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# The Ovarian Cycle of the Bitch: Plasma Estrogen, LH and Progesterone

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## ABSTRACT

Radioimmunoassays of estrogen and LH, and radioligand assays of progesterone were conducted on plasma samples collected serially and frequently during pregnant and nonpregnant cycles of Beagle bitches. Measurements of LH daily throughout proestrus and estrus in 20 cycles yielded mean (± SEM) basal levels of 1.4 ± 0.1 ng/ml, preovulatory elevations for 1–3 days, and peaks (7.5 ± 0.8 ng/ml) on the first day of estrus, or on the previous day. The day of the LH peak was designated as Day 0 for each cycle. Preovulatory increases in plasma progesterone could not be temporally dissociated from elevations in LH. Mean levels on Days −1, 0 and +1 were 0.8 ± 0.1, 1.6 ± 0.2 and 2.6 ± 0.2 ng/ml, both increases being significant ($P<.001$). Progesterone increased rapidly throughout estrus, reached 19.1 ± 2.5 ng/ml on Day 10 and a maximum of 22.9 ± 2.7 ng/ml on Day 25, and remained elevated until Day 30 at 19.9 ± 2.7 ng/ml. Mean plasma estrogen rose during proestrus; levels for Days 10, 3 and 1 before the LH peak were 26 ± 4, 43 ± 4, and 62 ± 4 pg/ml, respectively. Peak levels ranged from 46 to 113 ng/ml and occurred 0, 1, or 2 days prior to the LH peak. Estrogen levels fell rapidly coincident with or immediately following the LH peak.

No differences were found during the luteal phases of 12 pregnant and 10 nonpregnant cycles in mean maximum progesterone levels (29.1 ± 3.8 *vs* 26.8 ± 2.8 ng/ml), the ranges of individual maximum levels, nor the time of their occurrence. Levels declined gradually after Day 30 in both groups. However, the time at which plasma progesterone fell below 1 ng/ml was more variable ($P<.001$) in nonpregnant (51–82 days) than in pregnant (61–65 days) cycles. Plasma progesterone invariably declined during the two days prepartum; mean levels for Days 2, 1 and 0 prior to parturition were 3.3 ± 0.4, 2.1 ± 0.4 and 1.0 ± 0.1, each change being significant ($P<.05$). Secondary increases in plasma progesterone were found between Days 20 and 40 in 9 of 12 pregnant cycles. No comparable increases were seen in nonpregnant cycles. Plasma estrogen levels during the luteal phase remained constant (9–15 pg/ml) in nonpregnant cycles ($n=5$), but were elevated ($P<.01$) in pregnant cycles ($n=8$) during the three weeks prepartum and fell at parturition. Mean estrogen levels on Days 28, 4 and 0 prepartum were 27 ± 3, 19 ± 2 and 11 ± 2 pg/ml, respectively.

The data suggest that in the bitch: (1) late proestrus increases in plasma estrogen initiate and/or potentiate the preovulatory release of LH; (2) early preovulatory increases in plasma progesterone may facilitate the release of peak levels of LH and the onset of behavioral estrus; (3) pregnancy-specific mechanisms exist ensuring both a maintenance of elevated plasma progesterone levels throughout gestation and their decline to less than 1 ng/ml at parturition.

## INTRODUCTION

The histological, anatomical and behavioral correlates of the canine ovarian cycle have been described in detail (Andersen, 1970a, b; Andersen and Simpson, 1973; Bell and Christie, 1971; Evans and Cole, 1931). Briefly, following a 3–5 month anestrus of presumed ovarian quiescence, the bitch enters a 1–2 week proestrus characterized by follicle growth, swelling of the vulva and discharge of blood from the vagina. During proestrus she discourages attempts by the male to mount. Ovulation and CL formation take place early in the ensuing estrus which lasts another 1–2 weeks. During estrus copulation is encouraged by standing for the male, displaying the vulva, and deviating the tail to one side. Metestrus, the remainder of the luteal phase lasts about 2 months, and is characterized by loss of mating behavior, maintenance of pregnancy and/or a progestational uterus, and slow retrogression of corpora lutea until anestrus reappears.

Recent reports on the plasma levels of LH, estrogen and progesterone during these stages have added considerably to our understanding of canine reproductive physiology. However, limitations within and discrepancies among some of these reports have precluded any generalizations concerning the endocrine correlates of reproductive processes in the bitch and comparisons with those of other species.

