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Relationship of Serum Estrone, Estradiol-17β and Progesterone to LH, Sexual Behavior and Time of Ovulation in the Bitch

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ABSTRACT

Serum estrone, estradiol-17β and progesterone were measured in bitches in which LH, sexual behavior and laparoscopically confirmed time of ovulation had been previously studied. Steroid values from 25 estrous periods were normalized to day of peak LH (Day 0). Estrone, estradiol-17β and progesterone concentration did not differ significantly in either dogs subjected to laparoscopy at 48 h intervals or those bitches undergoing no laparoscopy.

During the preovulatory interval, mean peripheral estrone concentrations were more variable than estradiol-17β; however, both estrogens tended to rise gradually throughout this time period. Overall maximal estradiol-17β concentration was detected on Day -2 with estrone peaking later (Day -0.5). Both estrogens declined ($P<0.05$) coincidentally with the LH surge. Progesterone from Days -14.0 through -1.5 was maintained at basal concentrations. An increase ($P<0.05$) in mean progesterone occurred on the day of the preovulatory LH peak which correlated with direct observation of apparent preovulatory follicle luteinization. Progesterone increased gradually from Day -0.5 through 8 and then varied for the remainder of the sampling period.

Analysis of individual cycles indicated that circulating serum concentrations of reproductive steroids fluctuated from day to day or within days of the proestrous-estrous interval. In dogs subjected to laparoscopy, initial evidence of the presence of visible preovulatory follicles was closely related to the first detectable elevations in both estrone and estradiol-17β. Either estrone or estradiol-17β concentrations or both surged sharply in 24 of the 25 cycles examined; however, only titers of estradiol-17β were elevated above baseline in all cycles prior to, or at the time of, the LH peak. In 16 of 25 cycles, estradiol-17β concentrations declined by 20 pg/ml or more 12–24 h prior to the onset of sexual receptivity. Bitches remained in estrus during periods of declining estrone and estradiol-17β and increasing progesterone levels.

These data integrate the hormonal, ovarian and behavioral events of the estrous cycle of the bitch and suggest that: 1) a preovulatory estradiol-17β surge exists and is likely responsible for triggering LH release; 2) estrone may play a supportive role to estradiol-17β in the endocrine control of LH secretion; 3) preovulatory changes in follicular morphology are distinct and can be correlated with a shift from estrone and estradiol-17β to progesterone secretion; 4) prolonged sexual receptivity in this species is supported in the presence of declining estrogen and continuously rising progesterone concentrations.

INTRODUCTION

The domestic bitch is monestrous, experiencing several months of sexual inactivity followed by a singular estrous period in a breeding season (Stabenfeldt and Shille, 1977). Unlike most domestic animals, the estrous cycle of the bitch is distinguished by a prolonged interval of proestrous-estrous activity. This unique characteristic has stimulated considerable interest in the behavioral-endocrinological-ovarian relationship of this species.

Concentrations of LH, estradiol-17β (or total estrogens) and/or progesterone in peripheral blood sampled once daily have been measured throughout the estrous cycle of the bitch (Bell et al., 1971; Christie et al., 1971; Jones et al., 1973; Smith and McDonald, 1974; Concannon et al., 1975; Edqvist et al., 1975; Nett et al., 1975; Austad et al., 1976; Mellin et al., 1976). However, these studies have often yielded differing conclusions on temporal relationships particularly among alterations in reproductive steroids, the preovulatory surge of LH and sexual behavior. These inconsistencies recently

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were reviewed and discussed in detail by Concannon et al. (1977) who suggested that many of the differences among investigations were due to the varied subjective criteria used in identifying the onset of true behavioral estrus. Another reason for these incongruities is that most studies have attempted to establish definitive reproductive hormonal relationships from blood samples obtained only 1 time per day. In addition, most investigations have been concerned with correlating hormonal concentrations and behavior without attempting to integrate these occurrences to ovarian follicle development, maturation and time of ovulation.

It is apparent that a more detailed description of the time course of these hormones is necessary to define precisely the functional interactions between the ovarian steroids and LH secretion as well as the relationship of these hormones to the onset of sexual receptivity and time of ovulation. The studies in our laboratory have been concerned with a comprehensive examination of these parameters in a group of 15 adult bitches. A recent report has described the relationship between LH concentration, reproductive behavior and time of ovulation in these animals (Wildt et al., 1978). In this study, blood samples were obtained from bitches twice daily during the proestrous-estrous interval. A preovulatory LH surge (mean peak serum concentration 45.1 ng/ml) which was prolonged over a 3.5–4 day interval was detected in all animals. The greatest incidence of laparoscopically confirmed ovulation (77.2% of follicles ovulating) occurred over the interval of 24–72 h following the LH peak. Considerable variability in length of proestrus (mean 8.7 ± 0.7 days) and estrus (mean 11.4 ± 0.9 days) was noted and it was not possible to relate the LH surge or onset of ovulation to a specific day of proestrus or estrus.

The present study continues to examine estrous cyclicity in this group of dogs. In this paper we report the time courses of serum estrone, estradiol-17β and progesterone concentrations and the relationship of these hormones to the previously studied changes in serum LH and the occurrences of sexual receptivity and ovulation. Of particular interest is the existence of a distinct preovulatory estradiol-17β surge, the observation of preovulatory follicle luteinization, increased progesterone secretion and the previously unpublished information on serum estrone concentrations.

MATERIALS AND METHODS

Experimental Protocol

A detailed discussion of the experimental protocol including information on animals, laparoscopic methods and the radioimmunoassay for serum LH has been presented previously (Wildt et al., 1978). In brief, 15 adult multiparous bitches were assigned to 1 of 2 groups. Group 1 consisted of 9 bitches (7 beagles, 1 pointer, 1 Labrador) previously subjected to a laparoscopic surgical technique for exposing the ovary which is normally encapsulated in an ovarian bursa (Wildt et al., 1977, 1978). In each of these dogs, one or both ovaries were completely visible at laparoscopic examination. The remaining 6 bitches (4 beagles, 1 pointer, 1 Labrador) were designated Group 2 and were treated similarly to Group 1, but were not subjected to laparoscopy during the study.

