BIOLOGY OF REPRODUCTION 19, 1113–1118 (1978)

Parturition and Lactation in the Bitch: Serum Progesterone, Cortisol and Prolactin

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ABSTRACT

Concurrent changes in serum progesterone, cortisol and prolactin associated with the prepartum period and lactation of 6 suckling and 1 nonsuckling Beagle bitches were determined by radioimmunoassay. Progesterone in each bitch fell sharply prior to parturition and remained low during lactation. Mean progesterone at approximately 120, 36, 20 and 10 h prepartum was 4.5 ± 0.6, 3.1 ± 0.4, 1.2 ± 0.4 and 0.6 ± 0.1 ng/ml, respectively. In 6 of 7 bitches, serum cortisol was elevated above mean prepartum levels (23 ± 1 ng/ml) during the day prior to the onset of parturition, reached peak levels of 63 ± 7 ng/ml at 8–24 h prepartum and fell to 19 ± 4 ng/ml at 8–12 h postpartum. Mean cortisol remained between 22 ± 1 and 27 ± 5 ng/ml during lactation and weaning. During the last week of pregnancy, serum prolactin levels (14–97 ng/ml) were variable within (50 ± 2 to 33 ± 8 ng/ml) and among (25 ± 2 to 80 ± 9 ng/ml) bitches and averaged 40 ± 7 ng/ml. In each bitch, prolactin increased by 195 ± 29% during the 16–56 h prepartum and reach peak levels (117 ± 24 ng/ml) at 8–32 (21 ± 3) h prepartum in 6 bitches and at 24 h postpartum in the remaining bitch. By 36 h after these peaks, prolactin levels were reduced to 37 ± 8 ng/ml before again increasing in response to suckling. Mean prolactin increased during the first week of lactation, peaked at 86 ± 19 ng/ml at 1.5 weeks of lactation, fell slowly to 43 ± 6 ng/ml at 5 weeks, shortly before weaning and then fell abruptly to 13 ± 2 ng/ml following weaning. The hormone changes observed were similar to those reported for several other species and suggest that the bitch may be a useful model for the study of maternal endocrine adjustments.

INTRODUCTION

The pregnant bitch may be a useful model for the study of maternal physiological adjustments and of the endocrine control of parturition and lactation. The dog has a relatively short gestation (59–68 days) as well as a body size and temperment amenable to serial blood sampling and experimental manipulation. Changes in progesterone, estrogen, corticoids and hematocrit have been described for the pregnant bitch (Jones et al., 1973; Smith and McDonald, 1974; Concannon et al., 1975, 1977). Acute changes in progesterone, total corticoids and rectal temperature associated with parturition have also been reported (Concannon et al., 1977). Profiles of maternal circulating prolactin associated with parturition and lactation have been determined for rats (Amenomori et al., 1970; Morishige et al., 1973), cows (Hoffman et al., 1973; Ingalls et al., 1973) and sheep (Davis et al., 1971; Chamley et al., 1973; Burd et al., 1976). To date, reports on maternal prolactin profiles in the dog have been limited to relatively infrequent measurements for a limited number of pregnancies and lactations and without benefit of concurrent measurement of other circulating hormones (Jones et al., 1976; Knight et al., 1977a; Graf et al., 1977). The present paper details concurrent changes in maternal serum levels of progesterone, cortisol and prolactin associated with parturition and lactation in the bitch.

MATERIALS AND METHODS

Concentrations of progesterone, cortisol and prolactin were determined by radioimmunoassay in sequential serum samples collected by cephalic venipuncture from each of 7 bitches from 6 days prior to expected parturition until the end of lactation. Prior to parturition, samples were collected daily at 0800

Accepted July 6, 1978
Received March 8, 1978

and 1600 h or at 0800, 1600 and 2400 h. Following parturition, samples were collected between 0800 and 1000 h 2–4 times a week or more frequently.

