week following Day 0 was higher ($P<0.05$) in the first cycle (15.8 ± 2.0 vs 9.4 ± 0.8 ng/ml). The durations of proestrus (7.7 ± 0.5 vs 3.9 ± 0.7 days) and estrus (11.0 ± 1.1 vs 5.4 ± 0.4 days) were longer ($P<0.01$) in the second estrous cycle. The data suggest that 1) in general, serum hormone concentrations were elevated during the second cycle compared with the first cycle; and 2) durations of proestrus and estrus were longer during the second cycle. It appears that the shorter duration of sexual receptivity at the first behavioral estrous cycle in the bitch may have a hormonal basis.

INTRODUCTION

Dogs have been used extensively in biomedical research as a model animal. The dog is a relatively large laboratory animal suitable for frequent blood sampling. Although the importance of the dog in biological research is well documented, until recently very little research has been conducted to elucidate the endocrinological events in canine reproduction.

The domesticated bitch is unique in its prolonged interval of proestrous-estrous activity and a long duration of behavioral estrus. Recent reports by Nett et al. (1975), Concannon et al. (1975) and Wildt et al. (1978, 1979) have characterized the basic endocrine parameters controlling the estrous cycle in the bitch. It appears that the proestrous period in the bitch is characterized by a steady rise in the levels of estrone (E_1) and estradiol-17β (E_2), reaching a peak immediately before the onset of the preovulatory surge of luteinizing hormone (LH) and declining sharply as the animals show signs of sexual receptivity. There is evidence of preovulatory luteinization of the mature follicles and secretion of significant amounts of progesterone (Wildt et al., 1979) and this rise in progesterone (P) coincidental with the fall in estrogen levels initiates the expression of behavioral estrus (Concannon et al., 1977). Thus, it is evident that preovulatory progesterone plays a very important role in bringing the animal into behavioral estrus; the bitch remains responsive to mating for a long duration in concert with a rising and steadily elevated concentration of progesterone.

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It has been well established that the first ovulation at the start of a new breeding season in the sheep, is generally not associated with estrous behavior. In addition, progesterone secreted from this first functional corpus luteum (CL) conditions the ewe so that full estrous behavior is apparently coincidental with the estrogen peak preceding the second ovulation (Robertson, 1977). As progesterone appears to play a significant role in the expression of behavioral estrus in the ewe, a similar mechanism is possible in the bitch at the start of its reproductive life.

In the domesticated bitch, depending on the breed and body weight, puberty usually occurs between 6–12 months of age (McDonald, 1975). Although no published data are available, the experience of dog breeders indicates that the proestrous-estrous duration at the first behavioral estrus is highly variable and the breeding performance is lower than that observed in later estrous cycles. The present study was conducted to define sexual behavior at the first and second estrous cycle of the bitch and its relationships to the circulating hormone concentrations controlling the expression of such behavior.

MATERIALS AND METHODS

Seven purebred black Labrador bitches were included in this study. These animals came from 2 litters born within a 2 week period so that half of the animals were full siblings. Starting at the age of 5 months, all animals were routinely checked twice weekly for the initiation of proestrous-estrous activity using 2 male dogs. Proestrus in the bitch is characterized by vulvular swelling and a sanguinous vaginal discharge. Starting at proestrus, all animals were checked for estrous activity using the studs once daily throughout the proestrous-estrous interval. A bitch was considered to be in estrus upon showing the criteria which included tail deflection, allowing the stud to mount and initiate coitus (Concannon et al., 1975). Copulation between the stud and the estrous bitch was not permitted. Once daily, blood samples were collected from each animal during the period of proestrus and estrus. A similar protocol was followed during the second estrous cycle of the same group of animals except that daily blood collections were performed once in the morning and once in the afternoon. The blood samples were allowed to clot, the serum separated by centrifugation and stored frozen until analyzed for hormone concentrations.

Serum concentrations of estrone were quantitated by radioimmunoassay using the procedure described by Wildt et al. (1979). All samples were analyzed in duplicate and the minimum detectable dose in this assay was 3.5 pg/tube. The interassay and intraassay coefficients of variation were 11.7% and 9.7% based on 8 and 11 estimates, respectively.

Serum estradiol-17β concentration was measured in duplicate by the method of Korenman et al. (1974). The minimum detectable dose in this assay was 2.5 pg/tube. The interassay and intraassay coefficients of variation were 9.4% and 7.1% based on 8 and 11 estimates, respectively.

Progesterone was quantitated in duplicate by the method of Koligian and Stormshak (1977). The minimum assay sensitivity was 30 pg/tube. The interassay and intraassay coefficients of variation were 8.9% and 8.7% based on 6 and 7 estimates, respectively.