Essentially identical plasma progesterone profiles have been reported for the luteal phases of pregnant and nonpregnant bitches (Parkes et

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al., 1972; Masken, 1972; Jöchle et al., 1973; Jones et al., 1973a, b, c). In contrast, Smith and McDonald (1974) found levels of serum progesterone higher in pregnant than in nonpregnant bitches during the second half of the luteal phase and undetectable following parturition. Reports of plasma progesterone concentrations below 1 ng/ml in mid-metestrus have led some investigators to conclude that there are "specific and peculiar hormonal patterns in the cycling and pregnant dog which are distinctively different from all other species reported on so far" (Jöchle et al., 1973). Preovulatory surges in circulating estrogen have been variably reported as undetected (Phemister et al., 1973), or as occurring on the day prior to the LH peak (Jones et al., 1973c), at 0-to 8 days prior to estrus (Bell et al., 1971) or on Day 2 of estrus (Hadley, 1973). Peak plasma levels of estrogen reported have ranged from 22 pg/ml (Hadley, 1973) to over 600 pg/ml (Phemister et al., 1973).

Hormone profiles obtained by sequential and frequent measurements of plasma estrogen, LH, and progesterone in pregnant and nonpregnant Beagle bitches, as they relate to the day of the LH peak or day of parturition are presented in the present paper. The data suggest that, within certain limits, the temporal changes in the reproductive hormones during the canine ovarian cycle are predictable, experimentally repeatable and not particularly unique.

## MATERIALS AND METHODS

### *Animals*

The bitches were 2–5 year old purebred Beagles born and maintained in indoor cages at the Cornell University Dog Farm. They were fed a commercial ration at 0700 or 1000 h and provided water *ad lib*. Pregnant bitches were provided a supplemental fortified meat diet during the last trimester of pregnancy. Ten ml blood samples were collected between 0800 and 1000 h by cephalic venipuncture into evacuated tubes containing 200 μl saturated sodium citrate solution as anti-coagulant. Plasma was obtained by centrifugation and stored frozen until assayed. Bitches were observed daily for the presence of a sanguinous discharge from the vagina and the day of discharge was recorded as the start of proestrus. Daily, or on alternate days, bitches were placed with intact or vasectomized males to determine the onset of estrus. Estrus was considered to commence on the day of the first observed successful copulation, or the first day the bitch no longer refused mounting by the male and also showed signs of vulval display or reflex deviation of the tail. Three of the 10 nonpregnant bitches included in this study were allowed single matings with intact males. Pregnancy could not be diagnosed by palpation in any of these animals. With few exceptions, blood samples were collected daily during proestrus and the first week of estrus, and 2 or 3 times weekly thereafter. Daily samples were also obtained from pregnant bitches during the week of parturition. Dates of observed estrus for 384 cycles in 80 bitches were used to estimate the mean interestrous interval.

### *Progesterone Assay*

Plasma levels of progesterone were determined by a competitive protein binding assay (Murphy, 1967; Neil et al., 1967), utilizing a rapid method of sample preparation (Johansson, 1969). Duplicate or triplicate aliquots of each sample (200–500 μl) were extracted with 5 ml of reagent grade petroleum ether (30–60°) for 60 min. After freezing the plasma, the petroleum ether extract was decanted into an assay tube and concentrated at the bottom by adding and evaporating two 0.5 ml petroleum ether rinses. In 10 separate studies, this procedure yielded repeatable (n=50) quantitative recovery of tritiated progesterone previously equilibrated with dog plasma (91 ± 1 percent), and low recovery of tritiated cortisol (2 percent) and corticosterone (1 percent).

The assay reagent contained 150 μl human plasma and 56 ng (1,2-$^3$H]-corticosterone (40 Ci/mM) per 100 ml distilled water. One ml reagent was placed in each of the sample or standard hormone (0–20 ng) replicate tubes. Following incubation of all tubes at 42 °C for 5 min, vigorous shaking on a horizontal shaker for 5 min, and incubation at room temperature for 20 min, the time intervals for subsequent steps were kept constant for each tube. These included incubation at 4 °C (10 min), addition of florisil (65 ± 2 mg) and shaking on a vortex mixer (30 sec) and final incubation at 4°C (10 min) before aliquoting for liquid scintillation counting.

Assayable progesterone levels ranged from 0.5 to 75 ng/ml plasma. The mean within assay coefficient of variation for samples was 11 percent. Twenty assays of the same 4 pools of dog plasma (1–18 ng/ml) yielded a mean between assay coefficient of variation of 14 percent. Results obtained for progesterone added to plasma (3, 6, 15 ng/ml) were linear and quantitative when corrected for initial content and mean recovery. Plasma from ovariectomized and ovariectomized, dexamethasone-treated bitches repeatably assayed less than 0.5 ng/ml. The values reported below were not corrected for recoveries and represent total extractable and assayable progestins. These include not only progesterone, but the metabolites 17α- and 20α-OH progesterone. These metabolites have not been thoroughly investigated in our laboratory, but Christie et al. (1971) reported that 17α-OH progesterone may account for as much as 15–20 percent of the progesterone assayed in dog plasma.