The 2–4-year-old Beagles were bred once on the first or second day of estrus and whelped litters without assistance 60–65 (62.7 ± 0.5) days post-coitum. Litters at parturition, at 2 days postpartum and at weaning were comprised of 3–13, 0–9 (n=7) and 5–9 (n=6) pups, respectively. Pups were weaned from bitches 33–35 days postpartum. During a 5–7 day period prior to weaning, pups were isolated from bitches from 1600–0700 h daily. Previous reports have provided details concerning animals, animal maintenance, serum preparation and the radioimmunoassays of progesterone and prolactin (Concannon et al., 1975, 1977; Knight et al., 1977b). For assay of cortisol, 100 μl of each serum sample was first diluted to 1 ml with saline. Duplicate 100 μl aliquots of the diluted serum were extracted with 3 ml methylene chloride in a manner similar to that described for extraction of total corticoids from dog plasma (Concannon et al., 1977). Cortisol in dried extracts was determined using the radioimmunoassay procedure described by Krey et al., (1975) with the exception that 150 μl of assay dilutent was added to sample and standard tubes alike. The assay was able to measure serum cortisol levels of 4–100 ng/ml. Using smaller sample volumes the assay was previously used to monitor elevations of serum cortisol to 70–170 ng/ml following administration of ACTH to dogs (unpublished data). The cortisol assay had mean within and between assay coefficients of variation of 3.1% and 5.3%, respectively, in 6 successive assays of a serum sample assayed in duplicate at volumes of 20 and 50 μl.

All data were standardized to the time of parturition. Each mean value is reported as the mean ± SEM. Due to the variation in sampling frequency among bitches, mean hormone levels were determined by first calculating daily means for each bitch, averaging the latter over the minimum intervals required to obtain a representative value for each bitch and finally calculating an overall mean from the daily means (or their averages) of each bitch.

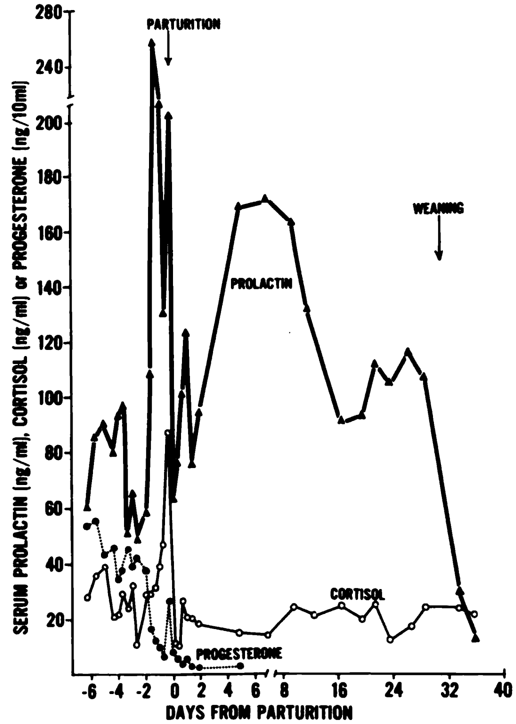

FIG. 1. Serum progesterone (●), cortisol (○) and prolactin (▲) during the prepartum period and lactation of an individual Beagle bitch.

RESULTS

Serum levels of progesterone, cortisol and prolactin during the prepartum and lactation periods varied considerably among individual bitches. Hormone profiles representative of changes observed within individual bitches are shown in Figs. 1–3. Profiles of individual bitches indicated acute changes in hormone levels which were not apparent in profiles derived from mean hormone levels. Mean levels for all bitches are shown in Fig. 4. Means from Day 2 postpartum through lactation do not include values for 1 bitch whose 3 pups died 1 day after whelping.

