Necessity of Sequential Pulses of Prostaglandin F2alpha for Complete Physiologic Luteolysis in Cattle

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ABSTRACT

The luteolytic effects of exogenous prostaglandin F2alpha (PGF) that did and did not simulate natural 13,14-dihydro-15-keto-PGF (PGFM) pulses were studied during mid-diestrus in 42 Holstein heifers. Plasma concentrations of PGF were assessed by assay of PGFM. In experiment 1, a single intrauterine injection of 4.0 mg of PGF into the uterine horn ipsilateral to the corpus luteum resulted in a precipitous progesterone decline, whereas sequential injections of 0.25 or 1.0 mg every 12 h resulted in a stepwise decrease (P < 0.05) following each injection. A progesterone increase occurred during the first 5 min before the luteolytic decrease but only for the 4.0-mg dose. From the results of experiment 2, a 2-h intrauterine infusion of a total of 0.5 mg of PGF was judged to best simulate a natural PGFM pulse. In experiment 3, simulation of sequential pulses at 12-h intervals resulted in a continuous precipitous decrease in progesterone to <1 ng/ml by the beginning of the fourth simulated pulse. In contrast, a single simulated pulse resulted in a 6-h progesterone decrease to a constant concentration for 3 days after treatment, followed by a return to control concentrations. The mean ± SEM interval between the pretreatment and posttreatment evaluations was shorter (P < 0.05) in the group with sequential simulated pulses (14 ± 1 day) than in the group with a single pulse (21 ± 1 day). Results indicated that excessive PGF doses may stimulate nonphysiologic progesterone responses and supported the hypothesis that sequential PGF pulses are required to stimulate natural luteolysis in cattle.

blood flow, cattle, corpus luteum, female reproductive tract, luteolysis, ovary, progesterone, prostaglandin F2alpha

INTRODUCTION

Secretion of prostaglandin F2alpha (PGF) by the uterus, augmented by intraluteal PGF production, terminates the luteal phase in many species, including cattle [1-5]. The minimal effective intrauterine (IU) dose of PGF (1-2 mg [6]) when given into the uterine horn ipsilateral to the corpus luteum (CL) in cattle is about one tenth of the minimal systemic dose (15 mg [7]). The IU effectiveness in cattle results from a unilateral utero-ovarian pathway [8, 9]. Surgical anastomoses of the uterine or the ovarian artery ipsilateral of an intact uterine horn to the corresponding vessel on the unilaterally hysterectomized side have demonstrated that a venoarterial unilateral pathway adequately accounts for the local luteolytic effect of a uterine horn on the CL in the adjacent ovary [10]. The transfer of the PGF involves passage from the uterine vein to the closely adherent ovarian artery [11]. In this regard, injection of a large dose (6 mg) of PGF into a uterine horn is followed by greater PGF concentrations in the ipsilateral ovarian artery than in the carotid artery [12]. Thus, the natural utero-ovarian route of delivery should be considered in studies of the mechanism of luteolysis that use exogenous PGF. A complexity in using the jugular vein for the PGF treatment and sampling is that 65% of PGF is metabolized during one passage through the lungs in cattle [13]. The main plasma metabolite of PGF is 13,14-dihydro-15-keto-PGF (PGFM) [14], and assay of PGFM has been used as an indicator of PGF release into the circulation [15].

Based on the PGFM concentrations, PGF is released from the uterus in pulses occurring approximately every 12 h in association with luteolysis in cattle [14-17]. The pulsatile release of PGF has been assumed to be an important aspect of luteolysis [3, 16]. However, the necessity for natural pulsatile delivery of PGF has not been demonstrated. Treatment of cattle with sequential pulses of PGF to simulate the natural pulse-delivery system has not been reported, to our knowledge. Sequential hour-long infusions of PGF into the ovarian artery of an autotransplanted ovary have been performed in ewes [18, 19]. Four sequential hour-long infusions in 19 h caused complete CL regression in one of four ewes, whereas five infusions in 25 h caused permanent regression in four of four ewes. Sequential hour-long infusions required considerably less PGF for luteolysis than continuous infusion. Results suggested that sequential pulses of PGF may be a component of the luteolytic process in ewes. However, the concentrations of PGF or PGFM and the extent to which each hour-long infusion simulated a natural PGF pulse were not given. Reported studies of the mechanisms associated with the luteolytic process in cattle that involved administration of exogenous PGF apparently did not account for the potential natural necessity of pulsatile delivery from uterus to CL.

