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Luteal function and blood flow during intravenous infusion of prostaglandin F2α in heifers

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Abstract

The effect of prostaglandin F2α (PGF) infusion for 3 h into the jugular vein on progesterone concentrations was studied in 24 Holstein heifers. Plasma concentrations of PGF were assessed by assay of 13,14-dihydro-15-keto-PGF (PGFM). The 3 h of PGF infusion were used to approximate the duration of the major concentrations of PGFM in a natural pulse. During infusion of 5, 10, or 15 mg/3 h, the concentrations of PGFM greatly exceeded the peak of a natural pulse. Plasma concentrations of progesterone decreased ($P < 0.05$) in the three PGF-treated groups by Hour 1 (Hour 0 = beginning of infusion). Progesterone increased between Hours 1 and 2, but the increase was significant ($P < 0.03$) only in the 5-mg group. Concentrations decreased more gradually between Hours 2 and 6 than between Hours 0 and 1 with no differences among PGF groups. The percentage of CL area with color-Doppler signals of blood flow were elevated similarly in the three PGF-treated groups at Hours 1 to 3 and by Hour 5 decreased to below the percentage at Hour 0. In a second experiment, approximating a natural PGFM pulse by intravenous infusion of PGF at a dose of 0.7 mg/3 h did not affect plasma progesterone concentrations. Results indicated that intravenous infusion of PGF for 3 h decreased the progesterone concentration when the total dose was equivalent to doses that have been shown to be completely luteolytic when given as a single systemic injection. However, intravenous infusion of a dose of PGF that approximately simulated a natural PGFM pulse did not effect progesterone concentration.

Keywords: blood flow, cattle, corpus luteum, luteolysis, prostaglandin F2α.

Introduction

The luteal phase is terminated in many species including cattle by uterine secretion of prostaglandin F2α (PGF), augmented by intraluteal PGF production (Arosh *et al.,* 2004; Weems *et al.,* 2006). In cattle, the minimal effective intrauterine dose of PGF (1 or 2 mg; Louis *et al.,* 1974) when given into the uterine horn ipsilateral to the corpus luteum (CL) is about 1/10 of the minimal systemic dose (15 mg; Lauderdale and Fokolowsky, 1979). The intrauterine effectiveness of PGF involves a unilateral venoarterial pathway, wherein the PGF is transferred from the uterine vein to the closely adherent ovarian artery (Hixon and Hansel, 1974; Mapletoft *et al.,* 1976; Ginther, 1981). The main plasma metabolite of PGF is 13,14-dihydro-15-keto-PGF (PGFM), and PGFM concentration is used as an indicator of PGF release into the circulation (Kindahl *et al.,* 1976). About 65% of PGF is metabolized to PGFM during one passage through the lungs (Davis *et al.,* 1985).

In association with luteolysis, PGF is released from the uterus in pulses occurring approximately every 12 h (Kindahl *et al.,* 1976; Mann and Lamming, 2006; Ginther *et al.,* 2007). The release of sequential PGF pulses has been assumed to be an important aspect of the luteolytic effect (Silvia *et al.,* 1991; Schams and Berisha, 2004; Mann and Lamming, 2006). However, the necessity for natural pulsatile delivery of PGF in cattle has not been demonstrated. Rather than pulsatile delivery of exogenous PGF, induction of luteolysis with PGF or a PGF analogue often is done with a single bolus injection, both for applied (Inskeep, 1973) and research (Shirasuna *et al.,* 2008) purposes.

Blood flow to the CL decreases dramatically in association with luteolysis (Niswender *et al.,* 1976). However, rather than a decrease in luteal blood flow as an initial step in the luteolytic process, color-Doppler studies in cattle indicated that luteal blood flow initially and transiently increases before a decrease in plasma progesterone from a single dose of PGF that induces precipitous and complete luteolysis (Acosta *et al.,* 2002; Ginther *et al.,* 2007). Studies with either 12-h or 24-h intervals between examinations indicated a temporal association between increased CL blood flow before the initiation of spontaneous luteolysis (Miyamoto *et al.,* 2005), but this conclusion was not confirmed in other similar studies (Ginther *et al.,* 2007).