Serum LH contents of serum samples in duplicate were analyzed by a double antibody radioimmunoassay described by Nett et al. (1975) and Chakraborty and Fletcher (1977). The minimum detectable LH concentration was 0.3 ng/ml in this assay system. The interassay and intraassay coefficients of variation were 12.2% and 9.8% based on 10 and 16 estimates, respectively.

All hormonal data were normalized to the day of preovulatory peak LH (Day 0) for both cycles. Assay data were analyzed using the Logit-Log method translated into Hewlett-Packard (Model 9830A) Basic by Grotjan and Steinberger (1977). Mean values are expressed as mean ± SEM and significant differences were determined by using Student's t test (Steel and Torrie, 1960).

RESULTS

A total of 138 serum samples for the first cycle and 267 samples for the second cycle was analyzed to determine the concentrations of E_1, E_2, LH and P.

Serum Level of Estrogens

Serum E_1 concentrations during the week preceding the preovulatory LH surge (week −1) were generally variable in both the first and the second estrous cycles (Fig. 1). The E_1 concentrations gradually increased reaching peaks of 34.0 ± 6.0 and 52.0 ± 16.0 pg/ml in the first and second estrous cycles, respectively, 1–2 days before the day of LH surge (Day 0). The E_1 concentrations declined thereafter, reaching basal concentrations in ∿7 days. The mean serum E_1 concentration during the week −1, however, was greater ($P<0.05$) in the second cycle (42.0 ± 3.0 pg/ml) compared with the first (29.0 ± 2.0 pg/ml, Fig. 2). Peak serum E_2 concentration (Fig. 1) was higher ($P<0.05$) in the second cycle compared with that of the first cycle (54.0 ± 8.0 vs 28.0 ± 7.0 pg/ml). Additionally, mean serum E_2 concentration during the week −1 was also greater ($P<0.05$) compared with that of the first cycle (47.0 ± 4.0 vs 22.0 ± 4.0 pg/ml) as shown in Fig. 2. The E_2 concentrations declined with the onset of the LH surge and reached basal concentrations

