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Dynamic progesterone responses to simulation of a natural pulse of a metabolite of prostaglandin $F_{2\alpha}$ in heifers

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ABSTRACT

The effects of prostaglandin $F_{2\alpha}$ (PGF) on circulating progesterone concentration were studied in four groups ($n = 4$) of Holstein heifers 9 d after ovulation. The progesterone response to simulation of a pulse of 13,14-dihydro-15-keto-PGF (PGFM) by a 2-h intrauterine (IU) infusion of 0.5 mg of PGF was compared with the response to a PGF-bolus IU injection of 4 mg. The beginning of infusion and time of injection were designated Minute 0. Progesterone concentration did not change significantly between Minute 0 and Hour 48 in control or IU vehicle-treated groups. In the PGF-bolus group, progesterone concentration increased ($P < 0.05$) between Minutes 0 and 10 and then decreased. In the PGF-infusion group, simulation of a PGFM pulse was not associated with an initial transient increase in progesterone. The first significant decrease ($P < 0.05$) in progesterone began at Minute 20 and continued until Hour 1. The progesterone concentration then began to rebound ($P < 0.05$) at Hour 1 and peaked at Hour 3 at almost the same concentration as at the start of PGF infusion. The progesterone again decreased after Hour 3 and increased again between Hours 24 and 48. In summary: (1) an initial transient increase in progesterone was not detected in association with an individual simulated pulse of PGF, indicating that the frequently reported pronounced transient increase after a bolus luteolytic dose of PGF is a nonphysiological response and (2) simulation of a PGFM pulse resulted in a distinct transient rebound in progesterone beginning at Hour 1 of the PGF infusion.

1. Introduction

Prostaglandin $F_{2\alpha}$ (PGF) is the natural luteolysin and is secreted from the endometrium in pulses in many animal species, including cattle (Silvia et al., 1991; McCracken et al., 1999; Schams and Berisha, 2004; Weems et al., 2006; Skarzynski et al., 2008). Based on a PGF metabolite (13,14-dihydro-15-keto-PGF; PGFM), PGF is secreted from the uterus in episodes of three or four pulses, 2 or 3 h in duration, and occurring about every 12 h in cattle (Kindahl et al., 1976a,b; Mann and Lamming, 2006; Ginther et al., 2007). A single luteolytic dose of PGF administered by either intrauterine (IU), intravenous (IV), or intraluteal routes causes an initial transient increase in progesterone prior to the decrease (Lamond et al., 1973; Hixon and Hansel, 1974; Skarzynski et al., 2003b). In a recent study (Ginther et al., 2009), progesterone increased within 5 min after a single IU bolus injection of 4 mg of PGF and thereafter decreased to <1 ng/ml (defined as complete luteolysis) within 36 h after treatment. An initial increase in progesterone was not detected in association with a simulated PGFM pulse (IU infusion of 0.5 mg of PGF during 2 h), and multiple pulses were required for complete luteolysis.

A rebound in progesterone concentration 2 or 3 h after treatment with a single dose of PGF or PGF analogue has been observed (Hixon and Hansel, 1974; Stellflug et al.,

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1977; Schallenberger et al., 1984; Baishya et al., 1994). In a recent study (Ginther et al., 2009), a transient rebound in progesterone concentration occurred at 2 or 3 h after the treatment in response to a PGF-bolus IU injection of 0.25 or 1.0 mg that induced partial luteolysis, but a rebound did not occur with a dose (4 mg) that induced complete luteolysis. In the 4-mg group, progesterone concentration did not rebound but maintained approximately the 1-h concentration until 3 h after treatment before beginning again to decrease. However, it is unknown whether a rebound in progesterone occurs during simulation of a PGFM pulse.

Reported conclusions on the mechanism of PGF-induced luteolysis that were based on a single dose of PGF or a PGF analogue are problematic, owing to the use of a pharmacological dose as indicated by an initial increase in progesterone prior to the decrease and lack of prominent rebound in progesterone following the decline. The potential importance and impact of the apparent differences between the two approaches for PGF administration necessitates an experiment in which the progesterone is compared within a single study between a bolus IU luteolytic PGF dose and a route and dose of PGF-infusion that simulates a natural PGFM pulse. The progesterone rebound may represent a physiological progesterone response to PGF that has not been considered in proposals on the nature of the luteolytic mechanism.