The purposes of the present experiments in heifers were: 1) to determine if a 3-h intravenous infusion of PGF initiated luteolysis when the total dose was equivalent to reported doses used for induction of acute and complete luteolysis with a single systemic bolus injection (Experiment 1), and 2) to determine if a

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3-h intravenous infusion of a PGF dose that simulated a natural PGFM pulse was luteolytic (Experiment 2). The effect of intravenous PGF infusion on luteal blood flow was also considered (Experiment 1).

Materials and Methods

Animals

Holstein heifers, aged 17 to 20 months, were used in two experiments. Selection of heifers was based on docile temperament and no apparent abnormalities of the reproductive tract, as determined by ultrasound examinations (Ginther, 1998). If more than one CL was present, the heifer was not used. Heifers remained healthy and in good body condition throughout the experiments. Heifers were sedated when indicated with a low dose of Xylazine hydrochloride (Xila-Ject, Phoenix Pharmaceutical Inc., St. Joseph, MO, USA; 14 mg/heifer, IM). Xylazine sedation produces color-Doppler hemodynamic effects on the internal iliac artery, but not on ovarian vascular perfusion, based on the vascular resistance index at the ovarian pedicle and percentage of CL area with color-Doppler signals of blood flow (Araujo and Ginther, 2009).

Infusions

Infusion of PGF (Dinoprost tromethamine; Lutalyse; Pfizer Animal Health, New York City, NY, USA) was done into the jugular vein (intravenous; IV). Infusion was done 8 to 10 d after ovulation, so that the CL was mature but before endogenous pulses of PGFM would be expected. The beginning of infusion was designated Hour 0. Infusion of PGF was done at a constant rate, using a variable-flow peristaltic mini-pump (Cat. No. 13-876-4; Fisher Scientific, Pittsburgh, PA, USA). The pump was calibrated to deliver the specified dose of PGF in 30 ml (Experiment 1) or 9 ml (Experiment 2) of vehicle. The IV infusion was made through an indwelling surgical tubing (Tygon tubing; id 0.040 in; Norton, Akron, OH, USA) placed and secured into a jugular vein. After the IV tubing was inserted, the heifer's head was no longer restrained or approached during the hours of infusion.

Natural PGFM concentration

The concentration of PGFM at the peak of a natural pulse was taken from an unpublished study and was used to judge the similarity to PGFM concentrations during PGF infusion. Six statistically identified natural pulses were used that were obtained from hourly sampling during 12-h windows during luteolysis, as described (Ginther *et al.,* 2007). The mean peak during the six pulses was 1065 pg/ml (standard deviation, 188 pg/ml).

Experiment 1

The purpose of this experiment was to determine whether a 3-h IV infusion of the minimal recommended single-injection systemic luteolytic dose of PGF (15 mg; Lauderdale and Fokolowsky, 1979) would be effective in inducing a decrease in progesterone and to determine the extent that the PGFM concentrations during infusion were similar to peak concentrations for a natural PGFM pulse. Since it seemed likely that the 15-mg dose would result in PGFM concentrations that exceeded a natural PGFM peak, an intermediate dose (10 mg) and a low dose (5 mg) also were used. Controls were infused with vehicle. Four experimental groups were used involving infusion 9 or 10 d after ovulation at doses of 0 (controls), 5, 10, and 15 mg (n = 4/group).

A duplex B-mode (gray scale) and pulsed-wave color-Doppler ultrasound instrument (Aloka SSD 3550; Aloka American, Wallingford, CT, USA) equipped with a linear-array 7.5-MHz transducer was used for transrectal scanning. In color-Doppler mode, the extent and direction of blood flow are indicated by color signals (Ginther, 2007), and the signals were used to display blood flow in the vessels of the CL. All Doppler scans were performed at a constant gain setting for color and a velocity setting of 6 cm/sec, except that the setting of 6 cm/sec was also compared to a setting of 10 cm/sec. The velocity setting represents the minimal rate of blood flow detected by the setting (Ginther, 2007). The effectiveness of the setting on minimizing detection of venous flow in the CL in these studies is unknown.