The experimental protocol was initiated in each bitch at the first evidence of a proestrous sanguineous vaginal discharge. Blood samples were collected by jugular venipuncture twice daily (0900 and 1600 h) during proestrus and the first 4–12 days of estrus. Samples were drawn once daily (0900 h) during the remaining days of estrus. Sexual behavior was assessed daily during the experimental period by exposing each bitch to 1 of 2 vasectomized male dogs. A bitch was considered to be in estrus when she exhibited the criteria described by Concannon et al. (1975) which included displaying the classical lordosis response and allowing the stud to mount and initiate coitus. Copulation between the stud and estrous bitch was not permitted. Bitches in Group 1 were subjected to laparoscopy at 48 h intervals during proestrus and estrus to view ovarian activity directly. A record was made of follicular development and particularly the morphological events associated with follicular rupture and corpus luteum formation.

Extraction of Serum Samples for Radioimmunoassay

Aliquots (2 ml) of each serum sample were extracted with 10 ml of diethylether (spectrograde, Fisher Scientific) by vortexing for 30 seconds in 20 ml glass scintillation vials. The ether layer was decanted from the aqueous layer following snap freezing of the aqueous layer in liquid nitrogen. The ether extract was evaporated to dryness and redissolved in 2 ml of absolute ethanol. Recoveries ranged from 79.0–92.0% as determined from tracer added prior to extraction. All steroid concentrations were corrected for mean recovery using recovery standards determined for that assay.

Estrogen Radioimmunoassays

An estrone assay was developed and validated. This assay involved evaporating duplicate 0.4 ml aliquots of the alcoholic solution of the ether extract to dryness at room temperature. The antiserum was anti-estrone-6-thyroglobulin serum (Lot #TH2) purchased from Miles Laboratories (Elkhart, IN). The alcoholic fractions were reconstituted with 0.1 ml of the PBSG buffer containing 0.01 M $NaH_2PO_4 \cdot H_2O$, 0.15 M NaCl, 0.1% sodium azide and 0.1% gelatin, pH 7.2 at 25°C. Standard solutions of estrone ranging from 5.0–250.0 pg/tube were prepared in 0.1 ml of PBSG

buffer. Approximately 25.0 pg of 2, 4, 6, 7-[^{3}H]-estrone (115 Ci/mMol) were added to the tubes in 0.1 ml of PBSG. The antiserum (dilution 1:4000) was added to the tubes in 0.1 ml of PBSG and incubated at room temperature for 2 h. Following incubation, 0.4 ml of a hydroxylapatite (HAP) slurry (30 g of dry HAP in 250 ml of 0.005 M $NaH_2PO_4 \cdot H_2O$, pH 7.2 at 25°C) was added as described previously by Trafford et al. (1976). The HAP was allowed to settle at least 12 h at 4°C. The supernatant fraction containing the free estrone was then aliquoted and counted in 2.5 ml of Formula 947 scintillation counting fluid in a Packard 2651 liquid scintillation spectrophotometer. The recovery of estrone added to pooled canine serum was 96.5% with a correlation coefficient of 0.97. The interassay and intraassay coefficients of variations were 11.7% and 9.7% based on 11 and 8 estimates, respectively. The minimum detectable dose of estrone was 3.5 pg/tube. The reagent blank was always less than 2.0 pg/tube and usually less than 1.0 pg/tube. The cross reactivity of the antiserum was checked with various steroids to insure its ability to quantitate estrone without chromatographic separation of the steroids.

Estradiol-17β was quantitated by the method validated by Korenman et al. (1974). Methods were similar to the previously described estrone assay with the following modifications. The antiserum used was anti-17β-estradiol-6-BSA (dilution 1:4500) prepared by the method of Niswender (1973) and the isotope was 2, 4, 6, 7-[^{3}H]-estradiol-17β (115 Ci/mMol). In this assay the antiserum was added in a volume of 0.05 ml in PBSG buffer. Standard solutions of estradiol-17β ranged from 5.0-250.0 pg/assay tube. Mean recovery of added estradiol-17β from pooled dog serum was 97.1% with a correlation coefficient of 0.98. The interassay and intraassay coefficients of variation were 9.4% (n = 11) and 7.1% (n = 8), respectively. The minimum detectable dose of estradiol-17β was 2.5 pg/tube. Reagent blank values were always less than 2.0 pg/tube and usually less than 1.0 pg/tube.

Progesterone Radioimmunoassay

Progesterone was quantitated by the method of Koligian and Stormshak (1977) using anti-progesterone-11-BSA antiserum. Progesterone standard solutions ranged from 30.0-2000.0 pg/tube. Duplicate aliquots of 0.025 ml and 0.05 ml of the alcoholic fractions were evaporated to dryness and reconstituted in 0.1 ml PBSG. Approximately 50.0—60.0 pg of 1, 2, 6, 7-[^{3}H]-progesterone (114 Ci/mMol) were added in 0.1 of PBSG. The progesterone antiserum (dilution 1:2500) was added in 0.2 ml of PBSG and after a 2 h incubation at room temperature, free from bound isotope was separated by the HAP method described above. The interassay and intraassay coefficients of variation were 8.9% (n = 7) and 8.7% (n = 6), respectively. Minimum assay sensitivity was 30.0 pg/tube progesterone.

Hydroxylapatite Procedure

Free from bound steroid was separated by the precipitation of antibody by hydroxylapatite (HAP). The effect of time of HAP exposure on the bound:free ratio of each radioimmunoassay was determined. Reaction mixtures containing the sample, isotope and antibody were allowed to equilibrate at room temperature. After cooling to 4°C, the HAP slurry was added. At specific times between 1 min and 24 h, the reaction mixture was centrifuged to sediment the HAP. Aliquots of the supernatant were used to determine the free cpm of hormone.

Evaluation of Data

A total of 25 proestrous-estrous intervals (Group 1 = 15, Group 2 = 10) were analyzed from the 15 bitches studied. The calculation of the standard curves and unknowns were performed using a 4 parameter logistic curve fitting program on a Hewlett-Packard Model 9830A computer (Grotjan and Steinberger, 1977). Serum LH concentrations were standardized so that the day of peak serum LH was designated as Day 0. Estrone, estradiol-17β and progesterone profiles were normalized by aligning serum hormone values in individual animals to the day of maximum LH concentration (Day 0). Unless otherwise indicated, values reported are means ± SEM. Statistical significance of data was tested using analysis of variance. Student's t test was employed to compare means.