### *Estrogen Assays*

These assays of "total extracted immunoreactive estrogen" were made on plasma samples collected after a 16–20 h fast. Samples were prepared by adding 100 μl of 1N NaOH to duplicate 2.0 ml aliquots, extracting with 9.5 mls reagent grade methylene chloride for 1 h, aspirating the bulk of the plasma phase, freezing the plasma residue, and decanting the extract into tubes for sample concentration prior to

assay. In 10 separate studies this procedure resulted in a mean recovery rate of 81 ± 0.7 percent (n=40) of [2,4,6,7-$^3$H]-estradiol-17$\beta$ previously equilibrated with dog plasma. The assay procedure and reagents were as described by Echternkamp and Hansel (1973), except that the buffer containing [2,4,6,7-$^3$H]-estradiol-17$\beta$ (105 Ci/mM, 150,000 dpm/ml) was added first. Following vigorous agitation for 1 minute and incubation at room temperature for 1 h, buffer containing the antiserum was added. The tubes were shaken gently, and incubated overnight at 4°C. Recovery of estradiol-17$\beta$ equilibrated with dog plasma (12, 25, 50, 100 pg/ml) in 5 assays was linear and quantitative (91 ± 3 percent, n=20) when corrected for initial content and mean recovery. Plasma sample mean within assay coefficient of variation was 13 percent. Mean between assay coefficient of variation was 26 percent for 3 plasma pools in 28 assays. Reagent blanks averaged 4.4 ± 1 pg/tube and ovariectomized dog plasma 8.9 ± 1 pg/ml. Values reported were not corrected for plasma or reagent blanks, but were corrected for extraction losses.

*LH Assay*

Plasma levels of immunoreactive canine LH were assayed in triplicate by the method of Boyns et al. (1972). The only modifications were substitution of highly purified bovine serum albumin for human serum albumin in the buffer, the addition of hypophysectomized dog plasma to standard hormone tubes, inclusion of heparin (1U/ml) in the buffer used in the first incubation, and the use of a sheep antiserum to rabbit globulin diluted 1/40 for the final incubation. The mean sample within assay and between assay coefficients of variation were 13 percent (21 assays) and 16 percent (6 assays of 4 pools), respectively.

*Data Presentation*

Plasma samples were collected at intervals frequent enough to allow an adequate representation of circulating progesterone levels during the follicular and luteal phases in 22 cycles (12 pregnent and 10 nonpregnant). In all but two of these cycles samples were assayed for LH during proestrus and estrus. The day of the LH peak is considered Day 0 of the cycle for all data presented. The day of the first significant increase in progesterone was considered to be Day 0 for the two cycles in which LH was not assayed. Hormone profiles of pregnancy, from 14 days prepartum onward, were further aligned on a common day of parturition (Day 64 of the cycle). The mean (± SD) length of gestation for this group was 62 ± 2.4 days from first breeding and 65 ± 0.9 from the day of the LH peak. Plasma estrogen profiles were determined for 13 entire cycles (8 pregnant and 5 nonpregnant), and for the periods of proestrus and estrus in 5 additional cycles. All samples comprising a particular hormone profile for any one cycle were measured within a single assay. All individual values reported are the means of duplicate or triplicate determinations and can be evaluated in terms of the within assay coefficient of variation for that hormone. All means values are reported as mean ± standard error of the mean and significant differences were determined by Student's t test, except as noted otherwise.

## RESULTS

*Cycle Intervals*

Figure 1 shows the distribution of interestrous intervals in the kennel. The bimodal distribution for both pregnant and nonpregnant bitches suggested a true normal range of 140–300 days. Intervals greater than 320 days most likely represent instances where the second observed estrus was subsequent to an intervening unobserved estrus. Progesterone levels were determined in weekly or bimonthly plasma samples obtained from 6 bitches with observed interestrous periods of 320–400 days. Elevated luteal phase progesterone levels appeared in each of these bitches, beginning 150 to 200 days following the previously observed estrus. Mean observed interestrous periods (excluding those greater than 340 days) were longer ($P<.001$) for pregnant than nonpregnant cycles (230 ± 3 days, n=122 *vs* 202 ± 5 days, n=51).

*Individual Cycle Hormone Profiles*

Profiles for 4 bitches are presented in Figure 2. These profiles illustrate the variation in sample frequency, hormone levels, and temporal changes encountered in calculating the mean hormone profiles presented below. Data during metestrus from bitch 08 (Fig. 2d) were excluded from the means because fetal resorption may have occurred in this animal.