Percentage of CL area with color-Doppler signals for blood flow was estimated from the blood-flow color displays of the real-time sequential two-dimensional planes of the entire CL in each experiment, as described for heifers (Ginther *et al.,* 2007). The color-flow signals at the periphery of the CL and within the CL were included in the percentage estimate. The validity for using percentage estimates of the area of the bovine CL with blood flow signals has been reported (Ginther *et al.,* 2007; Araujo and Ginther, 2009) The reported validations were done by comparing percentage estimates with the number of color pixels in a still image and by comparing percentage estimates with the spectral resistance index. The resistance index is an indicator of the extent of vascular perfusion distal to the point of assessment (Ginther, 2007).

The percentage of CL area with color-Doppler signals of blood flow was estimated each hour from Hours -1 to 6. The ultrasonographer was unaware of group. A blood sample was taken from the coccygeal

vein and CL blood flow was estimated before insertion of the IV infusion tubing (Hour -1). After insertion of the tubing, the heifer was undisturbed for an hour before the infusion began at Hour 0. A blood sample was taken, and concentrations of PGFM and progesterone were assayed in the plasma. Luteal blood flow was quantitated hourly at Hours 0 to 6. The comparison between color-Doppler velocity settings of 6 cm/sec versus 10 cm/sec for percentage of CL area with color-Doppler signals of blood flow was done in the three PGF-treated groups. For this purpose, all three groups that received PGF were combined; there was no difference in percentage of CL area with blood flow among the three groups. The scanner's tracing function in B-mode was used to determine cross-sectional area (cm^2) of the CL, as described (Ginther *et al.,* 2007). The length of the interovulatory interval between pretreatment and posttreatment ovulations was determined.

Experiment 2

The purpose of this experiment was to examine the luteolytic effect of IV infusion of a dose of PGF that was expected to result in PGFM concentrations approximating a natural PGFM pulse. The dose to be infused (0.7 mg/3 h) was extrapolated from the mean PGFM concentration (7540 pg/ml) from infusion of the lowest dose (5 mg) in Experiment 1 and the peak concentration during natural pulses (1065 pg/ml). The extrapolated dose was determined by the following mathematical calculations: 5 mg x 1065 pg/ml divided by 7540 pg/ml = 0.7 mg.

The protocol for IV infusion was the same as for Experiment 1, except that infusions were done 8 d after ovulation and only a PGF-treated group and a control group were used (n = 4/group). Controls were infused with 9 ml/3 h of vehicle, and the PGF-treated heifers were infused with 0.7 mg in 9 ml/3 h of vehicle, beginning at Hour 0. Concentrations of PGFM were determined from blood samples collected hourly from Hours 0 to 6 but only for the PGF-treated group. Progesterone concentration was determined for both groups from Hours -1 to 6 and every day until ovulation. Other end points were the interval from the pretreatment ovulation to the posttreatment ovulation and the interval from the pretreatment ovulation to a progesterone decrease of <1 ng/ml.

Blood samples and hormone assays

Blood samples were collected into heparinized tubes and centrifuged (2000 x *g* for 10 min), and plasma was decanted and stored (-20°C) until assay. Plasma progesterone concentrations were measured using a solid-phase radioimmunoassay kit containing antibody-coated tubes and ^{125}I-labeled progesterone (Coat-A-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA, USA), as described (Ginther *et al.,* 2007). The intraassay CV and sensitivity ranged between 5.8 to 11.6% and 0.02 to 0.03 ng/ml, respectively, for all experiments. Blood samples for PGFM assay were collected and immediately placed in ice cold water for 10 minutes before centrifuging and storing at -20°C. The plasma samples were assayed for PGFM by a modification of a radioimmunoassay procedure (Meyer *et al.,* 1995; Mattos *et al.,* 2004) that was adapted and validated in our laboratory for bovine plasma and has been described (Ginther *et al.,* 2007). The intraassay CV and sensitivity ranged between 7.2 to 12.0% and 33.3 to 69.4 pg/ml, respectively, for both experiments.