RESULTS

The results of the cross reactivity study involving the estrone antibody are presented in Table 1. The percentage cross reaction with estradiol-17β was less than 1.0%. Other steroids also displayed low degrees of cross reactivity thus indicating that the specificity of this antiserum is sufficient to justify its use without prior chromotographic separation.

The relationship of HAP exposure time to the bound:free ratio for the progesterone radioimmunoassay is illustrated in Table 2. No changes in the bound:free ratio were observed over a 24 h interval. Similar results were obtained with the estrone and estradiol-17β radioimmunoassays.

Mean concentrations of estrone, estradiol-17β and progesterone were calculated and analyzed in dogs subjected to laparoscopy

TABLE 1. Cross reaction of estrone-6-thyroglobulin antiserum with other steroids.

Steroid	% Cross reaction
Estrone	100.0
Estradiol-17β	<1.0
Estriol	<0.1
Testosterone	<0.1
Dihydrotestosterone	<0.1
Progesterone	<0.1
17-OH Progesterone	<0.1

TABLE 2. Effect of time of exposure to hydroxylapatite (HAP) on the bound:free ratio of the progesterone radioimmunoassay.

	Time[a]							
	1 min	10 min	1 h	2 h	4 h	8 h	18 h	24 h
b/f[b]	0.46	0.49	0.48	0.47	0.48	0.49	0.48	0.47

[a]Specified time intervals after which the reaction mixture was centrifuged to sediment HAP and cpm determined from aliquots of supernatant.

[b]Bound to free ratio.

(Group 1) and those undergoing no laparoscopy (Group 2). No significant differences ($P>0.05$) were detected between Groups 1 and 2 or among the 3 breeds of dogs with respect to relative fluctuations in individual hormone patterns. The values from both groups were pooled to obtain an overall profile of estrone, estradiol-17β and progesterone based on the total of 25 cycles examined. Figure 1 illustrates the endocrine relationship of these reproductive steroids to serum LH and the interval of the greatest incidence of ovulation (24–72 h following peak LH). Table 3 indicates the specific mean serum concentrations (±SEM) of the reproductive hormones at each sampling period.

The estrone:estradiol-17β ratio throughout the experimental period approximated 1.4. Two weeks prior to the LH peak (Day 0) basal estrone and estradiol-17β concentrations ranged from 26.3–29.3 pg/ml and 14.0–19.0 pg/ml, respectively. Both hormones appeared to begin to rise at approximately Day -11 to -10. During the sampling interval, estrone varied considerably, but peripheral concentrations were significantly ($P<0.05$) elevated on Days -3, -1, -0.5 and 0 compared to levels measured on the first 2 days of proestrus (Days -14 to -13). Overall, during the pre-LH surge interval estrone tended to increase gradually with this hormone, eventually peaking at 57.0 ± 9.9 pg/ml on Day -0.5. Mean estradiol-17β increased gradually during this period and was greater ($P<0.05$) by Day -8 than when measured on Days -14 to -13. Maximal estradiol-17β concentrations were detected over an interval from Days -3.5–0 with a peak value of 42.8 ± 5.9 pg/ml measured on Day -2. Both estrogens declined coincidentally with the onset of the LH surge and prior to the initiation of ovulation. Mean estrone titers declined significantly ($P<0.05$) between Days -0.5 and Day 1. Estrone then tended to decrease further through Day 3, ranged between 23.3 and 34.2 pg/ml from Days 3.5–11.5, after which estrone was variable, but approached early preovulatory baseline concentrations. Estradiol-17β levels decreased significantly ($P<0.05$) from the peak observed 48 h prior to the LH surge through Day 2. Consequently during the period of peak ovarian follicle maturation and the early stages of the ovulation interval, titers of this hormone continued to decline. Estradiol-17β plateaued between 20.1 and 27.9 pg/ml on Days 2–8 and then decreased to nadir.

Basal progesterone concentrations from Days -14.0 through -1.5 varied over a narrow range from 0.8–2.9 ng/ml. An increase ($P<.05$) in progesterone occurred from 2.3 ng/ml on Day -1.5 to 4.4 ng/ml on Day 0. This initial significant elevation in progesterone corresponded with laparoscopic morphological evidence of preovulatory follicle luteinization prior to ovulation. Pictorial evidence of these anatomical events has been previously presented (Wildt et al., 1978). Mean progesterone values gradually increased through Day 8 and then varied between 13.9 and 31.0 ng/ml through the remainder of the sampling period.

A more specific analysis of the relationship between these reproductive parameters was obtained by examining the data from individual animals. Figures 2–5 illustrate typical hormonal patterns and lengths of the estrous period from 4 bitches. Additional information on the ovulation interval has been included on 2 bitches subjected to laparoscopy (Figs. 2, 3).

Examination of individual cycles indicated that circulating serum concentrations of reproductive steroids fluctuated considerably from day to day or within the days of the proesterous-estrous interval. This oscillatory pattern of steroid secretion was observed in all 25 cycles. There appeared to be more daily variation in titers of estrone than estradiol-17β.