*Plasma LH*

Preovulatory increases in plasma LH levels were observed in each of 20 cycles in which samples throughout proestrus and estrus were assayed. Peak LH values ranged from 2.9 to

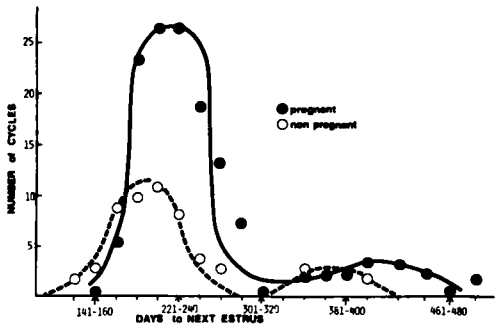


FIG. 1. Distribution of estrus-to-estrus intervals observed in Beagle bitches.

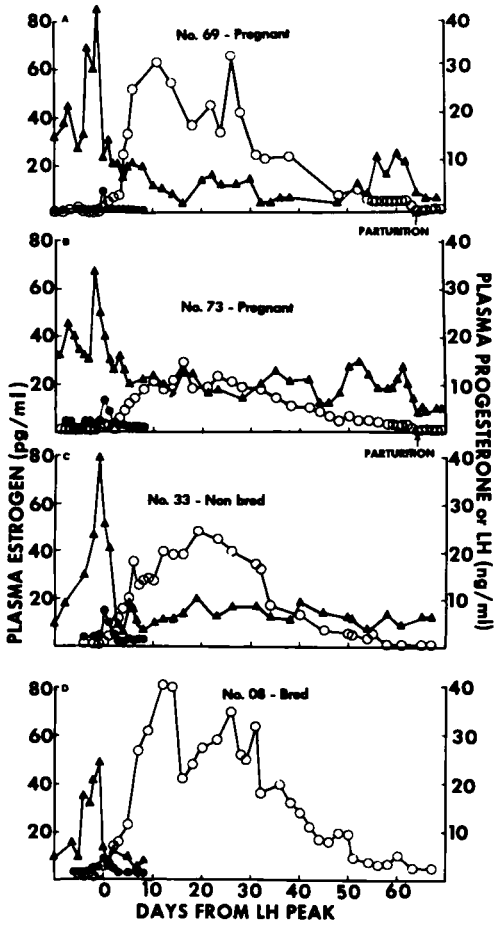


FIG. 2. Profiles of plasma estrogen (▲), LH (●) and progesterone (○) during 4 individual Beagle ovarian cycles.

17.5 ng/ml, and averaged 7.5 ± 0.8 ng/ml (Fig. 3b). Basal levels averaged 1.4 (0.7–2.0) ng/ml. Peak levels occurred from 2 days before to 4 days after the first observed copulatory activity. In contrast, using reflex tail deviation and vulval display as indicators of estrus, all LH peaks occurred on the first day of estrus or on the previous day. Plasma LH was elevated above baseline for two or more consecutive days in 10 cycles, and for a single day in the other 10 cycles.

*Progesterone*

Mean plasma progesterone levels for pregnant and nonpregnant cycles are depicted in Figure 4. Mean levels for all cycles increased rapidly following the LH peak, rising during estrus from 0.6 ± 0.1 ng/ml in late proestrus to