The objectives of the present experiments were the following: 1) to determine if low IU doses of PGF require sequential treatment for the completion of luteolysis (experiment 1), 2) to develop a dose and convenient method of delivery of exogenous PGF that would result in an approximation of a natural PGFM pulse (experiment 2), and 3) to simulate PGFM pulses to test the hypothesis that sequential PGF pulses are required for the stimulation of complete luteolysis (experiment 3). Apparent nonphysiologic responses to various doses of PGF also were considered.
MATERIALS AND METHODS

Animals

Animals were handled in accord with the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Research. Holstein heifers, aged 17–20 mo, were used in three experiments. Heifers remained healthy and in good body condition throughout each experiment. Animals were selected with docile temperament and near-identical ultrasonographic characteristics of the reproductive tract, as determined by ultrasound examinations [20]. If more than one CL was present, the heifer was not used. The animals were acclimated to the handling procedures for at least 2 wk before experimentation. When indicated, heifers were sedated with a low dose of xylazine hydrochloride (14 mg/heifer i.m. of Xilact-Ject; Phoenix Pharmaceutical Inc., St. Joseph, MO). Xylazine sedation reportedly [21] produces hemodynamic effects when assessed in a major artery (internal iliac) but does not affect local vascular perfusion in the ovaries, based on the vascular resistance index at the ovarian pedicle and percentage of CL area with color Doppler blood flow signals.

Catheterizations

Injection or infusion of PGF (dinoprost tromethamine [Lutalyse]; Pfizer Animal Health, New York, NY) into the uterine horn ipsilateral to the CL (IU) was performed during mid-diestrus in all experiments. The time of injection or the beginning of infusion was designated as Hour 0. Infusion of PGF was performed at a constant rate using a variable-flow peristaltic minipump (catalog No. 13-876-4; Fisher Scientific, Pittsburgh, PA). The pump was calibrated to deliver the specified dose of PGF in 3 h in ml of vehicle (experiment 2) or in 2 h in ml (experiment 3). The intravenous (IV) infusion for one group in experiment 3 was made through an indwelling surgical tubing (Tygon tubing [inner diameter, 0.040 inches]; Norton, Akron, OH) 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. The IV injections (experiment 1) and IU infusions (experiments 2 and 3) were made into the lower horizontal portion of the spiraled uterine horn (segment 3 [20]) ipsilateral to the CL. For IU injection and infusion of PGF, the tubing (polytetrafluoroethylene AWG Tubing-TFT 15 NT [inner diameter, 0.059 inches and outer diameter, 0.083 inches]; Parker Hannifin Corporation, Fort Worth, TX) was initially inserted through an artificial insemination pipette into segment 1, the horn was straightened transrectally, and the tubing was pushed into segment 3. The tubing was secured by digital compression of the horn while the pipette was withdrawn. Maintenance of the IU location was confirmed before and at the end of infusion by transrectal ultrasonography. A session was defined as a 5-h or 6-h period after the injection or the beginning of infusion.

Ultrasonography

A duplex B-mode (gray scale) and pulse wave color Doppler ultrasonic instrument (Aloka SSD 3550; Aloka American, Wallingford, CT) equipped with a linear array 7.5-MHz transducer was used for transrectal scanning of the ovaries to determine the day of ovulation and cross-sectional area of the CL. The scanner’s tracing function in B mode was used to determine cross-sectional area (in centimeters squared) of the CL, as previously described [17]. A decrease in progesterone to <1 ng/ml was used as an indicator of complete luteolysis [16]. The length of the interval from ovulation to progesterone level <1 ng/ml and the experimental interovulatory interval were determined in experiments 1 and 3.