Serum progesterone 5 days prior to parturition ranged from 2.6–7.8 ng/ml and averaged 4.5 ± 0.6 ng/ml. Progesterone fell precipitously prior to parturition in each bitch. Levels fell below 1.5 ng/ml between 12 and 40 h prior to birth of the first pup. Mean progesterone at 40–32, 24–16 and 12–8 h prepartum was 3.12 ± 0.40, 1.19 ± 0.36 and 0.55 ± 0.07 ng/ml, respectively. Progesterone remained below 0.5 ng/ml throughout lactation with the exception

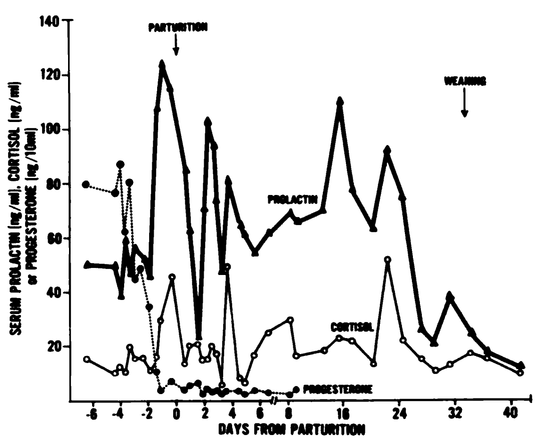

FIG. 2. Serum progesterone (●), cortisol (○) and prolactin (▲) during the prepartum period and lactation of an individual Beagle bitch.

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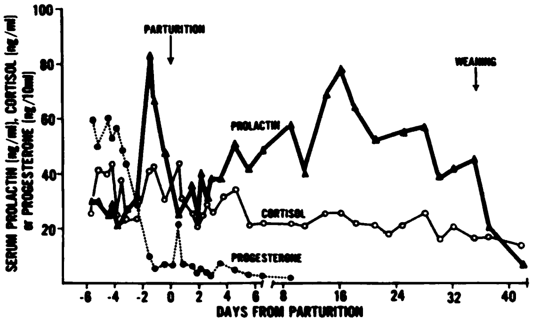

FIG. 3. Serum progesterone (●), cortisol (○) and prolactin (▲) during the prepartum period and lactation of an individual Beagle bitch.

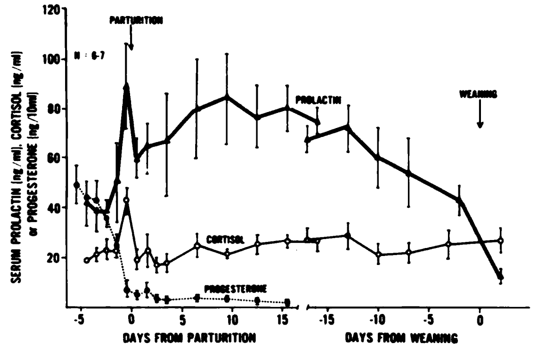

FIG. 4. Mean (± SEM) serum progesterone (●), cortisol (○) and prolactin (▲) during the prepartum period and lactation of Beagle bitches.

of brief (<12 h) elevations to 1.1−2.7 ng/ml in 3 of 7 bitches during the first 36 h postpartum.

Serum cortisol levels in samples collected at 8−16 h intervals 2−4 days prepartum ranged from 11−43 ng/ml and averaged 22.9 ± 1.2 (n=57) ng/ml. During that period mean levels for individual bitches ranged from 14.5 ± 1.3 (n=8) to 30.7 ± 3.2 (n=8) ng/ml with mean levels for all bitches at different sampling times ranging from 20.1 ± 2.3 to 24.5 ± 2.7 ng/ml. In 6 of 7 bitches, a distinct increase in cortisol occurred prior to parturition, with peak levels of 42−87 (62.5 ± 7.2) ng/ml occurring 8−24 (12.7 ± 2.6) h prepartum. Cortisol levels were subsequently reduced (19.3 ± 3.6 ng/ml) at 8−12 h postpartum. Mean cortisol remained between 21.8 ± 1.2 and 26.7 ± 5.4 ng/ml throughout the lactation and post lactation sampling periods (Fig. 4).