Statistical analyses

Data were examined for normality with the Shapiro-Wilk test. Data that were not normally distributed were transformed to natural logarithms or ranks. Individual end points were analyzed for time effects (hour or day), and comparisons involving groups were analyzed for main effects (group, time) and the interaction. In Experiment 2, the progesterone concentrations at Hour 0 were different ($P < 0.04$) between the controls (7.9 ± 1.1 ng/ml) and PGF-treated group (5.6 ± 0.3 ng/ml). Therefore, the concentrations for each heifer were transformed to the percentage change from Hour 0. Data were analyzed by SAS MIXED procedure (version 9.1.3; SAS Institute, Inc), using a REPEATED statement with autoregressive structure to account for autocorrelation between sequential measurements. Student's paired *t*-tests were used to locate differences between two times within a group when a significant time effect was found. When a group-by-time interaction was significant or approached significance, the interaction was further examined by study of the time effect in each group or combinations of groups. Tukey's multiple-range test was used when comparisons were made within a time among more than two means. Single-point data (e.g., length of interovulatory interval) were examined by one-way analysis of variance and significant group effects further tested by Duncan's multiple range test or Student's unpaired *t*-tests. A probability of $P \leq 0.05$ indicated that a difference was significant and a probability between $P > 0.05$ and $P \leq 0.1$ indicated that significance was approached. Data are presented as the mean ± SEM, unless otherwise indicated.

Results

Probabilities for main effects and an interaction that were significant or approached significance and for

differences between selected means are shown in the figures.

Experiment 1

For Hours -1 to 6 encompassing the 3-h PGF infusion, a blood-flow velocity setting of 6 cm/sec resulted in a greater (main effect of setting) estimate of percentage of CL area with color-Doppler signals of blood flow (68.3 ± 1.9%) than the setting of 10 cm/sec (56.6 ± 1.9%; Fig. 1). The interaction of group (two settings) and hour was not significant.

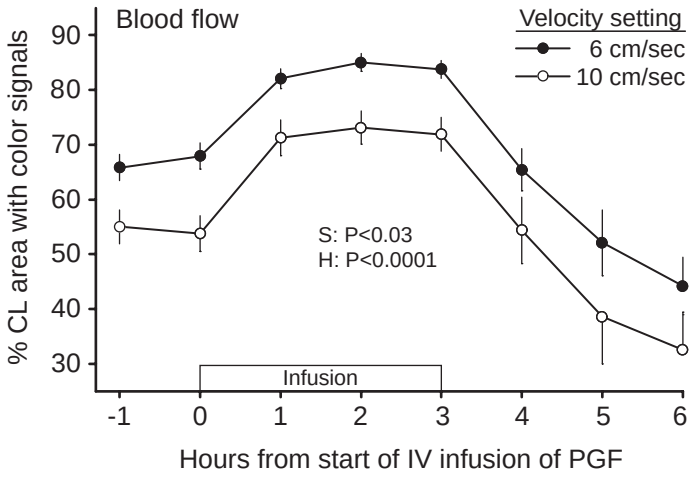

Figure 1. Mean (±SEM) percentage of CL area with color-Doppler signals of blood flow at two velocity settings in association with 3 h of intravenous infusion of PGF (n = 4/setting). The velocity setting for the color-Doppler instrument indicates the lowest blood velocity detected by the indicated setting. Main effects (S = setting; H = hour) that are significant are shown. Experiment 1.

Intravenous infusion of a total of 5, 10, or 15 mg of PGF during 3 h progressively increased the PGFM concentrations as the doses increased (Fig. 2). The concentration (7540 ± 595 pg/ml) after 1 h of infusion in the group with the lowest dose was greater ($P < 0.0001$) than the peak concentration (1065 ± 77 pg/ml) in six natural pulses.