In individual cycles monitored by laparoscopy, the first distinct rise in estrone and estradiol-17β concentrations above baseline was

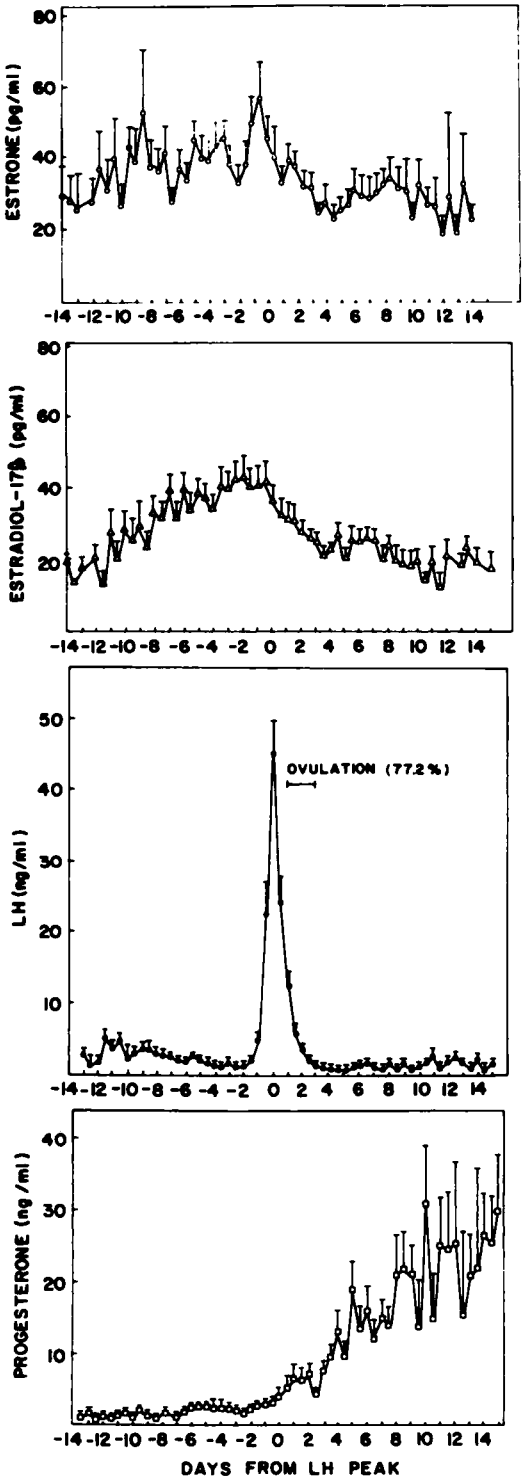

FIG. 1. Mean (±SEM) serum estrone, estradiol-17β, LH and progesterone profiles based on 25 proestrous-estrous periods in the bitch. Bracket (⊢⊣) indicates interval of the greatest incidence of confirmed ovulation (77.2% of follicles ovulating).

closely related to the first visual evidence of follicles on the ovarian surface. When compared to early proestrous basal concentrations, detectable elevations in both estrogens were observed within ±48 h of the first day of discovered follicular development in all 15 Group 1 cycles.

Overall, in 16 of the 25 cycles examined, both estrone and estradiol-17β rose sharply and fell coincidentally with or near the LH surge. Four of the 25 cycles produced an estrone but not an estradiol-17β surge and 4 cycles produced an estradiol-17β but not an estrone surge. In 1 cycle, both estrone and estradiol-17β were elevated above basal level, but neither rose sharply at this time. Thus, overall, in 24 of 25 cycles analyzed, either estrone or estradiol-17β surges or both were detected near the LH surge. Estrone rose sharply 0–72 h prior to peak LH (Figs. 2, 3, 5) with surge concentrations ranging from 42.0–185.0 pg/ml. In 5 of the 25 cycles, estrone was not elevated near the period of increased LH and either peaked during early proestrus or late estrus (Fig. 4). In general, circulating concentrations of estradiol-17β increased gradually over the 10–11 day interval preceding the ovulatory rise in LH (Figs. 2, 3, 5). In 20 of the cycles, the rise in this hormone ended in a distinct surge (peak range 48.0–107.0 pg/ml) within 72 h preceding the LH peak.

There was no clear relationship between changes in estrone concentration and sexual behavior. In 14 of the 25 cycles, maximum levels of estrone occurred during estrus. In the remaining animals, estrone concentration peaked 1–3.5 days prior to the onset of sexual receptivity. Estradiol-17β titers varied with respect to estrous behavior and, in general, were not sustained at comparatively high levels during estrus. In individual cycles, estradiol-17β concentrations peaked and then gradually declined (Fig. 5), fluctuated dramatically (Figs. 3, 4) or decreased sharply and remained near basal level (Fig. 2) throughout the estrous period. In 16 of the 25 cycles examined, estradiol-17β decreased 20 pg/ml or more approximately 12–24 h prior to the onset of sexual receptivity.

Results from individual animals indicated that progesterone concentrations remained relatively stable prior to the onset of the LH surge. Previous examinations of the LH profiles in these animals indicated that an episodic burst of LH occurred in 13 of the 25 cycles examined

TABLE 3. Mean (±SEM) serum estrone (E_1), estradiol-17β (E_2-17β), luteinizing hormone (LH) and progesterone (P) concentrations based on 25 proestrous-estrous periods in the bitch.