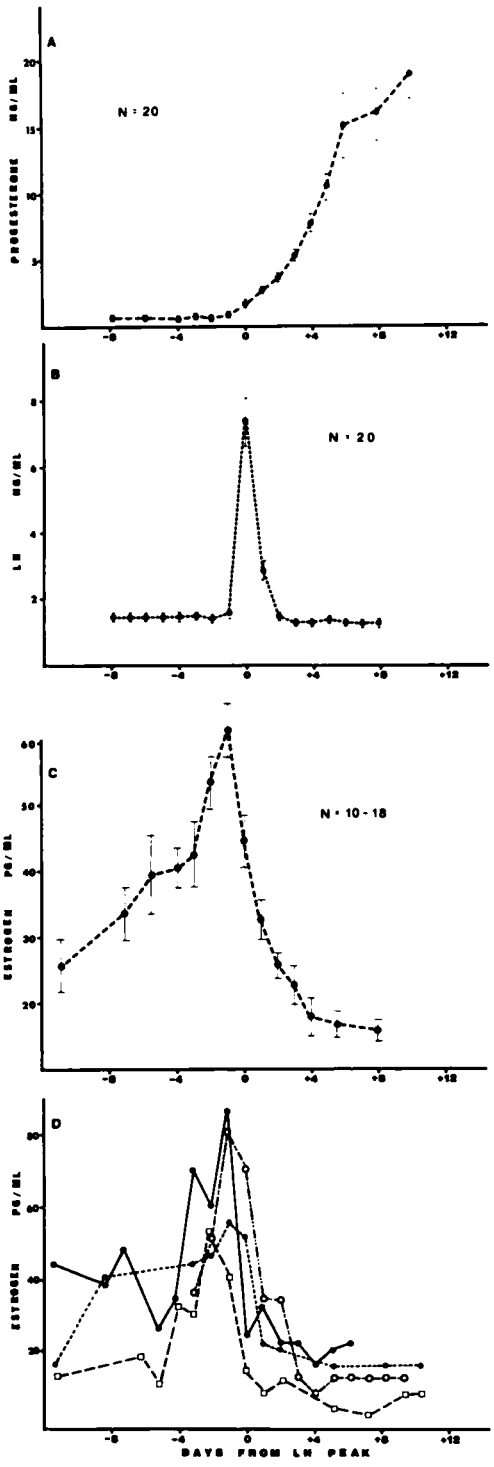


FIG. 3. Mean plasma levels of progesterone (A), LH (B), estrogen (C), and individual estrogen profiles (D) of Beagle bitches in relation to the day of observed LH peaks.

a concentration of 19.1 ± 2.5 ng/ml by Day 10. Levels remained elevated until Day 30 (19.9 ± 2.7 ng/ml), with a maximum mean level of 22.9 ± 2.7 ng/ml on Day 25. No difference could be found between pregnant and nonpregnant cycles for mean maximum progesterone levels (29.1 ± 3.8 *vs* 26.8 ± 2.8 ng/ml), their ranges (11.0–53.3 *vs* 15.5–40.5 ng/ml), or the time of their occurrence (8–29 days *vs* 12–28 days). Mean levels from 20 cycles in which daily concurrent progesterone and LH measurements were obtained depict the changes in circulating progesterone levels in relation to the LH peak (Fig. 3a). Mean progesterone levels on Days −1, 0, and 1 were 0.8 ± 0.1, 1.6 ± 0.2, and 2.6 ± 0.2 ng/ml respectively; both daily increases were significant ($P<.001$).

Plasma progesterone slowly decreased from Day 30 onward. Mean levels during pregnant (Fig. 4a) and nonpregnant (Fig. 4b) cycles were similar throughout most of metestrus. The somewhat higher mean levels observed between Days 35 and 56 in the pregnant cycles were not significantly different ($P>.05$) from those in nonpregnant cycles. However, distinct differences in plasma progesterone patterns were evident upon examination of individual profiles. Progesterone levels for 4 pregnant and 4 non-pregnant cycles are shown in Figs. 5 and 6,

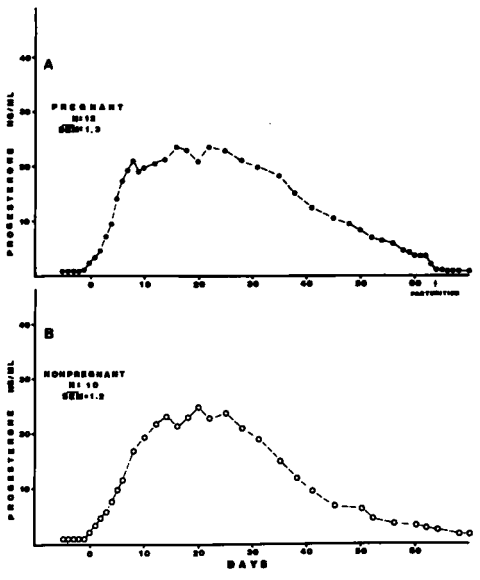


FIG. 4. Mean plasma progesterone levels in pregnant (A) and nonpregnant (B) Beagle bitches. Values for the 14 days prepartum are aligned to a common Day 64 parturition.

respectively. Increases in plasma progesterone occurred between Days 20 and 40 (Fig. 5) in 9 of the 12 pregnancies studied. Obvious second trimester increases were not evident (Fig. 2b, for example) in the other 3 pregnancies. In contrast, no progesterone increases at the corresponding time were found (Fig. 6) in any of the 10 nonpregnant bitches studied. One bitch that was bred but failed to whelp had a plasma progesterone pattern similar to that seen in the pregnant group (Fig. 2d) suggesting that implantation was followed by fetal resorption.