Each main effect and the interaction of group by hour were significant for progesterone during Hours -1 to 6 (Fig. 2). Concentrations did not change significantly in the controls when analyzed separately, whereas there was an hour effect and no group effect or interaction for the three PGF groups. Averaged over the PGF groups, concentrations decreased ($P < 0.05$) between Hours 0 and 1, increased between Hours 1 and 2, and then decreased. The mean increase between Hours 1 and 2 primarily reflected a significant increase ($P < 0.03$) in only the 5-mg group. The transient increase in the 5-mg group between Hours 1 and 2 resulted in similar concentrations between Hour 0 and 2. Differences in length of the interovulatory interval approached significance ($P < 0.1$). The interval was shorter ($P < 0.05$) in the 10-mg (15.8 ± 1.4 d) and 15-mg (16.0 ± 1.5 d) PGF groups than in the controls (21.7 ± 1.7 d), but the 5-mg group (19.5 ± 1.5) was not different from the other groups.

The percentage of CL area with blood-flow signals showed a significant hour effect and a group-by-hour interaction (Fig. 2). When analyzed separately, the percentages for the control group did not change significantly, whereas the percentages for the three PGF-treated groups showed a significant hour effect with no group effect or interaction. Averaged over the PGF groups, the percentage of CL area with blood flow increased ($P < 0.05$) between Hours 0 and 1, did not change during Hours 1 to 3, decreased ($P < 0.05$) after Hour 3, and was lesser ($P < 0.05$) at Hour 5 than at Hour 0. The area (cm^2) of the CL did not change during Hours -1 to 6 and was not different among groups (not shown).

Experiment 2

The concentration of PGFM during Hours 1 to 3 from PGF infusion of a dose of 0.7 mg/3 h reached 1450 ± 45 pg/ml with a decrease to 240 ± 47 pg/ml by Hour 4 (Fig. 3). The percentage change from Hour 0 for progesterone concentrations during Hours -1 to 6 showed a main effect of hour from a gradual increase averaged over groups with no effect of group or a group-by-hour interaction. The progesterone concentrations for 0 to 11 d after treatment showed an effect of day with no effect of group or a group-by-day interaction (Fig. 4). The interval from the pretreatment to posttreatment ovulations and from treatment to progesterone <1 ng/ml were not different between groups (not shown).