Experiment 1: IU Injection

This experiment was performed to determine if a dose of PGF that was ineffective as a luteolytic treatment when given in a single IU injection 9 or 10 days after ovulation would be effective when given sequentially. Simulation of the natural PGFM pulse was not expected, based on a previous study [22] of circulating PGF concentration following IU injection. Doses of PGF were chosen that were expected to be ineffective, borderline, and effective for circulating PGF concentration following IU injection. Doses of PGF were 0.25, 1.0, and 4.0 mg (n = 23). The four experimental groups were controls (0.0 mg of PGF, vehicle only) and the PGF doses of 0.25, 1.0, and 4.0 mg (n = 4) given in 1 ml of vehicle. Sequential treatment sessions were begun every 12 h for sessions 1 to 4, except that only session 1 was used for the 4.0-mg group, owing to expected immediate completion of luteolysis. Collection of blood samples was performed hourly at Hours 0–6 for each treatment session through an indwelling cannula in a jugular vein. In addition, a sample was collected at Minutes 0, 2, 5, 10, 15, 30, 45, and 60 after treatment with PGF but only for session 1. Concentrations of PGFM were determined for the frequent blood samples collected during the first hour of session 1 and hourly at Hours 0–6 for sessions 1–4. Progesterone was assayed for the samples collected at Minutes 0, 5, 15, 30, and 60 for the first hour of session 1 to determine if the reported immediate posttreatment increase in progesterone [22] occurred in each PGF-treated group. Progesterone was also assayed for samples collected at 6-h intervals during 0–48 h after the beginning of session 1, thereby encompassing all of the four sessions. Thereafter, progesterone was assayed every 12 h until 24 h after morphologic regression seemed complete, based on ultrasonographic assessment of CL area (in centimeters squared) and CL color Doppler blood flow signals that involved <20% of the CL area [17].

Natural PGFM pulses were characterized for comparison with the experimental PGFM concentrations from the IU injections of PGF. Hourly samples were taken during a 12-h window, beginning 15 days after ovulation in four heifers. The 12-h window was used each day until CL regression was apparent by color Doppler ultrasonography. Six pulses of PGFM were identified during luteolysis (rapidly decreasing progesterone) by statistical comparison of the values for a suspected pulse against the intraassay coefficient of variation (CV), as previously described [17]. The mean ± SD for the peak of the pulses was determined as an indicator of the variation among natural pulses.

Experiment 2: Dose Titration for IU Infusion

This was a preliminary dose titration study for guidance on the dose of PGF to be used for IU infusion in experiment 3. Doses of 0.125, 0.25, 0.5, 1.0, and 2.0 mg/3 h were infused (n = 2) on Day 8. Blood samples for PGFM assay were collected every hour at Hours 0–6 and for progesterone at Hours 0, 6, 24, and 48. Data were assessed by inspection. However, for guidance in the selection of a dose of PGF that best simulated a spontaneous PGFM pulse, the observations (n = 18) comprising the three highest values for the six natural pulses from experiment 1 were compared with the mean PGFM concentrations for the IU infusion comprising Hours 0, 2, 10, 15, 30, 45, and 60 (mean ± SD). The dose that resulted in best simulate the PGFM concentrations and the shape of the data profile of the natural pulses and the doses that caused an apparent transient decrease in progesterone were considered in selecting a dose for experiment 3.

Experiment 3: Sequential IU Infusions

The primary objective of this experiment was to determine if sequential simulated pulses after ovulation would induce complete luteolysis, whereas a single simulated pulse would produce only a transient or no decrease in progesterone. A total 0.5-mg dose of PGF (0.25 mg/h for 2 h) was used, based on the results of experiment 2. A 2-h infusion was selected for delivery of the PGF dose, rather than the 3 h used in experiment 2, based on the judgment that the 2-h infusion would adequately simulate a natural pulse. An IV group was included to compare IV vs. IU infusion. The experimental groups involved vehicle IU for a single session (IU1) and a single dose of PGF of 0.5-mg IV for a single session (0.5-mg IV1), 0.5-mg IU for a single session (0.5-mg IU1), and 0.5-mg IU for four sequential sessions (sessions 1–4) at 12-h intervals (0.5-mg IU4; n = 4/group). For analyses that were limited to session 1, the 0.5-mg IU1 and 0.5-mg IU4 groups were combined (n = 8).