Progesterone profiles of pregnant and nonpregnant bitches also differed in the times and rates at which plasma levels declined to nondetectable levels. In each of the pregnant cycles plasma progesterone declined rapidly during the two days prior to parturition (Fig. 5). Mean progesterone levels for Days −3 to +1 from parturition (Day 64, Fig. 4a) were 3.4 ± 0.4, 3.3 ± 0.4, 2.1 ± 0.4, 1.0 ± 0.1 and 0.8 ± 0.1, respectively. The declines on each of the two prepartum days were significant ($P<.05$). In contrast, plasma progesterone levels in nonpregnant cycles (Fig. 6) always showed a gradual decline, reaching levels below 1 ng/ml as early as Day 51 and as late as Day 82. The variation in the number of days plasma progesterone was elevated above 1 ng/ml in individual cycles was significantly greater ($P<.001$) in nonpregnant than pregnant bitches (Table 1).

*Plasma Estrogen*

Mean plasma estrogen levels during proestrus and estrus for 18 cycles are shown in Fig. 3c. Mean levels were elevated at the start of proestrus (26 ± 4 pg/ml), continued to increase slowly through Day −3 (43 ± 4 pg/ml) and then increased rapidly reaching a peak of 62 ± 4 pg/ml. Plasma estrogen levels fell rapidly during estrus, reaching levels below those of early proestrus by Day 4 of the cycle (18 ± 3 pg/ml).

Patterns obtained for individual bitches are shown in Figure 3d and Figure 2. Peak levels ranged from 46 to 113 pg/ml, averaged 70 ± 4 pg/ml, and occurred at 0, 1 or 2 days before the LH peak (n=3, 11, 4 respectively). In many cases subsequently reduced estrogen levels were observed concomitant with the LH peak. Figure 7 summarizes the hormone profiles determined during proestrus and estrus as related to other changes observed during these periods.

Estrogen levels during metestrus in pregnant and nonpregnant cycles are shown in Figure 8.

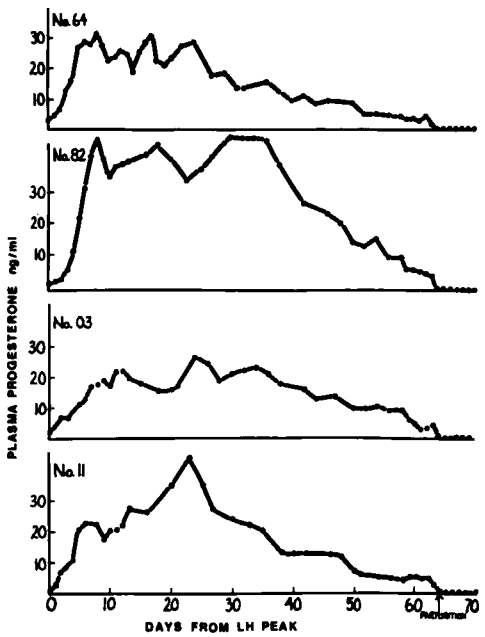


FIG. 5. Plasma progesterone profiles during 4 Beagle pregnancies. Values for the 14 days prepartum were aligned to a common Day 64 parturition.

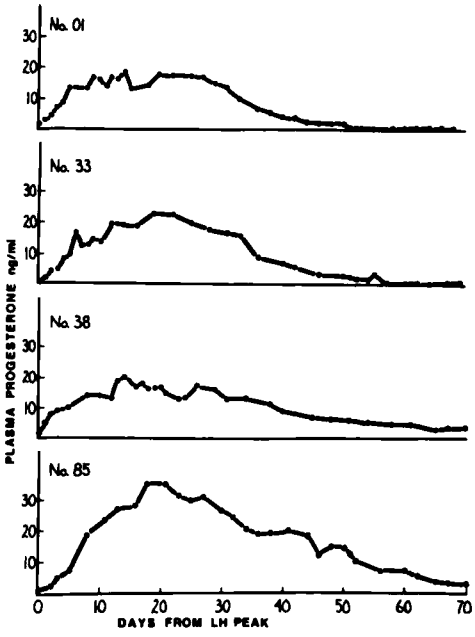


FIG. 6. Plasma progesterone profiles during 4 nonpregnant Beagle cycles.

Levels remained relatively constant in nonpregnant cycles (9–15 pg/ml) but were increased in pregnant cycles by Day 36 (27 ± 3 pg/ml), remained elevated until two days prepartum (19 ± 2 pg/ml, and fell rapidly to non-pregnant levels by the day of parturition (11 ± 2 pg/ml). Estrogen levels were higher ($P<.01$) in pregnant than nonpregnant cycles from Days 44 to 60. Successive differences ($P<.05$) were not found earlier in gestation.