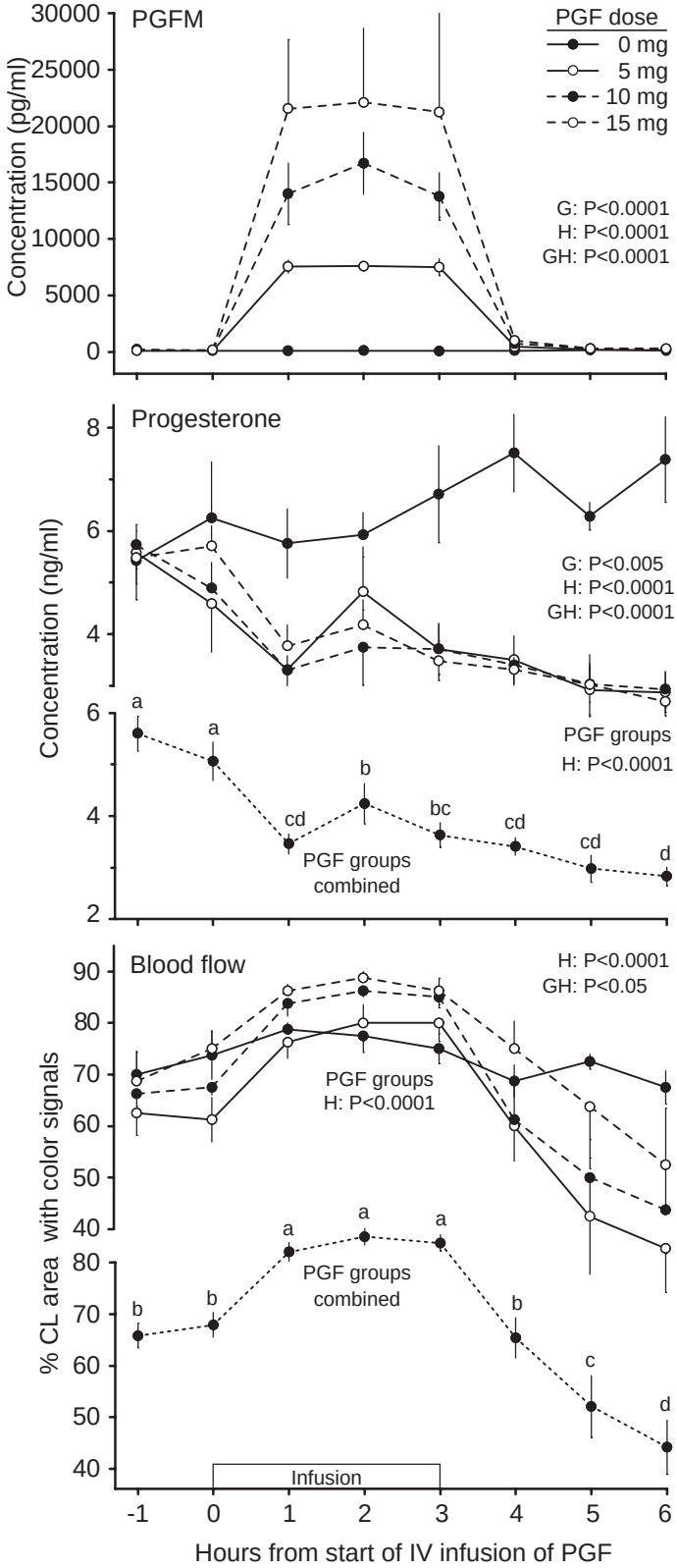

Figure 2. Mean (±SEM) concentrations of PGFM and progesterone and percentage of CL with color-Doppler signals of blood flow in association with 3 h of intravenous infusion of 0, 5, 10, or 15 mg of PGF (n = 4/dose). Main effects (G = group; H = hour) and interaction (GH) that are significant for all four groups or only for the PGF-treated groups are shown. a–d = means that are different (P < 0.05) among hours averaged for the three PGF treated groups. Experiment 1.

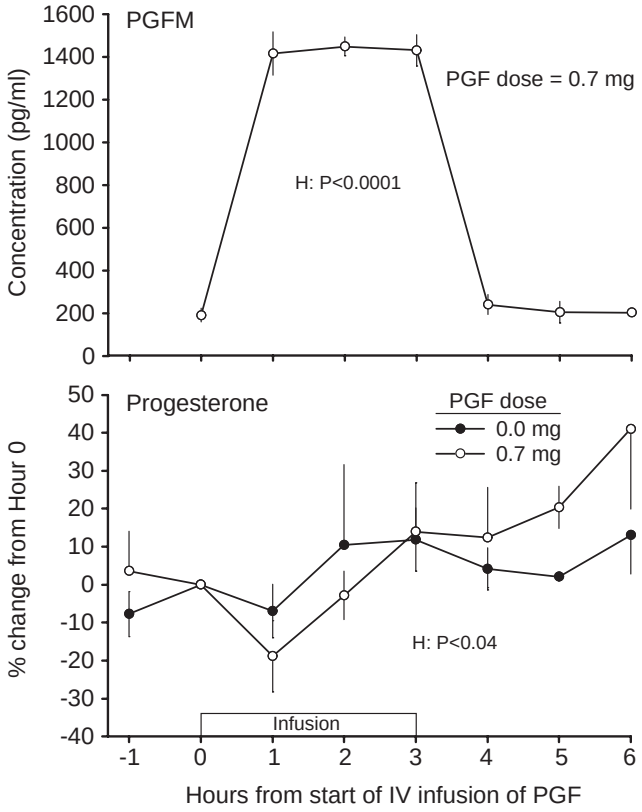

Figure 3. Mean (±SEM) concentrations of PGFM and percentage change in concentrations of progesterone in association with 3 h of intravenous infusion of 0.0 or 0.7 mg of PGF (n = 4/dose). H = main effect of hour. The group effect and the interaction of group-by-hour were not significant for progesterone. Experiment 2.