Blood samples for PGFM and progesterone assay were collected at Minutes 0, 2, 5, 10, 15, 30, 45, and 60 for the 0.5-mg IU1 and 0.5-mg IU1 groups and were used to compare the IU rate of absorption into the circulation with increases from direct IV infusion. Samples for PGFM and progesterone were collected from all groups at Hours 0, 1, 2, 3, 4, and 5. Additional samples for progesterone only were collected at Hours 12, 24, 36, and 48 and daily thereafter until ovulation. Cross-sectional area (in centimeters squared) of the CL was determined at Hours 0, 12, 24, 36, and 48. Data for CL area were converted to percentage change from Hour 0, owing to apparent differences among groups before infuson began. Concentrations of PGFM for the six natural pulses from experiment 1 were used to judge the success in simulating a natural pulse by 2-h IU infusion of PGF.

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 125I-labeled progesterone (Coat-A-Count Progesterone; Diagnostic Products Corporation, Los Angeles, CA), as previously described [17]. The intraassy CV and sensitivity ranged from 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 min before centrifuging and storing at −20°C. The plasma samples were assayed for PGFM by a modification of a radioimmunoassay procedure [24, 25] that was adapted and...
Experiment 1: IU Injection

Circulating concentrations of PGFM after an IU injection for each PGF group (doses of 0.25, 1.0, and 4.0 mg) increased in 2 min, reached maximum in 10 min, and then decreased to near the base concentration in 1 h (Fig. 1). The maximum concentration at 10 min for the 0.25-mg dose (1799 ± 137 pg/ml) was greater (P < 0.0001) than that at the peak of the six natural pulses (1065 ± 77 pg/ml; Fig. 2). The mean ± SD for the peak of the natural PGFM pulses was 1065 ± 188 pg/ml (range, 879-1339 pg/ml). The PGFM concentrations at Hours 0–6 in each of the four sessions within each of the 0.0-, 0.25-, and 1.0-mg groups did not differ among sessions (data not shown).

Concentrations of progesterone showed significant main effects of group (dose) and time (minutes or hours) and a group-×-time interaction for the first 60 min, for the 6 h of session 1, and for every 6 h for 48-h posttreatment, except that the interaction only approached significance (P < 0.1) for the 6 h of session 1 (Fig. 3). When each group was analyzed separately for each time period (60 min, 6 h, and 48 h), the time effect was not significant for the 0.0-mg group, and only the main effect of time (minutes or hours) was significant (P < 0.001) for the three groups that received PGF. The differences (P < 0.05) among minutes or hours averaged over the three PGF groups are shown (Fig. 3). Although the interaction was not significant, an increase (P < 0.05) between Minutes 0 and 5 occurred exclusively for the 4.0-mg group. Averaged over the three PGF groups, progesterone decreased progressively between Minutes 15 and 60. The decrease in progesterone between Hours 0 and 1 was significant (P < 0.0005) for each PGF group, but the increase between Hours 1 and 2 was significant (P < 0.03) only for the 0.25- and 1.0-mg groups. When the 0.25-mg and 1.0-mg groups for every 6 h for 48 h were analyzed after removing the 0.0-mg and 4.0-mg groups, only the hour effect was significant (P < 0.001). The hour effect represented significant decreases combined for the 0.25- and 1.0-mg groups between Hours 0 and 6 (P < 0.03), Hours 12 and 18 (P < 0.0004), and Hours 24 and 30 (P < 0.002) but not between Hours 6 and 12 and Hours 18 and 24. Owing to the mean decrease in progesterone to <1 ng/ml within 48 h after PGF treatment in the three PGF groups, the subsequent progesterone concentrations are not shown. However, the intervals from the pretreatment ovulation to a progesterone decrease to <1 ng/ml and to the posttreatment ovulation were shorter (P < 0.05) for each of the groups that received PGF than for the 0.0-mg group (Table 1).

