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Plasma concentrations of 13,14-dihydro-15-keto prostaglandin F_2-alpha (PGFM), progesterone and estradiol in pregnant and nonpregnant diestrus cross-bred bitches

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Abstract

The canine corpus luteum (CL) typically sustains elevated plasma progesterone concentrations for 2 months or more, with a peak approximately 15–25 days after ovulation, followed by a slow decline. The processes involved in the slow, protracted regression of the CL over the remaining 1.5–2-month period in nonpregnant bitches and until shortly prepartum in pregnant bitches are not well characterized. The rapid luteolysis that occurs immediately prepartum appears to be a result of a prepartum rise in peripheral PGF. The potential role of PGF in the slow regression process in the several weeks preceding parturition and in nonpregnant bitches after 15–25 days after ovulation is not known. Therefore, plasma concentrations of 13,14-dihydro-15-keto-prostaglandin F_2-alpha (PGFM), progesterone (P_4) and estradiol (E_2) were determined and compared in bitches during nonpregnant diestrus ($n = 9$) or pregnancy ($n = 8$). During the gradual decrease in plasma concentrations of progesterone in both groups, the P_4 pattern appeared unrelated to changes in either E_2 or PGFM concentrations. The PGFM pattern was different between diestrus and pregnant bitches ($P > 0.01$); there was an apparent progressive but slow increase in PGFM in pregnant bitches from Days 30 to 60, followed by a large increase prior to parturition; concentrations declined immediately postpartum. However, there were no increases in PGFM during the same interval in nonpregnant bitches. Mean estradiol concentrations were sporadically elevated during the last third of pregnancy and less so in nonpregnant diestrus; there was no acute prepartum increase in estradiol associated with the PGFM increase. In summary, although there were no apparent changes in peripheral $PGF_2\alpha$ concentration involved in regulating the slow protracted phase of luteal regression in nonpregnant bitches, modest increases in PGFM may play a role in ovarian function after mid-gestation in pregnant bitches. Furthermore, the acute prepartum rise in PGFM was not dependent on any concomitant increase in estradiol concentrations.

Keywords: Bitch; Luteal regression; PGFM; Progesterone; Estradiol

1. Introduction

The canine corpus luteum (CL) is a transient endocrine gland characterized by rapid growth, differentiation and luteinization of granulosa and thecal cells

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of the ovulatory follicles [1,2]. Even in the absence of pregnancy, the canine CL remains functional until around Day 60 (ovulation = Day 2) of diestrus [3–6]. In dogs, corpora lutea typically sustain elevated plasma progesterone concentrations for 2 months or more, with a slow decline in progesterone following a peak at approximately Days 15–25. The processes involved in the slow, protracted regression of the CL over the remaining 1.5–2-month interval in nonpregnant bitches and until shortly prepartum in pregnant bitches, are not well characterized. The rapid luteolysis that occurs immediately prepartum appears to be a result of a prepartum rise in peripheral PGF. The potential role of PGF in the slow luteal regression preceding parturition and in nonpregnant bitches is not known. Progesterone (P_4) and estradiol (E_2) patterns have been determined and compared during diestrus and pregnancy [3–6]. However, PGFM concentrations have been reported only during the prepartum period [7]. There are no reports comparing PGFM concentrations throughout nonpregnant diestrus compared to pregnancy.

A luteolytic factor of uterine origin is unlikely to be involved with regulation of luteal function and cycle length in dogs, based on results in hysterectomized bitches [8,9]. Although an abrupt luteolysis at the end of gestation has been reported, the preceding slow decline in progesterone is reported to be slower than that observed in nonpregnant diestrus [6], perhaps due to a luteotrophic effect of elevated prolactin concentrations during the second half of gestation. To better understand the process of slow luteal regression in the bitch, the present study undertook to measure plasma concentrations of 13,14-dihydro-15-keto prostaglandin F_2-alpha (PGFM), progesterone and estradiol in cross-bred pregnant versus nonpregnant diestrus bitches.

2. Materials and methods

2.1. Dogs

Seventeen adult cross-bred bitches, from 5 to 40 kg body weight, were maintained in individual cages, under natural light, fed a commercial diet twice a day, and given ad libitum access to water. There were eight pregnant bitches and nine nonpregnant diestrus bitches. The bitches had been previously identified as proestrus on the basis of vulvar swelling, serosanguineous vaginal discharge, or both. After the initial observations, daily vaginal cytology was performed until 80–90% superficial cells were detected [10,11]. Bitches were then artificially inseminated or naturally bred every 48 h until the end of estrus. Several male dogs were used for breeding. Pregnancy diagnosis was performed by ultrasonography (Pie Medical Scanner 480) at 25 days after the first breeding. The bitches intended for the nonpregnant group were not bred. Vaginal cytology was performed in all bitches during estrus cycle until the first day of cytological diestrus [10,11]. The day of the preovulatory LH peak (Day 0) was subjectively estimated as 8 days prior to the onset of diestrus vaginal smears [12]. Parturition occurred spontaneously in three bitches (Days 64, 65 and 65, respectively). The remaining pregnant bitches were ovariohysterectomized on Days 63 or 64. All procedures were performed following the Animal Welfare Research Guide of FMVZ, UNESP, Botucatu Campus.

2.2. Blood sample collection

Blood samples were obtained by jugular venipuncture and immediately centrifuged (600 × *g* for 15 min). Plasma was stored in microvials and frozen (−20 °C) pending assay. Sampling was done every 5 days, starting at Day 10. Blood samples from pregnant bitches were obtained until Day 63 ($n = 2$), 64 ($n = 3$) or immediately postpartum ($n = 3$). Diestrus samples were collected until Days 65 ($n = 3$), 75 ($n = 3$), or 85 ($n = 3$). In addition, daily samples were then obtained during the 10 days before the end of the sampling period at Days 63 or 64 postpartum in pregnant bitches; or at Days 65, 75 or 85 in nonpregnant bitches. Blood collection frequency was further increased to 6-h intervals during the last 72 h preceding the end of the general sampling period, and for an additional 24 h to conclude the study. For the present study, estradiol concentrations were determined from Days 40 to 66 in both groups; PGFM and progesterone were assayed in all samples through Day 66.

2.3. PGFM assay

The technique was validated using methodology previously used in bovine plasma [13]. Serial dilutions of canine and bovine plasma were assayed to estimate any volume effect (30–300 μL) on assay efficacy, and the recovery of different concentrations of PGFM added to control bovine and canine plasma samples was assessed. Dilutions of rabbit PGFM anti-serum [13] ranged from 1:4000 to 1:12,000. Prostaglandin-free canine plasma (PFCP or plasma control) was obtained from three bitches treated with flunixin meglumine (Banamine, Schering-Plough®), 1 mg/kg IM, once daily for 3 days [14]. Plasma pools were stored in 50 mL plastic vials (Falcon®) at −20 °C until assayed.