Day[a]	E_1 (pg/ml)	E_2-17β (pg/ml)	LH (ng/ml)	P (ng/ml)	Day	E_1 (pg/ml)	E_2-17β (pg/ml)	LH (ng/ml)	P (ng/ml)
-14.0	29.3 ± 8.4	19.0 ± 2.5	2.0 ± 0.9	1.1 ± 0.4	0.5	40.4 ± 8.0	32.5 ± 4.3	24.2 ± 3.4	5.0 ± 2.0
-13.5	28.0 ± 7.0	14.0 ± 0.0	2.3 ± 0.3	0.9 ± 0.2	1.0	33.0 ± 4.3	31.5 ± 4.7	12.3 ± 1.8	6.4 ± 2.1
-13.0	26.3 ± 9.0	18.0 ± 1.7	2.6 ± 0.8	1.5 ± 0.6	1.5	39.4 ± 4.5	31.6 ± 4.0	5.8 ± 1.2	6.4 ± 1.7
-12.5	27.3 ± 7.5	19.0 ± 2.4	1.4 ± 1.1	0.8 ± 0.1	2.0	37.1 ± 4.8	27.9 ± 3.4	3.3 ± 0.8	7.1 ± 1.4
-12.0	28.3 ± 6.0	20.5 ± 3.0	1.4 ± 0.4	1.1 ± 0.3	2.5	32.3 ± 3.8	25.7 ± 2.4	1.8 ± 0.4	4.5 ± 0.7
-11.5	37.0 ± 10.5	12.5 ± 2.5	4.9 ± 1.3	0.8 ± 0.2	3.0	31.9 ± 3.9	25.0 ± 2.4	1.2 ± 0.4	7.7 ± 1.3
-11.0	31.0 ± 8.2	27.0 ± 6.5	3.5 ± 0.9	1.3 ± 0.4	3.5	24.7 ± 2.2	21.1 ± 2.4	1.1 ± 0.3	9.6 ± 1.8
-10.5	40.0 ± 11.0	19.5 ± 5.5	4.6 ± 1.2	1.4 ± 0.4	4.0	27.9 ± 4.5	22.5 ± 2.7	0.9 ± 0.2	13.2 ± 3.0
-10.0	26.6 ± 5.2	28.0 ± 5.5	2.2 ± 0.8	0.9 ± 0.3	4.5	23.1 ± 3.6	27.0 ± 4.1	0.7 ± 0.2	9.7 ± 2.0
-9.5	43.4 ± 4.8	24.7 ± 6.5	2.9 ± 0.9	2.0 ± 0.8	5.0	25.3 ± 3.2	20.1 ± 3.1	0.6 ± 0.2	19.0 ± 4.0
-9.0	39.3 ± 8.9	29.0 ± 7.3	3.4 ± 1.1	1.4 ± 0.3	5.5	27.2 ± 3.8	25.5 ± 3.9	1.1 ± 0.4	13.5 ± 3.3
-8.5	53.2 ± 17.5	22.0 ± 5.4	3.4 ± 1.2	0.9 ± 0.2	6.0	31.2 ± 5.2	25.3 ± 3.1	1.2 ± 0.5	16.0 ± 3.4
-8.0	37.6 ± 7.8	32.9 ± 4.5	2.9 ± 0.8	1.3 ± 0.2	6.5	29.6 ± 5.0	26.0 ± 3.0	1.6 ± 0.6	12.3 ± 2.6
-7.5	36.2 ± 6.3	31.6 ± 4.9	2.7 ± 0.7	1.4 ± 0.2	7.0	28.9 ± 5.5	25.1 ± 3.7	0.8 ± 0.2	15.2 ± 2.6
-7.0	41.8 ± 6.8	39.0 ± 5.1	2.4 ± 0.5	1.3 ± 0.2	7.5	30.3 ± 5.3	20.1 ± 3.6	0.8 ± 0.3	14.0 ± 2.1
-6.5	27.7 ± 3.3	31.5 ± 4.1	2.0 ± 0.4	1.8 ± 0.6	8.0	32.7 ± 3.8	23.3 ± 3.1	1.5 ± 0.7	21.2 ± 5.7
-6.0	36.9 ± 5.4	39.3 ± 4.4	1.8 ± 0.4	2.9 ± 0.5	8.5	34.2 ± 5.7	19.5 ± 4.5	0.5 ± 0.1	21.9 ± 5.3
-5.5	34.2 ± 4.5	33.8 ± 4.4	2.5 ± 0.5	2.9 ± 0.7	9.0	31.5 ± 5.0	18.7 ± 2.8	1.4 ± 0.6	21.1 ± 4.0
-5.0	44.9 ± 5.2	38.4 ± 4.0	1.9 ± 0.6	2.9 ± 0.4	9.5	30.8 ± 8.1	18.3 ± 3.8	0.6 ± 0.3	13.9 ± 6.5
-4.5	40.4 ± 5.9	37.4 ± 4.7	1.3 ± 0.3	2.8 ± 0.8	10.0	23.3 ± 3.5	19.6 ± 2.9	1.0 ± 0.3	31.0 ± 8.0
-4.0	39.4 ± 6.4	34.3 ± 3.7	1.1 ± 0.3	2.8 ± 0.9	10.5	32.5 ± 6.5	14.4 ± 1.6	1.5 ± 0.5	14.9 ± 6.0
-3.5	42.4 ± 7.8	40.1 ± 5.9	1.0 ± 0.2	2.2 ± 0.4	11.0	27.2 ± 4.0	20.0 ± 3.3	2.4 ± 1.5	25.2 ± 6.7
-3.0	46.3 ± 4.3	39.3 ± 5.2	1.6 ± 0.4	2.0 ± 0.4	11.5	26.7 ± 7.4	12.2 ± 4.2	0.7 ± 0.4	24.6 ± 8.1
-2.5	38.4 ± 4.9	42.7 ± 5.6	1.0 ± 0.2	1.8 ± 0.3	12.0	18.8 ± 4.5	21.0 ± 3.8	1.7 ± 0.7	25.7 ± 11.0
-2.0	32.8 ± 4.1	42.8 ± 5.9	1.1 ± 0.3	2.1 ± 0.3	12.5	29.5 ± 22.5	19.9 ± 3.4	2.1 ± 1.0	15.5 ± 11.5
-1.5	38.4 ± 6.0	40.3 ± 5.3	1.8 ± 0.4	2.3 ± 0.6	13.0	19.5 ± 5.3	18.8 ± 3.1	1.6 ± 0.5	21.3 ± 5.4
-1.0	50.3 ± 7.4	40.9 ± 5.3	4.8 ± 1.1	2.7 ± 0.4	13.5	32.7 ± 13.8	22.7 ± 3.2	0.8 ± 0.6	22.0 ± 14.0
-0.5	57.0 ± 9.9	41.5 ± 6.0	22.4 ± 4.4	3.1 ± 0.8	14.0	22.6 ± 3.0	19.0 ± 4.2	2.1 ± 0.9	26.6 ± 5.7
0.0	45.5 ± 5.4	36.2 ± 4.7	45.1 ± 4.4	4.4 ± 0.9	14.5	26.1 ± 9.6	20.8 ± 3.7	0.4 ± 0.2	25.8 ± 6.2
					15.0	21.1 ± 4.2	17.8 ± 4.1	1.4 ± 0.7	30.0 ± 7.7

[a]Day of peak serum LH = Day 0.