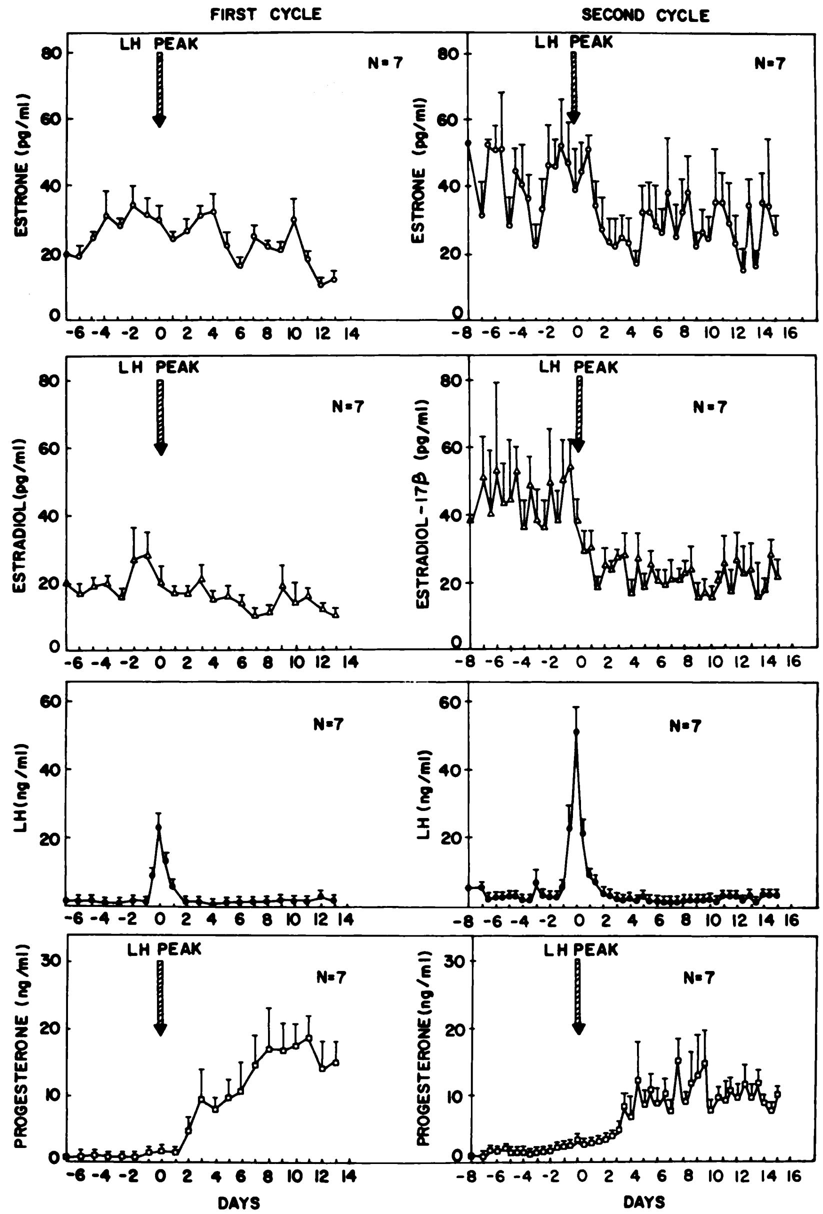

FIG. 1. Relationship of estrone, estradiol-17β, LH and progesterone at the first and second estrous cycle of the Labrador bitch.

during the second week (week +2) following Day 0.

Serum LH Levels

An initial rise in serum LH concentrations was observed during proestrus in the second cycle (Fig. 1) as the mean LH concentration during week −1 (4.8 ± 0.9 ng/ml) was greater ($P<0.05$) compared with that of the first week (2.5 ± 0.7 ng/ml, Fig. 2). The mean preovulatory LH surge (Day 0) reached a peak concentration of 51.6 ± 6.9 ng/ml in the second cycle (Fig. 1) compared with 23.1 ± 4.2 ng/ml at the first cycle. These values were also different ($P<0.01$).

An episodic burst of LH was detected in 6 of 7 animals between 2–7 days before Day 0

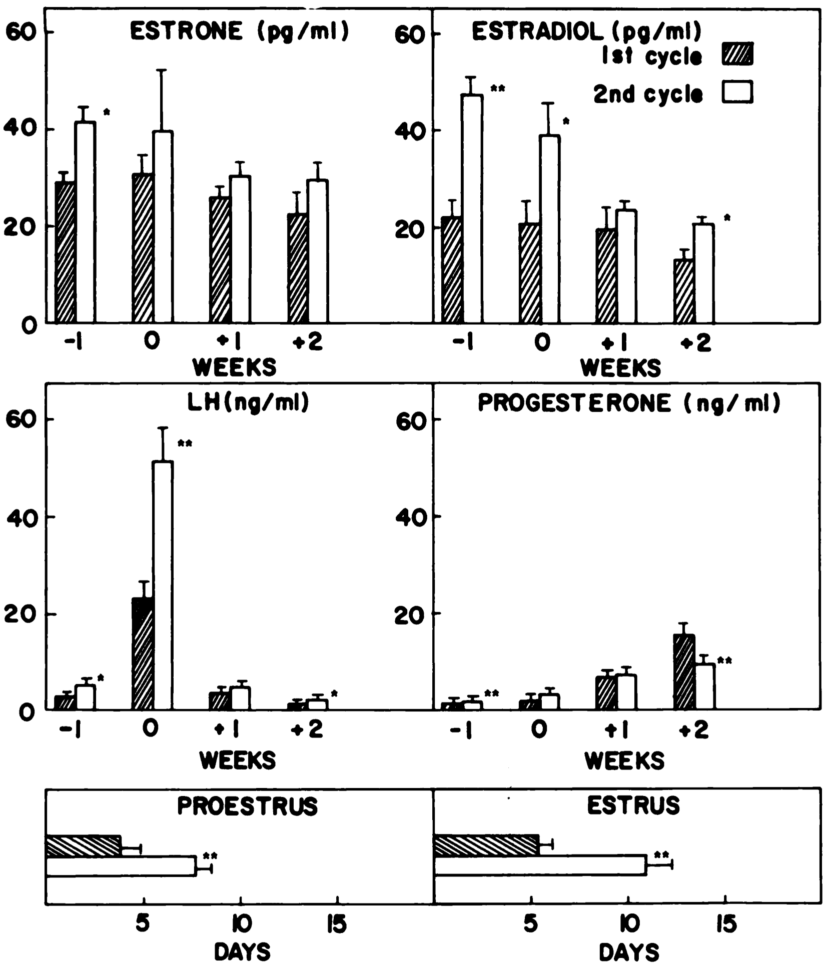

FIG. 2. Comparison of hormone levels and estrous behavior at the first and second estrous cycle of the Labrador bitch (*P<0.05, **P<0.01).

during the second estrous cycle, but this phenomenon was observed in only 1 animal during the first estrous cycle (Fig. 1). During the preovulatory surge, LH remained significantly elevated above baseline concentrations for 4.5–5 days during the second cycle compared with a much shorter duration of 2.5–3 days during the first cycle.