## DISCUSSION

The physiological responses to the slightly elevated estrogen levels at the onset of proestrus (26 pg/ml) are considerable, and include hyperemia of the reproductive tract, vulval edema and uterine bleeding suggesting that the sex steroid target tissues of the bitch are very sensitive to small changes in circulating estrogen. The increase in plasma estrogen levels during early proestrus is most likely the consequence of resumed vesicular follicle growth reported to occur at this time (Andersen and Simpson, 1973). The finding of maximum plasma estrogen levels (46–113 pg/ml) during late proestrus in each of the cycles assayed, and their occurrence 2, 1, or 0 days prior to the LH peak, suggest that in the bitch, as in other species, an increase in plasma estrogen is a major component in the triggering mechanism for the preovulatory release of LH.

The occurrence, in individual cycles, of peak estrogen level as early as 2 days prior to the LH peak and of subsequently reduced levels coincident with the LH peak suggest that attainment of maximal estrogen levels in the bitch is not dependent on the preovulatory surge of LH. More likely, peak estrogen secretion is the result of unchecked follicular growth initiated at the start of proestrus. Moreover, the temporal relationships between the changing levels of estrogen, LH and progesterone (Fig. 7) suggest

TABLE 1. Days progesterone levels remained above 1 ng/ml in beagle ovarian cycles.

| Cycle | Days | | |
|---|---|---|---|
| | No. | Mean ± SEM | Range |
| Pregnant | 12 | 63.6 ± .3 | 61-65 |
| Nonpregnant | 12 | 68.0 ± 2.9[a] | 51-82 |

[a]Different ($P<.001$) from pregnant; Chi-square test for homogenity of variance (Steel and Torrie, 1960).

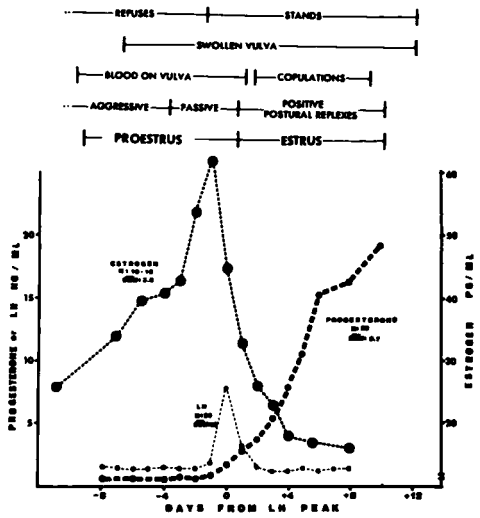


FIG. 7. Mean plasma levels of estrogen, LH and progesterone during proestrus and estrus in Beagle bitches. The vertical bars represent the mean time of onset and termination of the parameter indicated.

that the initial preovulatory increase in plasma LH, while stimulating total steroidogenesis and progesterone secretion, inhibits estrogen synthesis and depresses plasma estrogen levels.

Plasma levels of estrogen found in the present study were 10 times lower than those reported by Masken (1972) and Phemister et al. (1973), were similar to those reported by Bell et al. (1971), and slightly higher than levels of estradiol-17$\beta$ found by Jones et al. (1973b, c).

Plasma LH concentrations measured were similar to those reported by others using the same assay system (Boyns et al., 1972; Jones et al., 1973a, b, c). The occurrence and approximate timing of the preovulatory LH peak are in agreement with those reports and others (Masken, 1972; Phemister et al., 1973; Smith and McDonald, 1974).

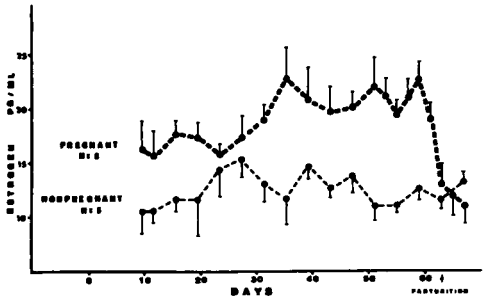


FIG. 8. Mean (± SEM) plasma estrogen levels during the pregnant and nonpregnant metestrus of Beagle bitches. Values for the 14 days prepartum are aligned on a common Day 64 parturition.

The presence of elevations in plasma LH lasting 1 to 3 days in samples collected daily for each of the cycles studied suggests that the preovulatory increase in the bitch has a duration of 18–48 h. Plasma LH peaks in the rat, rabbit, hamster, ewe and cow are of much shorter duration (Butcher et al., 1974; Dufy-Barbe et al., 1973; Bast and Greenwald, 1974; Goding et al., 1969; Hansel and Echternkamp, 1972). Prolonged elevation times for LH comparable to those in the bitch have been reported for the rhesus monkey (Weick et al., 1973).