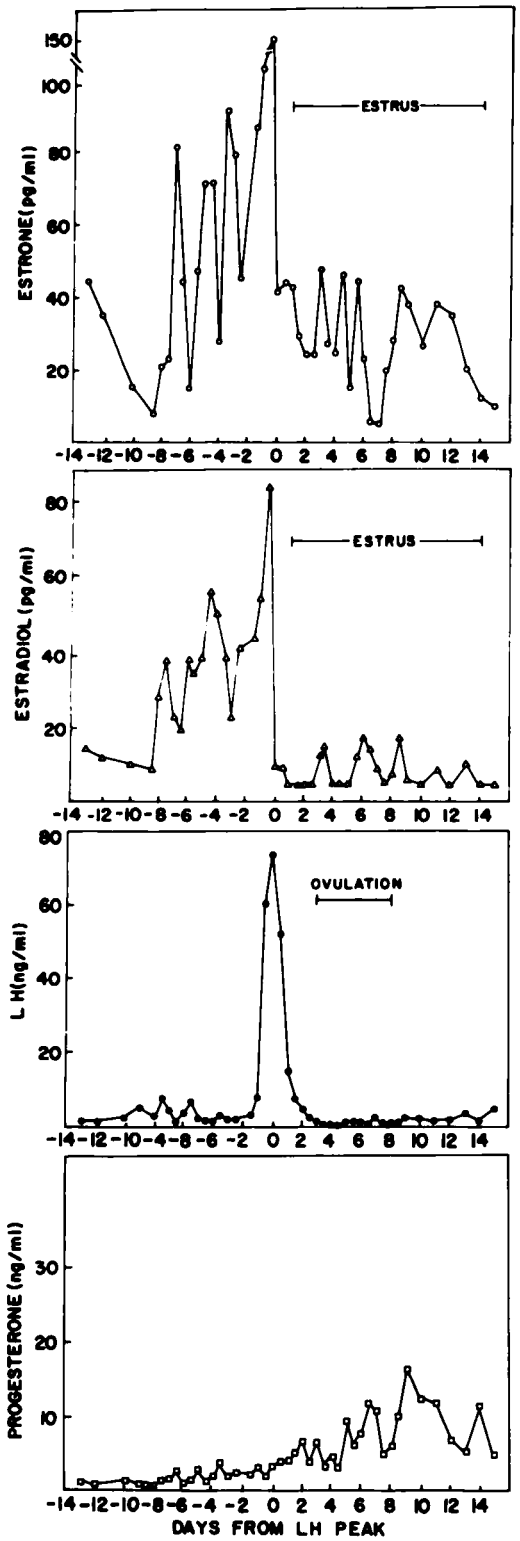

FIG. 2. Reproductive hormone profiles in relation to sexual receptivity and observed time interval of ovulation for bitch #5336.

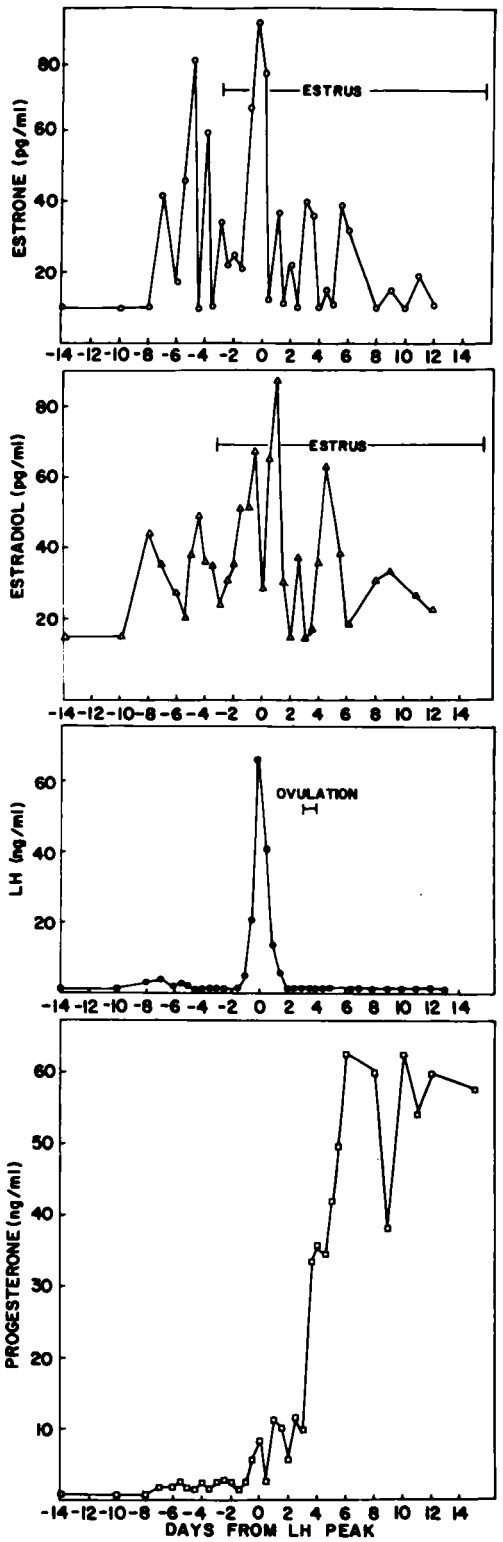

FIG. 3. Reproductive hormone profiles in relation to sexual receptivity and observed time interval of ovulation for bitch #6372.