Experiment 2: Dose Titration for IU Infusion

The mean concentrations of PGFM for Hours 0–6 and of progesterone for Hours 0, 6, 24, and 48 from IU infusion of PGF for Hours 0–3 are shown (n = 2; Fig. 4). Compared with the observations comprising the mean of the three highest values for the six natural pulses (746 ± 86 pg/ml), the concentration was greater for the 2.0-mg (2018 ± 136 pg/ml; P = 0.0008) and 1.0-mg (1810 ± 68 pg/ml; P = 0.006) groups, was not significantly different for the 0.5-mg group (806 ± 49 pg/ml), and was lesser for the 0.25-mg (413 ± 23 pg/ml; P = 0.02) and 0.125-mg (250 ± 41 pg/ml; P = 0.002) groups. Progesterone concentrations decreased to <1 ng/ml by Hour 24 in the 2.0-mg group, decreased transiently to approximately 3 ng/ml in the 1.0-mg and 0.5-mg groups, and decreased slightly or did not seem to decrease in the 0.25-mg and 0.125-

![Figure 1](https://example.com/fig1.png)

![Figure 2](https://example.com/fig2.png)
mg groups. The decrease and then increase in progesterone concentration seemed to be most prominent for the 0.5-mg group. The 0.5-mg dose was selected for experiment 3 after considering both the progesterone responses and the differences in PGFM concentrations between experimental and natural pulses.

Experiment 3: Sequential IU Infusions

The IV infusion of PGF during session 1 (0.5-mg IV1) resulted in an immediate (≤2 min) increase in PGFM to a concentration that was maintained until the end of infusion at Hour 2 and then decreased rapidly (Fig. 5). The IU infusion during session 1 (0.5-mg IU) was associated with a gradual increase in circulating PGFM between Minutes 2 and 45 compared with the immediate and greater increase in the IV group. The concentration in the two combined 0.5-mg IU groups at Hour 1 of session 1 was maintained until Hour 2 and decreased thereafter. However, the concentration was lower (P < 0.02) at Hours 1 and 2 but was greater (P < 0.009) at Hour 3 than that for IV infusion.

Progesterone concentrations during the first hour of infusion in session 1 showed a similar reduction in concentrations between Minutes 0 and 60 for the IV and IU infusion routes (main effect of minute; Fig. 5). For progesterone concentrations during Hours 0–5 of session 1, the hour effect and the interaction of group (0.0-mg IU, 0.5-mg IV, and 0.5-mg IU) and hour were significant. When groups were analyzed separately, only the combined 0.5-mg IU groups showed an effect of hour (P < 0.0002). The interaction was attributable primarily to a decrease in progesterone between Hours 0 and 1 in the 0.5-mg IU groups (P < 0.006).

The two main effects (group and day) and the interaction (group × day) for progesterone concentrations during 0.5-day and 1-day intervals were significant (Fig. 6). The interaction represented primarily lower concentrations in the 0.5-mg IU4 group (infused during sessions 1–4) on Days 1–5 than in the other groups and an intermediate and transient reduction on Days 0.5–3 in the 0.5-mg IU1 group (infused only for session 1). The apparent reduced concentrations in the IV1 group on Days 6–9, compared with the 0.0-mg IU1 and 0.5-mg IU1 groups, were not significant. As expected, the concentrations decreased similarly between 0 and 0.5 days after the session 1 treatment in the 0.5-mg IU1 group in Days 6–9, compared with the 0.0-mg IU1 and 0.5-mg IU1 groups, were not significant. At Days 0.5–3, concentrations were lower (P < 0.0001) in the IU4 group than in the 0.0-mg IU1 and 0.5-mg IU1 groups on Days 0.5–3 but not thereafter. The intervals from the pretreatment ovulation to the day when progesterone level was >1 ng/ml and to the posttreatment ovulation were shortest for the sequential treatments (IU4 group), with no differences among the other three groups (Table 1).