Some of the variation among reports in the time of occurrence of the LH peak in relation to the onset of estrus may be due to differences in the criteria used to characterize estrus. Daily observations of sexual behavior throughout proestrus and estrus in approximately 200 cycles have shown that two distinctly different behavioral changes take place. Behavior observed early in proestrus is similar to that observed throughout most of the year. The responses to a male approaching from the rear are turning, confrontation, rearing, growling and biting. Later in proestrus, however, such behavior is increasingly suppressed. The responses then observed are attempts to escape, crouching, and eventually complete passivity, allowing the male to mount and occasionally submitting to intomission and ejaculation. This is followed in 1–3 days by the abrupt appearance of sexually positive elements in the bitch's behavior pattern. These include presentation of the hindquarters to the male, reflex deviation of the tail, elevation of the perineum and display of the vulva in a manner comparable to lordosis in rodents. The appearance of this sexually positive behavior represents the best criterion for estimating the onset of true estrus.

Viewed in reference to these behavioral changes, the observed hormone profiles (Fig. 7) suggest that increasing levels of plasma estrogen during proestrus depress the normal behavior pattern of the bitch, culminating in complete passivity at the end of proestrus when estrogen levels are maximal. Moreover, since the reflex behavior elements characteristic of estrus appeared coincident with or immediately following the LH peaks, it is possible that they are effected by the initial preovulatory increases in plasma progesterone found at this time.

The possibility that progesterone metabolites may have contributed to these initial

increases must be considered. However, more recent studies have verified the preovulatory rise in plasma progesterone as measured by a specific radioimmunoassay which does not crossreact with 17α OH-, or 20α OH-, progesterone (unpublished observations).

The bitch ovulates approximately 24–48 h after the LH peak following a 2–3 day period of luteinization of the granulosa (Phemister et al., 1973). The early increases in plasma progesterone may reflect preovulatory luteinization induced by LH stimulation. Preovulatory increases in progesterone have been reported in the ovarian venous or peripheral plasma of several polyovulatory laboratory species (Labhetswar et al., 1973; Shaikh and Harper, 1972; Butcher et al., 1974) and in the peripheral plasma of the monovulatory rhesus monkey (Weick et al., 1973).

In the rat small amounts of exogenous progesterone are synergistic with estrogen in inducing ovulation (Everett, 1961) and sensitizing the pituitary to LH-RH (Aiyer, 1974). In the bitch the times of the initial increases in progesterone and LH could not be dissociated (Fig. 3). Therefore the data, albeit based on once daily sampling, do not preclude the possibility that the initial increases in plasma progesterone parallel the initial increments in LH and synergize with the previous increases in estrogen to effect the release of peak levels of LH from the pituitary.

The general similarity found in mean progesterone levels during pregnant and nonpregnant cycles (Fig. 4), and the variability between animals in peak levels and the time of their occurrence, are in agreement with the reports of Masken (1972) and Jones et al. (1973a, b, c). However, distinct differences were found between gravid and nongravid cycles in the length of time measurable amounts of progesterone were detected in the plasma (Table 1; Figs. 5 and 6). This parameter was extremely variable among nonpregnant animals ranging from 51 to 82 days, lengths similar to those reported by Christie et al. (1971). But, in agreement with the results of Smith and McDonald (1974), elevated plasma progesterone levels were invariably maintained through gestation (Table 1) in the pregnant bitches, and in each instance declined to nondetectable levels at parturition. These observations suggest that in the bitch there are one or more mechanisms specific to pregnancy ensuring both a progesterone block to prevent premature parturition and a withdrawal of progesterone to facilitate normal parturition. The maintained progesterone and increased estrogen levels observed during gestation, and the prepartum decline in both, are not unique to the bitch, and have been observed in many mammalian species (Wagner et al., 1974; McCormack and Greenwald, 1974; Baranczuk and Greenwald, 1974).

The postimplantation increases in plasma progesterone and estrogen (Figs. 5 and 8) may be of ovarian or placental origin, or both. Smith and McDonald (1974) have suggested that a placental gonadotropin acts on the corpora lutea of the pregnant bitch. But Jones et al. (1973a) were unable to obtain evidence that any material immunologically cross-reactive with canine LH increased in the plasma of pregnant bitches. The abrupt declines in circulating progesterone and estrogen at the time of placental dislocation and parturition are suggestive of placental steroid secretion, and progesterone has been identified in canine placentas obtained just prior to parturition (Telegdy et al., 1963). Abortions following ovariectomy during pregnancy (Sokolowski, 1971; Andersen and Simpson, 1973) demonstrate the requirement for ovarian progesterone secretion through day 50–55 of gestation. However, the possible roles of gonadotropin and/or steroid secretion by the placenta in the normal maintenance of the terminal stages of pregnancy in the dog require further study.

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