Two hypotheses were tested in the present experiment. (1) An initial pronounced transient increase in progesterone does not occur in association with an individual pulse of PGF, based on the response to a PGF dose and method of delivery that simulates a natural PGFM pulse. (2) Simulation of a PGFM pulse by a 2-h IU infusion of PGF is associated with a rebound in progesterone concentrations characterized by a decrease and an increase during infusion and a decrease after infusion.

2. Materials and methods

2.1. Heifers and treatments

Heifers were handled according to the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Agricultural Research. The heifers were Holsteins and were 16–20 months of age. The heifers were maintained in a shelter with access to outdoor paddocks. They were provided with *ad libitum* access to water, trace-mineralized salt, and a mixture of grass and alfalfa hay. All heifers were in good health and body condition. Animals were selected with docile temperament and no apparent abnormalities of the reproductive tract, as determined by ultrasonic examinations (Ginther, 1998). If more than one corpus luteum (CL) was present, the heifer was not used. The animals were accustomed to the handling procedures for at least 2 weeks prior to experimentation. The ovaries were monitored daily by transrectal ultrasonography to detect ovulation and formation of a CL. The day of disappearance of the dominant follicle was taken as the day of ovulation.

Heifers were sedated before placement of an indwelling catheter into a jugular vein with 14 mg/heifer (IM) of xylazine hydrochloride (Xila-ject, Phoenix Pharmaceuticals Inc., St. Joseph, MO, USA). Xylazine sedation reportedly (Araujo and Ginther, 2009) 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.

Four experimental groups were used ($n = 4$/group): controls (no treatment), saline-infusion (6 ml of PBS infused IU during 2 h), PGF-infusion (0.5 mg of PGF in 6 ml of PBS infused IU during 2 h), and PGF-bolus (4 mg of PGF injected IU). The 2-h IU infusion of 0.5 mg PGF was used to simulate a natural PGFM pulse as previously demonstrated (Ginther et al., 2009). The bolus IU injection of 4 mg PGF was used to represent a dose of PGF that stimulates an initial progesterone increase before a complete decrease (luteolysis). The PGF product was dinoprost tromethamine (Lutalyse; Pfizer Animal Health, NY, USA). According to the manufacturer, the product contains the naturally occurring PGF (dinoprost) as the tromethamine salt. The doses described herein refer to an equivalent of natural PGF.

2.2. Protocol

The experiment was begun 9 d after ovulation. Heifers were randomized within replicates of the four groups. The operator was unaware of the treatment given to heifers in the saline-infusion and PGF-infusion groups. A variable-flow peristaltic mini-pump (Catalogue No. 13-876-4; Fisher Scientific, Pittsburgh, PA, USA) was calibrated for IU infusion of 6 ml of vehicle (PBS) or a 0.5 mg total dose of PGF in 6 ml PBS during 2 h at a constant infusion rate (Ginther et al., 2009). After aseptic preparation of the perineum and vulval area, an AI instrument with sterile sheath was inserted through the vagina and into the uterine horn (segment 1; Ginther, 1998) ipsilateral to the CL ovary. The AI instrument was then removed leaving the sheath in place. A tubing (PTFE AWG Tubing-TFT 15 NT; id, 1.5 mm; od, 2.1 mm; Parker Hannifin Corp., Fort Worth, TX, USA) that was connected to the silicone tubing from the mini-pump was passed through the sheath into the horn, the horn was straightened, and the tubing was passed into the lower horizontal cranial portion of the uterine horn (segment 3). The tubing was secured in place by digital compression of the horn while the sheath was withdrawn. The sheath was split with a blade to remove it from the tubing. The position of the tubing was confirmed by transrectal digital palpation and ultrasonic scanning both at the beginning and end of the infusion. For the PGF-bolus IU injection, the tubing and placement of the tubing into the cranial uterine horn ipsilateral to the CL were the same as for the PGF infusion, and 4 mg PGF (0.8 ml volume) was injected rapidly with a syringe. The study ended at Hour 48, except that the day of posttreatment ovulation was determined. The interovulatory interval was the number of days from pretreatment ovulation to posttreatment ovulation.

2.3. Blood samples and hormone assays

Blood samples were collected at Minutes 0, 2, 4, 6, 8, 10, 15, 20, 25, 30, and 45 and Hours 1, 2, 3, 4, 5, 6, 24, and 48 (Minute 0 = beginning of infusion or time of injection).