Serum prolactin concentration in samples collected at 8−16 h intervals from the fifth to the third day prepartum ranged from 14 to 97 ng/ml and averaged 40.1 ± 7.4 ng/ml. During the same period, mean prolaction levels for individual bitches ranged from 24.5 ± 2.0 to 80.4 ± 8.6 ng/ml and had coefficients of variation ranging from 0.13 (50 ± 2.4 ng/ml) to 0.63 (33.1 ± 8.0 ng/ml). In each bitch, sequential increases in serum prolaction were initiated 16−56 (36.6 ± 5.4) h prepartum and reached peak levels (117 ± 24 ng/ml) within 32 h of parturition. Those peak prolactin levels in individual bitches represented increases of 73−327 (195 ± 29)% over the respective mean levels 3−5 days prepartum. In 6 of 7 bitches, peak prolactin levels occurred 8−32 (21.3 ± 3.4) h prepartum. In the remaining bitch, prolactin continued to increase during active labor and peaked 24 h after the onset of parturition. This bitch also showed the most rapid onset of parturition following the prepartum decline in progesterone (Fig. 5a). The inclusion of the prolactin levels of this bitch in the daily means (Fig. 4) markedly inflated the magnitude of the means and standard errors for prolactin on Days 1 and 2 postpartum. In each of the remaining bitches, prolactin levels fell during the 36 h following parturition to levels (35.6 ± 8.2 ng/ml) near or below mean levels present 3−5 days prepartum.

Throughout the remainder of lactation, prolactin in each bitch remained above mean levels observed 3−5 days prepartum. Mean prolactin levels increased during the first week of lactation and reached a secondary peak of 86 ± 19 ng/ml on Day 10. Mean prolactin declined slowly during the 3rd and 4th weeks of lactation and was 43.4 ± 5.7 ng/ml during the week prior to weaning (Fig. 4). The lowest

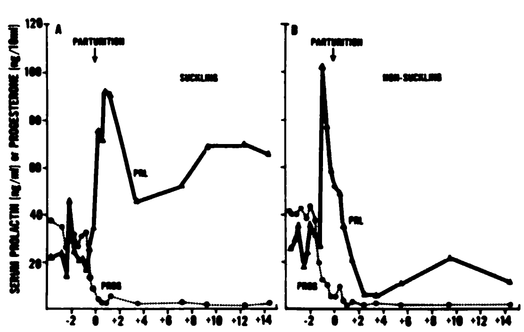

FIG. 5. Serum progesterone (●) and prolactin (▲) during and following parturition (A) in a bitch that underwent parturition early in relation to the fall in progesterone and prior to the time of observed peak prolactin levels and (B) in a bitch that did not suckle a litter.

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prolactin levels for each bitch were observed following weaning and averaged 13.0 ± 2.4 ng/ml. In the 1 bitch that did not suckle pups, prolactin fell continously from 102 ng/ml 1 day prepartum to 6 ng/ml 4 days postpartum and levels during the 2 weeks postpartum remained below those observed 3–5 days prior to parturition (Fig. 5B).

DISCUSSION

The decline in progesterone before parturition was consistent and similar to data reported previously for bitches sampled less frequently (Concannon et al., 1975, 1977). Such results support the idea that progesterone withdrawal may play a major role in the mechanism of parturition induction in the bitch.

The prepartum elevations in serum cortisol in 6 of 7 bitches are in agreement with previously reported prepartum elevations in total plasma corticoids (Concannon et al., 1977). The erratic nature of these prepartum elevations in cortisol, their failure to parallel consistently in time the decline in progesterone and the occurrence of normal parturition in one bitch in the absence of an obvious prepartum rise in cortisol all suggest that elevated cortisol in the maternal circulation is not a prerequisite to normal parturition in the bitch. It is possible that prepartum elevations in the maternal circulation merely reflect much larger increases at the fetoplacental-uterine level and that the latter are more intimately involved in the mechanisms of prepartum luteolysis and initiation of labor as has been suggested for the goat and other species (Thorburn et al., 1977). Although the bitches showed no visible signs of stress related to handling or blood sampling, it is possible that stress related to these procedures might have induced sporadic elevations in cortisol levels.