For percentage change in CL area (in centimeters squared), both main effects and the interaction were significant (Fig. 7). When groups were analyzed separately, a significant hour effect occurred in both the single (0.5-mg IU1; P < 0.004) and sequential (0.5-mg IU4; P < 0.0001) IU infusion groups. The hour effect represented a decrease (P < 0.05) between Hours 0 and 12 for each group and subsequent decreases only for the sequential 0.5-mg IU4 group, as shown. At Hours 24, 36, and

FIG. 3. Mean ± SEM concentrations of progesterone during the first hour, 6 h, and 48 h after IU injection of PGF (n = 4). The 4.0-mg IU1 group was injected only at the beginning of session 1 (solid arrow on x-axis), and the remaining groups were injected at the beginning of each of sessions 1–4 at 12-h intervals (solid and open arrows). Main effects (group [G] and minute [M] or hour [H]) and interactions (GM or GH) that were significant or approached significance are shown. Means that are different (P < 0.05) among minutes and hours averaged for the three PGF-treated groups (two top panels) or for the two groups with lowest doses of PGF (bottom panel) are indicated by a–f. An increase (P < 0.05) between Minutes 0 and 5 was found only in the 4.0-mg group, and an increase (P < 0.05) between Hours 1 and 2 was found only in the 0.25-mg and 1.0-mg groups. Experiment 1.
48, percentages were lesser ($P < 0.05$) in the sequential group than in the single group and were lesser ($P < 0.05$) in the single group than in the IV and 0.0-mg groups.

**DISCUSSION**

The increase to maximum in circulating PGFM concentrations within 10 min after the IU injection of each dose of PGF (0.25, 1.0, and 4.0 mg) is consistent with previous studies [22, 26]. The maximum concentrations greatly exceeded the peak of a natural PGFM pulse, even for the smallest dose. Although the rapid absorption rate from the uterus resulted in PGFM concentrations that did not simulate a natural pulse, some information was obtained on the role of exposure of the CL to sequential bursts of PGF. The highest dose (4.0 mg) induced luteolysis (mean progesterone level, $1\text{ ng/ml}$) within 36 h, as expected [23]. However, the 0.25- and 1.0-mg doses induced stepwise CL regression, in keeping with the sequential treatment session every 12 h. The stepwise regression was shown by the progesterone decrease during the 6 h of each of sessions 1, 2, and 3 and the absence of a decrease in the 6-h periods between sessions. The decrease in progesterone to $<1\text{ ng/ml}$ occurred after three sessions of PGF injection, suggesting that only three consecutive bursts of PGF were needed for the completion of luteolysis. Similarly, progesterone decreased to $<1\text{ ng/ml}$ after three simulated PGFM pulses in experiment 3. However, a study with different numbers of sessions would be needed for a firmer conclusion on the number of required exposures to PGF. The stepwise regression of the lower doses was as effective as the precipitous progesterone decrease of the high dose, as indicated by the similar decrease to a mean of $<1\text{ ng/ml}$ within 36 h for the two lower doses and the high dose. The results suggested that natural sequential delivery of PGF is important for luteolysis, but this conclusion is tentative in that the sequential bursts did not resemble natural pulses.

Examination of the posttreatment progesterone profile after the injected low IU doses of PGF indicated that a progesterone decrease did not begin until 15 min after treatment, followed by a continuous rapid decrease until 1 h. However, concentrations increased during the next hour, followed by an apparent slower gradual decrease until 6 h. These are novel observations that require confirmation but suggest that the initial response to a burst of PGF is a transient rapid decrease in progesterone, followed by a slower rate of decrease until another PGF burst occurs. In response to simulating a PGFM pulse, the progesterone decline occurred only between 0.5 and 1.0 h after initiation of the simulated pulse, even though the PGF infusion continued for another hour. In mares [27], plasma progesterone concentrations decreased after the peak of each individual natural PGFM pulse. However, in a limited study [17] in heifers, progesterone levels did not differ significantly during the 7 h associated with individual PGFM pulses. These observations indicate a need for further study of the progesterone response to each natural PGF pulse in cattle.