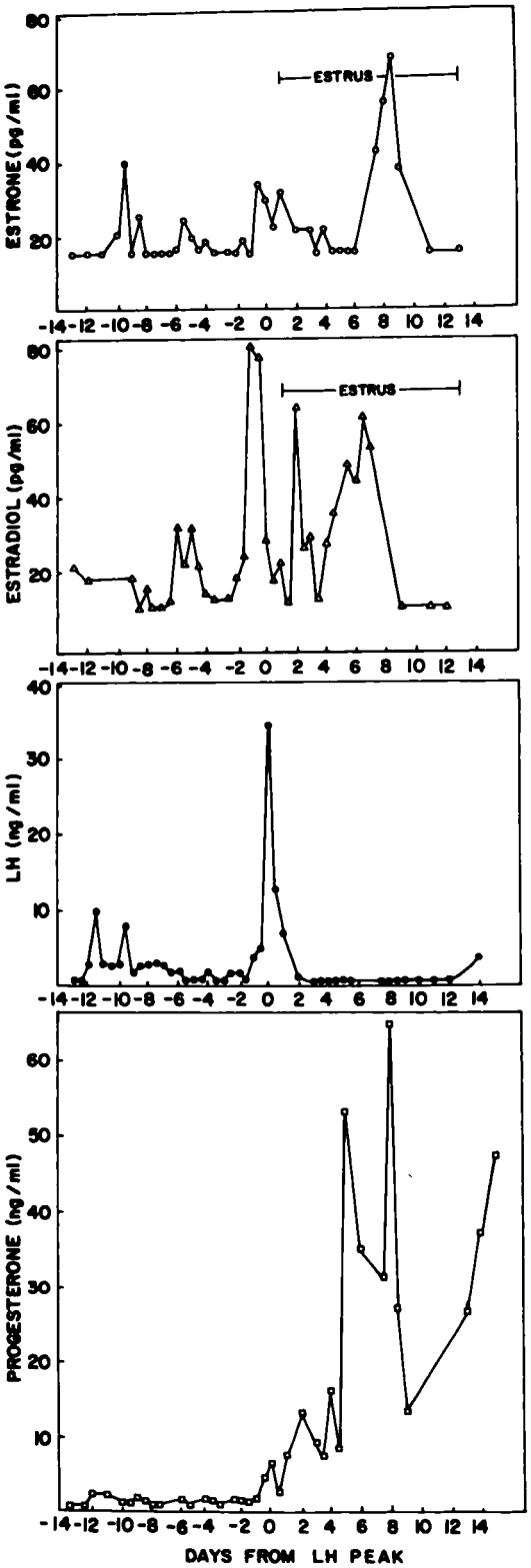

FIG. 4. Reproductive hormone profiles in relation to sexual receptivity for bitch #7561.

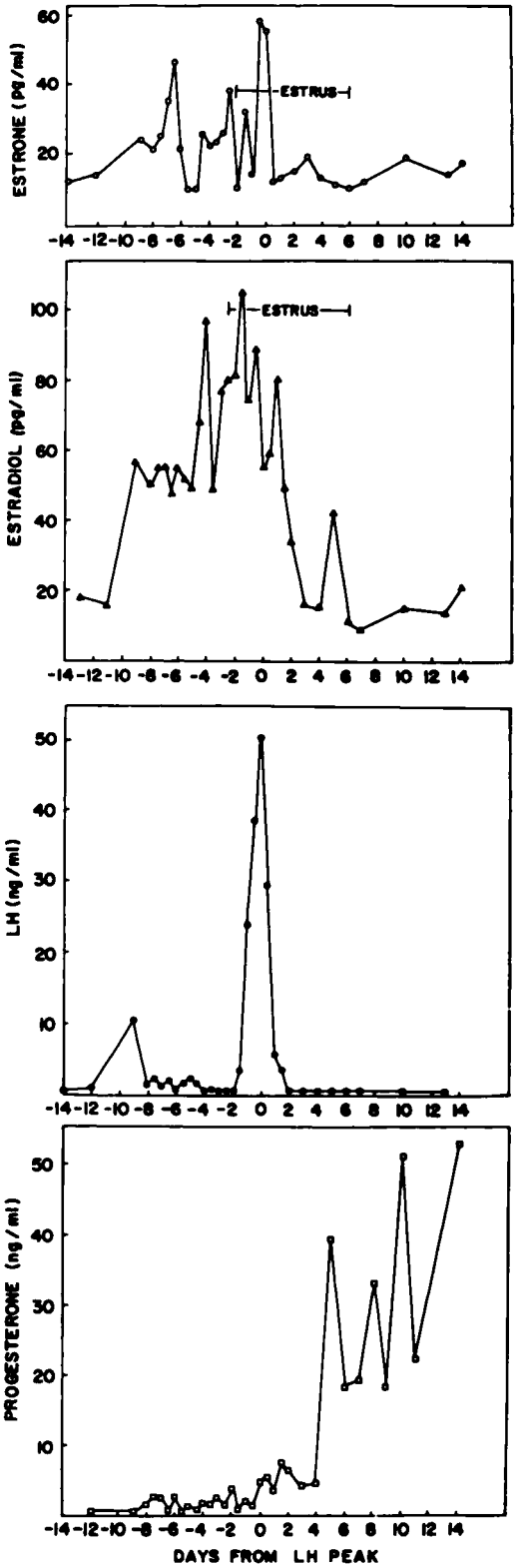

FIG. 5. Reproductive hormone profiles in relation to sexual receptivity for bitch #4669.

(Wildt et al., 1978). This episodic rise in LH averaged 6.0 ± 0.9 ng/ml in these 13 cycles and occurred 3–9.5 days prior to the major LH peak. In 7 of these 13 cycles, a slight detectable rise in progesterone (0.5–2.5 ng/ml) occurred concomitantly or within 24 h following the episodic burst of LH. This early rise in progesterone was not sustained and, in each case, titers of this hormone returned to nadir. In all individual animals, progesterone concentration increased coincidentally with the onset of the LH surge. Progesterone increased most rapidly on Days 0.5–6. The initial 96 h of this interval corresponded with gross morphological changes associated with preovulatory follicular luteinization and the period of ovulation. Peak progesterone concentration (range 8.0–93.0 ng/ml) occurred in 3 of 25 (12.0%), 13 of 25 (52.0%) and 9 of 25 (36.0%) of the cycles between Days 0–5, 5.5–10 and 10.5–15, respectively. Postovulation progesterone titers had some degree of daily fluctuation, but remained elevated to the end of the sampling period. Bitches maintained a state of sexual receptivity in the presence of gradually rising and peak concentrations of progesterone (Figs. 2–5).

DISCUSSION

Previous results have indicated that anesthesia and laparoscopy performed at 48 h intervals during the proestrous-estrous interval had no effect on basal levels or onset and peak concentration of serum LH in the bitch (Wildt et al., 1978). Results from the present study indicated these anesthetic and surgical procedures also had no effect on temporal alterations or peripheral concentrations of estrone, estradiol-17β and progesterone. These data suggest that the routine procedures associated with laparoscopy have no discernable effect on the endocrine system of this species.