Basal prolactin levels observed following weaning were similar to those measured in bitches prior to implantation and during anestrus (Knight et al., 1977a, b). Prolactin levels during the week prior to the prepartum surge were approximately 3 times higher than basal levels present after weaning (Fig. 4) suggesting that levels are elevated late in pregnancy. However, it is difficult to estimate to what extent elevated prolactin levels observed during the week prior to parturition are specific to pregnancy since we did not measure prolactin in sequential samples from nonpregnant bitches during the late luteal phase.

The dramatic prepartum surges in prolactin support a similar observation by Jones et al. (1976) in a single bitch. Initiation of the prepartum prolactin surge concomitant with or immediately after the start of the prepartum fall in progesterone supports the suggestion that prolactin release in the bitch may be stimulated by progesterone withdrawal (Graf et al., 1977). Abrupt prepartum prolactin surges in association with concomitant abrupt declines in progesterone have also been observed in rats (Morishige et al., 1973), cows (Hoffmann et al., 1973; Ingalls et al., 1973) and sheep (Chamley et al., 1973; Burd et al., 1976).

The extent to which the prepartum surge in prolactin plays an active role in parturition remains unclear. About the time of parturition, cortisol peaks occurred concomitant with or just subsequent to the prolactin peaks. Reported increases in fetal plasma prolactin in late pregnancy and their correlation with fetal adrenal growth in man and with fetal plasma cortisol in sheep have prompted the suggestion that prolactin may play a role in stimulating fetal adrenocorticosteroid secretion prior to parturition (Challis et al., 1977). However, the suppression of plasma prolactin levels by the administration of 2 Br-α-ergokryptin during late pregnancy in dairy cattle did not interfere with the occurrence of normal prepartum increases in plasma corticoids, decreases in plasma progesterone or parturition (Hoffmann et al., 1973).

The possibility that the prepartum fall in progesterone, surge in prolactin and rise in cortisol are all initiated by a single mechanism should be considered. Prostaglandin (PG) $F_{2\alpha}$ has been shown in cattle to suppress progesterone, increase cortisol and release prolactin (Louis et al., 1973, 1974). $PGF_{2\alpha}$ administered repeatedly in low doses was luteolytic and abortifacient in the bitch (Concannon and Hansel, 1977) suggesting the possibility that $PGF_{2\alpha}$ could be an endogenous luteolysin secreted in increasing amounts prepartum as it is in goats (Thorburn et al., 1972).

The abrupt declines in cortisol and prolactin immediately postpartum appear to reflect a loss of the feto-placental stimuli initiating parturition with the secondary postpartum increase in prolactin being dependent on the suckling stimulus. If prolactin release in the bitch is stimulated by progesterone withdrawal, the postpartum fall in prolactin may reflect the cessation of progesterone withdrawal as nadir

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progesterone levels are reached. The elevated but slowly declining levels of prolactin during lactation are in agreement with those previously observed in a limited number of lactating bitches (Knight et al., 1977a). These elevated and fluctuating prolactin levels are apparently dependent on stimuli provided by suckling pups since levels fell abruptly to nadir values following weaning (Fig. 4) and elevated levels characteristic of lactation were not observed following the prepartum prolactin surge in the bitch that did not nurse a litter (Fig. 5B). The fluctuations in prolactin levels may have been due to the fact that blood sampling times were fixed and not related to the onset of the suckling stimulus.

The transient postpartum decline in prolactin and the subsequent increase during lactation in the bitch are similar to those reported for ewes (Davis et al., 1971). In the rat, prolactin levels also fall to low levels during delivery (Saunders et al., 1977) with a subsequent increase during lactation being dependent on the presence of suckling pups (Amenomori et al., 1970; Saunders et al., 1977).

The present results indicate that the changes in progesterone, cortisol and prolactin during parturition and lactation in the bitch are not unlike those reported for several other species and suggest that the pregnant bitch may be a useful model for studying the endocrine mechanisms associated with parturition and lactation.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of R. G. Cowan, M. E. Lanieu and F. Michel and the animal care provided by H. Saroka, D. Shattuck and F. Terwilliger. The antiserum to progesterone was provided by Dr. K. Kirtone, The Upjohn Company This research was supported by NIH-NICHD Contract No. 72–2725.

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