**TABLE 1.** Mean ± SEM effects of PGF treatment at mid-diestrus on the intervals from pretreatment ovulation until progesterone decrease to $<1\text{ ng/ml}$, and from pretreatment ovulation until posttreatment ovulation.

<table>
<thead>
<tr>
<th>Experiment (route)$^a$</th>
<th>Dose</th>
<th>No. of sessions$^b$</th>
<th>No. of days from pretreatment ovulation until progesterone is $&lt;1\text{ ng/ml}$</th>
<th>No. of days from pretreatment ovulation until posttreatment ovulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Injected IU)</td>
<td>0.0  mg</td>
<td>1</td>
<td>18.9 ± 1.4$^c$</td>
<td>21.2 ± 1.5$^c$</td>
</tr>
<tr>
<td></td>
<td>0.25 mg</td>
<td>4</td>
<td>12.0 ± 0.0$^d$</td>
<td>13.3 ± 0.9$^d$</td>
</tr>
<tr>
<td></td>
<td>1.0  mg</td>
<td>4</td>
<td>11.3 ± 0.4$^d$</td>
<td>13.2 ± 0.8$^d$</td>
</tr>
<tr>
<td></td>
<td>4.0  mg</td>
<td>1</td>
<td>10.5 ± 0.3$^d$</td>
<td>15.2 ± 0.5$^d$</td>
</tr>
<tr>
<td>3 (Infused IU or IV)$^b$</td>
<td>0.0 mg (IU)</td>
<td>1</td>
<td>18.8 ± 1.2$^c$</td>
<td>21.7 ± 1.7$^c$</td>
</tr>
<tr>
<td></td>
<td>0.5 mg (IV)</td>
<td>1</td>
<td>16.3 ± 1.8$^c$</td>
<td>19.5 ± 1.3$^c$</td>
</tr>
<tr>
<td></td>
<td>0.5 mg (IU)</td>
<td>1</td>
<td>17.5 ± 0.5$^c$</td>
<td>20.8 ± 1.1$^c$</td>
</tr>
<tr>
<td></td>
<td>0.5 mg (IU)</td>
<td>4</td>
<td>10.2 ± 0.6$^d$</td>
<td>13.5 ± 0.6$^d$</td>
</tr>
</tbody>
</table>

$^a$ n = 4 heifers/group.

$^b$ No. of sequential treatment periods at 12-h intervals.

$c,d$ Within each experiment and interval, means without common superscripts are significantly different ($P < 0.05$).

$^e$ Infusion dose was given during 2-h interval.

![FIG. 4. Mean ± SEM PGFM and progesterone concentrations associated with a 3-h IU infusion of PGF (n = 2). The 0.5-mg dose was chosen for use in experiment 3, owing to the production of a simulated PGFM pulse that was similar to a natural pulse from experiment 1 and progesterone concentrations that seemed to decrease and then increase. Experiment 2.](https://academic.oup.com/biolreprod/article-abstract/80/4/641/2557566)
Inspection of published characteristics and profiles of bovine PGFM pulses [14, 16, 17] suggested that a simple constant infusion rate of PGF for 2 or 3 h would be adequate for simulating the function of a single natural PGF pulse, based on the 2- or 3-h period of major concentrations in natural PGFM pulses. A second assumption was that the mean characteristics of a natural PGFM pulse need not be closely duplicated, as indicated by the wide variation among peaks of PGFM pulses. A third assumption was that the elevated nadir between PGFM pulses during the luteolytic period [17] did not represent an essential component of the luteolytic mechanism and is supported by an increase in CL blood flow during each pulse and not between pulses [17]. Improved simulation of the mean shape of natural PGFM pulses and the nadirs between pulses would require minipumps with programmable changes in flow rates. In addition, pumps that are attachable to the animal may minimize the adverse effects of prolonged animal restraint. Such a refined approach was beyond the goal of the present investigations, but the results may encourage more refinement in future studies.