Serum Progesterone Levels

Serum progesterone concentrations are shown in Fig. 1 and mean weekly concentrations compared in Fig. 2. An initial rise in progesterone concentration during proestrus (week −1) was detected in the second cycle but not in the first. The mean progesterone concentration during this period was higher (P<0.05) in the second cycle (1.6 ± 0.1 ng/ml) compared with that of the first cycle (1.0 ± 0.3 ng/ml). After the peak serum LH concentration (Day 0), serum progesterone concentrations increased sharply in both cycles (Fig. 1). Although there was no significant difference in the progesterone concentrations during week +1, mean concentration during week +2 in the first cycle (15.8 ± 2.0 ng/ml) was higher (P<0.05) compared with that of the second cycle (9.4 ± 0.8 ng/ml).

Proestrous-Estrous Behavior

The relative lengths of proestrus and estrus for the first and second cycles are shown in Fig. 2. The duration of proestrus was shorter (P<0.01) at the first cycle (3.9 ± 0.7 days) compared with that of the second (7.7 ± 0.5 days). A similar trend was also observed in the duration of estrus, in which the estrous period during the second cycle (11.0 ± 1.1 days) was longer ($P<0.01$) compared with the estrous period in the first cycle (5.4 ± 0.4 days).

Both the age at which these animals exhibited the first behavioral estrus and the interval between the first and the second behavioral estrus were variable. The age at first estrus ranged from 11 to 16 months with a mean age of 13.9 months. The interestrual period between the first and the second cycle ranged from 4.4 to 7.5 months with a mean of 5.4 months.

DISCUSSION

Serum hormone concentrations observed during the second behavioral estrous cycle were similar to those reported in adult multiparous bitches by Wildt et al. (1978) and Nett et al. (1975). The concentrations of E_2 and LH were, however, significantly depressed during the first behavioral estrous cycle in the present study. An early episodic release of LH before the preovulatory LH surge has been implicated with a rise in preovulatory progesterone level and it has been suggested that this increased progesterone secretion initiates the onset of sexual receptivity in the bitch (Concannon et al., 1975; Wildt et al., 1979). Although this early LH release and increase in progesterone concentration was observed in 6 of 7 animals during the second cycle, only 1 animal showed evidence of such release during the first cycle. This appears to have resulted in a significantly shorter duration of sexual receptivity during the first estrous cycle.

A rising level of circulating estrogen concentrations is important in initiating the proestrous-estrous period in the bitch and has been suggested to trigger the preovulatory LH release in the bitch as in many other species (Concannon et al., 1975; Nett et al., 1975; Wildt et al., 1979). The significantly lower circulating level of estrogens may have contributed to the reduced LH release observed during the first behavioral estrous cycle. It does not appear that the lower preovulatory peak serum LH concentration during the first estrous cycle (23.1 ng/ml) was due to infrequent (once daily) sampling, because based on the same radioimmunoassay system and using the same collection frequency Nett et al. (1975) observed a higher level of preovulatory serum LH (35.5 ng/ml) in the multiparous bitch.

The mean serum progesterone concentration during proestrus (week −1) of the second cycle was significantly higher compared with that during the first cycle. In contrast, mean progesterone concentration during week +2 of the first cycle was significantly higher compared with that of the second cycle. Presence of episodic LH release during proestrus may have caused the preovulatory (week −1) rise in progesterone during the second cycle; however, no such explanation is apparent for the higher progesterone concentration during week +2 of the first cycle. Lack of comparable data on hormone concentrations during the first behavioral estrus precludes any definite conclusion and further investigation is necessary to evaluate the possible physiological significance of these observations.

The age at first behavioral estrus in this study ranged from 11 to 16 months compared with 6 to 12 months reported earlier by McDonald (1975). Although the variability of the age of onset of puberty was similar, animals in the present study generally exhibited behavioral estrus at a later age. It is possible that breed differences may exist (beagle vs Labrador) with regard to onset of puberty. In addition, the possibility of silent ovulatory cycles prior to onset of first observed estrus in the bitch comparable to that observed in the sheep at the start of the seasonal estrous cyclicity, cannot be ruled out.

The length of the proestrous-estrous period during the first cycle was significantly shorter compared with that of the second cycle. It has previously been suggested (Wildt et al., 1979; Concannon et al., 1977) that the episodic release of LH prior to the preovulatory LH surge initiates the preovulatory rise in progesterone which is responsible for the expression of behavioral estrus. Lack of such episodic LH release during the first cycle may explain this observation. It is suggested that such a short duration of sexual receptivity may reduce the chances of mating and pregnancy at the first behavioral estrous cycle in the bitch.

Based on these findings, it appears that the hormonal mechanism controlling estrous cycle may not be fully integrated and operative at the time of the first behavioral estrus in the bitch. Lower hormone concentrations during the first estrous cycle could result in a shorter period of sexual receptivity and lower reproductive performance.

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RECOMMENDED REVIEWS

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