The relative average basal and peak concentrations of estradiol-17β and progesterone were in the ranges previously reported for the female dog. The mean maximum of 42.8 pg/ml estradiol-17β was less than the average peaks of 79.7 and 68.9 pg/ml reported by Austad et al. (1976) and Nett et al. (1975), respectively, but greater than the mean concentrations of 17.2 and 30.0 pg/ml reported by Jones et al. (1973) and Edqvist et al. (1975), respectively. Previous investigations have indicated that, in general, maximum progesterone values (19.9–55.0 ng/ml) occurred between 6 and 10 days after the LH peak (Christie et al., 1971; Smith and McDonald, 1974; Concannon et al., 1975; Nett et al., 1975; Austad et al., 1976; Mellin et al., 1976). In the present study, progesterone gradually rose for 8 days after the LH peak, remained elevated, but then fluctuated over a range of approximately 18 ng/ml.

There are no previous reports concerning the peripheral concentrations of estrone in the bitch during the proestrous-estrous period. The study of Metzler et al. (1966) indicated that both estrone and estradiol-17β were secreted by the canine ovary while other circulating estrogens including estradiol-17α, estriol and 16-epiestriol were probably metabolites. Significant alterations in serum estrone suggested that this hormone was related to the ovarian and behavioral activity associated with proestrus and estrus. The reason for the considerable variability in estrone titers during the sampling period is unclear. Examination of individual cycles indicated that often increases and decreases in estradiol-17β titers were generally followed by similar fluctuations in estrone titers 12–36 h later. Since estradiol-17β serves as a precursor for the biosynthesis of estrone, it is likely that the assayed values of the latter hormone consisted of ovarian secreted estrone as well as estrone derived from estradiol-17β. Consequently, variations in metabolic processes such as cellular conversion rates of these estrogenic steroids could be responsible for producing some of these temporal deviations in estrone.

Steroid profiles from individual cycles generally resembled the overall averages. However, the present data were the first to emphasize that unlike LH, temporal fluctuations in estrone and estradiol-17β occurred throughout the proestrous-estrous period and, with respect to progesterone, occurred 8–15 days after the LH peak. In contrast, Austad et al. (1976) observed little variation in estradiol-17β in samples obtained at 24 h intervals. Nett (1975) measured LH, progesterone and estradiol-17β in blood samples at 20 min intervals during the second day of estrus. These investigators noted little change in the former 2 hormones while estradiol-17β varied as much as 68.5 pg/ml during the bleeding interval.

There have been conflicting reports on the presence of a pre-LH "estrogen surge" in the bitch. Independent studies measuring total estrogens during proestrus and estrus have reported either a well defined rise in estrogen

(Concannon et al., 1975; Mellin et al., 1976) or no distinguishable estrogen surge (Bell et al., 1971; Phemister et al., 1973). Nett et al. (1975), measuring estradiol-17β, found peak elevations of this hormone occurring just prior to or concomitantly with the LH surge. In the present study, there was evidence that estradiol-17β was a component of the controlling mechanism for the preovulatory release of LH. In addition, estrone cannot be totally eliminated as a component of the endocrine stimulus for LH release. However, unlike estradiol-17β which was elevated in all cycles, estrone was sustained near basal levels in 4 animals near the preovulatory rise and fall in LH. Thus, it is likely that estrone plays a supportive and not superior role to estradiol-17β in the triggering mechanism for LH release in the bitch.

Estradiol-17β and, in general, estrone were declining or already at basal levels during the late preovulatory stages of follicular development. This observation was related to the distinct anatomical alterations in ovarian follicle morphology detected by laparoscopy at this time. Coincidentally with the onset of the prolonged LH surge and prior to detection of ovulation, which depended on the observation of distinct stigmata, follicles began to take on classical characteristics of luteinization as previously described (Wildt et al., 1977). The present results indicated that these morphological changes could be correlated in individual dogs with a shift from estradiol-17β and estrone to progesterone secretion. The early rise in the latter hormone confirms our previous observation that this altered follicular morphology was associated with preovulatory luteinization in the bitch. In a previous study utilizing a limited number of dogs, no evidence was found for a discrete preovulatory rise in progesterone (Christie et al., 1971). In contrast, two independent laboratories reported initial elevations in progesterone during the LH surge and prior to ovulation (Phemister et al., 1973; Concannon et al., 1975, 1977). The present results agree with those of the latter investigators and indicate that the bitch exhibits a preovulatory rise in progesterone occurring coincidentally with, but not prior to, the onset of the LH surge. In this context, the bitch resembles the rat (Hashimoto et al., 1968), hamster (Leavitt and Blaha, 1970; Lukaszewska and Greenwald, 1970), rabbit (Shaikh and Harper, 1972), monkey (Weik et al., 1973) and human (Neil et al., 1967).

Amoroso (1949) suggested that progesterone may play an important synergistic role in the development and maintenance of canine reproductive behavior patterns. More recently Concannon et al. (1975) noted estrous behavior in bitches only after detecting an increase in plasma progesterone in the presence of declining estrogen levels. In addition, sexual receptivity can be induced following administration of progesterone to ovariectomized bitches to which estrogen had been administered previously (Beach and Merari, 1970; Concannon et al., 1977). Consequently, it has been speculated that estrous behavior in this species is elicited by a synergism between falling titers of estrogen and rising progesterone levels and that estrus is terminated when estrogen is no longer falling and progesterone increasing (Concannon et al., 1977). Our results confirm this general hypothesis, but emphasize that the temporal fluctuations in these hormones would make it difficult to predict accurately the precise duration of estrus based on specific concentrations of these reproductive steroids. It appears that estrogen in the bitch may serve as a priming agent to facilitate receptivity upon the addition of progesterone. The present study noted initial elevations in progesterone associated only with the coincident rise in LH. Consequently, progesterone release commences at a precise time during the cycle, whereas estrogen secretion appears more variable and is dependent on the gradually developing ovarian follicle. Thus it appears that a behavioral mechanism has evolved in the bitch in which progesterone aids in the expression of sexual receptivity.

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