The simulated PGFM pulses from IU infusion of PGF approached the characteristics of a natural pulse, especially after considering the leeway indicated by the variation in the peak of natural pulses. In addition, the IU infusion (in contrast to IV infusion) did not result in a precipitous decrease in CL blood flow, as indicated by the lack of change in CL area during the luteolytic period [17].
to the IV route) more closely simulated the gradual increase and decrease that occurs on each side of a natural PGFM peak. The precipitous progesterone decrease from a single 2-h IU infusion of 0.5 mg of PGF and the return to a concentration similar to the control concentrations by 3 days contrast with the continuous decrease from sequential IU sessions at 12-h intervals. The rapid 24-h decline in progesterone from the sequential PGF infusions and the slower rate of decline during the next 12 h are consistent with the reduction rate during spontaneous luteolysis when data are normalized retrospectively to a decrease to <1.0 ng/ml of progesterone [17]. The comparatively slower rate of the mean decline in the controls than in the sequentially treated group (experiment 3) is attributable to the beginning of luteolysis at different times among control individuals.

The relationships in progesterone concentrations among groups were consistent with the reduced area (in centimeters squared) of a cross section of the CL during Hours 24, 36, and 48 in the sequential IU group and an intermediate area reduction in the single IU group. No reduction in CL area was detected in the controls or the IV group. In addition, the progesterone and CL results were consistent with a reduced interval from ovulation to a progesterone concentration of <1 ng/ml and to the posttreatment ovulation in the group with sequential simulation of pulses. These intervals in the other two PGF-treated groups were similar to the intervals in the control group. Therefore, the results well supported the hypothesis that sequential PGF pulses are required for the natural completion of luteolysis in cattle.

The transient increase in progesterone concentrations that occurred within 5 min after IU injection of a dose of PGF that caused luteolysis with a single treatment (4.0 mg) agrees with findings that an initial increase in progesterone occurred within 5 or 10 min after systemic, IU, or intraluteal PGF administration of a single luteolytic dose [12, 22, 28, 29]. However, in the present investigations, an initial increase in progesterone before the decrease associated with luteolysis was not detected with doses of PGF that required sequential IU treatment for complete luteolysis (0.25 or 1.0 mg; experiment 1) or in association with simulated PGFM pulses. This observed dose-sensitive phenomenon apparently represented a nonphysiologic response to unnatural doses and delivery of PGF. Although further study is needed, it seems that many of the reported studies during the past few decades on the nature of the luteolytic process in cattle may have resulted in dubious interpretations, owing to potential artifactual or pharmacologic responses to unnatural doses or unnatural delivery of PGF to the CL. A dose and method of delivery that approximated the endogenous system were included in the present investigations. Nevertheless, some reservation is required, owing to the unnatural time of treatment during mid-diestrus.

In conclusion, sequential injections of 0.25 and 1.0 mg of PGF every 12 h and a single injection of 4.0 mg were made into the uterine horn ipsilateral to the CL during mid-diestrus. Each dose induced luteolysis in a mean of 36 h. However, the high dose induced an initial precipitous decline in progesterone, followed by a more gradual but continuous decline. In contrast, the sequential lower doses induced stepwise CL regression, in keeping with the sequential treatments. An approximate simulation of natural PGFM pulses was performed by constant IU infusion of a total of 0.5 mg of PGF during 2 h, beginning every 12 h. Simulation of a single pulse induced a transient decrease in progesterone that returned to pretreatment concentrations in 4 days, with no effect on the interval to posttreatment ovulation. Sequential infusion of PGF to simulate PGFM pulses induced a continuing decrease in progesterone and shortened the interval to ovulation by approximately 7 days. Results supported the hypothesis that sequential PGF pulses are required for completion of luteolysis in cattle. In addition, it was noted that an unnatural dose or route of delivery of exogenous PGF may produce a progesterone response that may not occur naturally such as the frequently reported immediate and transient increase in circulating progesterone before the luteolytic decrease.

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