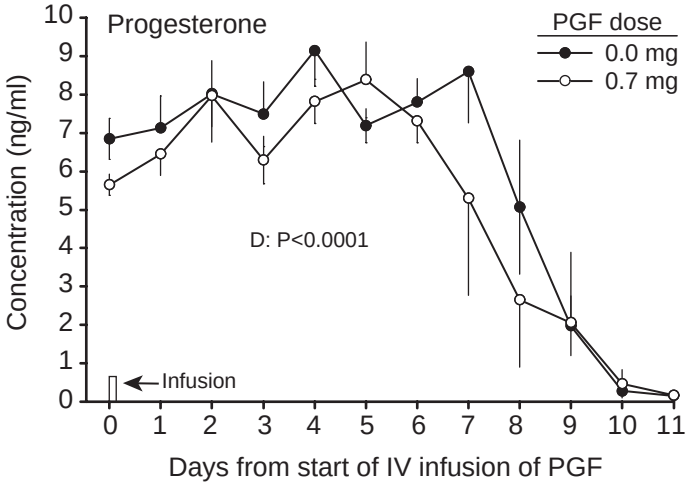

Figure 4. Mean (±SEM) concentrations of progesterone for 0 to 11 d after intravenous infusion of 0.0 or 0.7 mg/3 h of PGF (n = 4/dose) 8 d after ovulation. D = main effect of day. The group effect and group-by-day interaction was not significant. Experiment 2.

Discussion

A velocity setting of 6 cm/sec produced a data profile of the percentage of CL area with color-Doppler signals that was similar to the profile for a setting of 10 cm/sec, as indicated by the absence of a setting-by-hour interaction. The greater percentage estimates of flowing blood by the setting of 6 cm/sec was expected,

given that the setting represents the slowest velocity that will produce blood-flow signals (Ginther, 2007). The setting of 6 cm/sec is the lowest setting for the present instrument and was used for Experiment 1, owing to the detection of flow in more vessels without a difference between settings in the shape of the data profiles.

Inspection of published characteristics and profiles of bovine PGFM pulses (Kindahl *et al.,* 1976; Mann and Lamming, 2006; Ginther *et al.,* 2007) suggested that a simple constant infusion rate for 3 h would be adequate for simulating the function of a single natural pulse, based on the 3-h period of major concentrations in natural PGFM pulses. A second assumption was that the mean characteristic of a natural pulse need not be closely duplicated, as indicated by the wide variation among peaks of PGFM pulses. In both experiments, the experimental pulses were approximately in the form of a plateau for Hours 1 to 3 with a decrease to near baseline by Hour 4. Doses of PGF (5, 10, and 15 mg) that reportedly vary from ineffective to effective in inducing luteolysis when given as an IV injection produced experimental PGFM pulses when given by IV infusion during 3 h that were many-fold more prominent than natural PGFM pulses. The lowest PGF dose (5 mg/3 h) produced a PGFM concentration that was approximately seven-fold greater than the peak concentration for the natural PGFM pulses.

The initial progesterone decrease during the first hour after the beginning of a 3-h IV infusion of 5, 10, or 15 mg was followed by a transient increase during the next hour and then a gradual decrease for the remainder of the 6-h session. This is a novel observation. The increase between Hours 1 and 2 was most obvious (significant) with the lowest dose (5 mg). This observed dose-sensitive phenomena would need confirmation and further study. A second previously unreported observation was a more rapid decrease in progesterone during Hours 0 to 1 than during Hours 2 to 6. The progesterone increase between Hours 1 and 2 and the more gradual decrease between Hours 2 and 3 occurred despite the continued infusion of PGF until Hour 3.

Infusion of each of the 5-, 10-, and 15-mg doses was equally effective in inducing a decrease in progesterone over Hours 1 to 6. However, the reduced length of the interval between ovulations was significant for the 10- and 15-mg doses but not for the 5-mg dose. In this regard, a systemic single injection of 5 mg of PGF did not increase the number of cattle returning to estrus in 2 to 5 d, whereas doses of 15, 25, and 35 mg did increase the number and were equally effective (Lauderdale and Fokolowsky, 1979). The results from IV infusion of PGF for 3 h at 8 d postovulation may have involved a resurgence of CL progesterone production after Hour 6, but this was not studied. Incomplete luteolysis was indicated by the 8-d intervals from treatment to ovulation with the 10- and 15-mg doses, which seems longer than the 2 to 5 d reported for bolus treatments that induce acute and complete luteolysis (Lauderdale and Fokolowsky, 1979). The extent of luteolysis (progesterone decrease and resurgence) after Hour 6 with these IV infusion doses would require specific study. A resurgence in progesterone production after the initial decrease from a borderline PGF dose has been well-demonstrated in mares (Gastal *et al.,* 2005; Bergfelt *et al.,* 2006).

The 1-h interval for determination of progesterone concentration and percentage of the CL area with color-Doppler signals of blood flow with the PGF doses of 5-, 10-, and 15-mg/3 h precluded study of the temporal relationships between the initial changes in progesterone and blood flow. The reported changes began in less than 1 h (Acosta *et al.,* 2002). On an hourly basis, an increase in CL blood flow during intravenous PGF infusion occurred simultaneously with the increase in PGFM and the decrease in progesterone. The blood-flow results are consistent with the report (Ginther *et al.,* 2007) that blood flow increased during the ascending arm of each natural PGFM pulse. The decrease in blood flow percentage by Hour 5 to less than the percentage at Hour 0 is consistent with the decrease in progesterone between Hours 0 and 6. An infusion of 30 ml of vehicle IV was not associated with a detectable increase in CL blood flow. In contrast, each dose of PGF increased the CL blood-flow to a similar extent among doses, so that the increase can be attributed to the PGF infusion rather than to the prolonged heifer restraint, the intravenous infusion procedure, and hourly ultrasonographic examinations.

The extrapolation that a PGF dose of 0.7 mg/3 h (Experiment 2) would approximate a natural PGFM pulse was only partially successful. The PGFM concentrations in the experimental pulses from the 0.7-mg dose were about 26% greater than for the peak of natural pulses. However, the more prominent simulated pulse does not detract from the finding that the single simulated pulse from IV infusion was ineffective in altering circulating concentrations of progesterone. The ineffectiveness could be attributed to the use of a single simulated pulse rather than sequential pulses. It seems more likely that the intravenous route for infusion of the PGF played the primary role in the ineffectiveness. In this regard, 65% of PGF reportedly is metabolized during one passage through the lungs in cattle (Davis *et al.,* 1985). Thus, the amount of PGFM in the jugular blood is a poor representation of the amount of PGF in the arterial blood when it arrives at the CL. The present results indicate that future development of a PGF infusion procedure that results in a simulated natural PGFM pulse should include sequential pulses and should consider the intrauterine route. The intrauterine route would be more compatible with the natural delivery of PGF from a uterine horn to ovary through a unilateral venoarterial pathway (Ginther, 1981).

In conclusion, an IV 3 h infusion of a total of 5, 10, or 15 mg of PGF 8 d after ovulation decreased the circulating concentrations of progesterone between Hour 0 (beginning of infusion) and Hour 1 in all three groups, followed by an increase between Hours 1 and 2 in the 5-mg group. Concentrations decreased in all PGF-treated groups between Hours 2 and 6, but the decrease was more gradual than for Hours 0 to 1. In all three PGF-treated groups, the percentage of CL area with color-Doppler signals of blood flow increased between Hours 0 and 1, plateaued during Hours 1 to 3, and then decreased during Hours 3 to 5 to below the percentage at Hour 0. Infusion IV of a total of 0.7 mg of PGF during 3 h produced a PGFM plateau during Hours 1 to 3 that was about 26% greater than the peak of a natural PGFM pulse. Progesterone concentrations were not affected. Results demonstrated that IV infusion of PGF doses for 3 h that were previously shown to be completely luteolytic from a single systemic injection were at least partially luteolytic as indicated by a progesterone decrease during 6 h after the start of infusion. A 3-h IV infusion of a dose of PGF that resulted in an approximation of a natural PGFM pulse was not luteolytic.

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