Prostaglandin F2α promotes ovulation in prepubertal heifers

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Abstract

The objective was to determine the effects of exogenous prostaglandin $F_{2\alpha}$ (PGF), with or without progesterone treatment, on first ovulation in prepubertal heifers. We tested the hypothesis that PGF has a luteolysis-independent ovulatory effect in cattle. Crossbred Angus heifers (12 to 14 mo old, 250 kg body weight, and an average body condition score of 3 out of 5) were examined by transrectal ultrasonography on two occasions, 11 days apart. Heifers in which a CL was not detected at either examination were considered prepubertal. Heifers were assigned randomly to three experimental groups: (1) PG group (N = 14); heifers were treated with a PGF analog (500 μg cloprostenol im) 5 days after the emergence of a spontaneous (i.e., naturally occurring, noninduced) follicular wave; (2) PPG group (N = 12); heifers were given an intravaginal progesterone-releasing insert (CIDR; Pfizer Animal Health, Montreal, QC, Canada), and a follicular wave was induced with 50 mg of progesterone + 2 mg of estradiol benzoate im, and a PGF analog was given at the time of CIDR removal, on Day 5 of the follicular wave (on average, 8.6 ± 0.5 days after CIDR insertion); and (3) control group heifers were given no treatment (N = 14). Heifers were examined daily by transrectal ultrasonography from the start of the experiment to confirmation that ovulation had occurred, or to 5 days after PGF injection (PG and PPG groups) or until dominant follicles of the next follicular wave reached 8 mm (control group). The percentage of heifers that ovulated within 10 days after wave emergence was higher in PPG (10/12; 83.3%) and PG (11/14; 78.5%) groups than in control (1/14; 7.1%; P < 0.0001). Ovulations occurred 69.6 ± 6 h and 93.8 ± 5 h after PGF treatment in PPG and in PG groups, respectively, whereas only one heifer in the control group ovulated 96 h after Day 5 of follicular wave (P = 0.13). In summary, PGF treatment was associated with ovulation in prepubertal heifers whether or not exogenous progesterone was used as a pretreatment. The hypothesis that PGF will induce ovulation by a luteolysis-independent mechanism was supported.
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1. Introduction

In beef cattle production systems, heifers that reach puberty early are more likely to become pregnant in their first breeding season [1,2], and have a higher lifetime productivity. Therefore, early puberty increases the profitability of cattle production. In that regard, onset of puberty can be hastened with exogenous progestins that modulate LH pulse frequency [3]. Exogenous progesterone acts by decreasing the number of hypothalamic estradiol receptors, resulting in increased LH pulse frequency after cessation of progesterone treatment [3,4]. In association with progesterone, most estrus synchronization hormonal

treatment protocols in cattle use prostaglandin $F_{2\alpha}$ (PGF) to regress the functional CL.

Prostaglandin $F_{2\alpha}$ is a biologically potent substance with multiple applications in the control of reproduction. In cattle, the most common uses are based on its luteolytic properties (i.e., estrus synchronization, regression of persistent CL, and induction of abortion or parturition) [5]. Although PGF (and its analogues) are primarily used as luteolysins, they have also been reported to affect ovulation, implantation, pregnancy maintenance, and postpartum physiology [6]. One possible mechanism of ovulation induction by PGF is by increasing pituitary responsiveness to GnRH, thereby increasing LH release in postpartum cows and prepubertal heifers [7]. Furthermore, experimental evidence in cattle indicates that during the periovulatory period, intrafollicular prostaglandin is essential for the process of ovulation [8]. Although the ovulatory effect of PGF in association with progesterone treatment or follicular aspiration has been demonstrated [9], apparently no studies have been published demonstrating whether PGF per se can induce ovulation in prepubertal heifers.

The present investigation was designed to determine if it is possible to induce ovulation in prepubertal heifers (in the absence of a functional CL) with an injection of exogenous PGF, with or without pretreatment with exogenous progesterone. We tested the hypothesis that treatment with PGF near the end of the growing phase of the dominant follicle induces ovulation by a luteolysis-independent mechanism in prepubertal heifers.

2. Materials and methods

The Committee for Ethics in Animal Experimentation from the Universidade Federal de Santa Maria approved all procedures performed in this experiment.

2.1. Animal treatments and ultrasonographic examinations

Forty prepubertal beef heifers (*Bos taurus*; Red Angus crosses) were used. The heifers were 12 to 14 mo of age, 240 to 270 kg body weight, average body condition score of 3 out of 5, and were maintained at 30° 08′27′′S and 53° 21′04′′ W on native pasture with free access to water and mineralized salt.

Before the beginning of the experiment, ovaries of the heifers were examined by transrectal ultrasonography on two occasions, 11 days apart, to confirm that the heifers were prepubertal (i.e., absence of CL at both examinations).

Prepubertal heifers were assigned randomly among three groups: (1) PG group (N = 14): 500 μg of PGF analogue (cloprostenol; Ciosin, Schering-Plough Animal Health Ind. Com., Ltda., Campinas, SP, Brazil) was given im 5 days after emergence (Day 0) of a spontaneous follicular wave (i.e., heifers were monitored daily by ultrasonography and treatment was given on Day 5 of a naturally occurring follicular wave); (2) PPG group (N = 12): an intravaginal progesterone-releasing device (CIDR, 1.9 g progesterone; Pfizer Animal Health, Montreal, QC, Canada) was placed in the vagina, 50 mg progesterone (Progesterona Rio de Janeiro, Laboratório Allignani Hnos SRL, Santa Fé, Argentina) and 2 mg of estradiol benzoate (Estrogin, Farmavet, São Paulo, SP, Brasil) were administered im at the same time to induce emergence of a new follicular wave (Day 0); 500 μg PGF im was given at the time of CIDR removal, on Day 5 of the follicular wave; and (3) control group (N = 14): no treatment was given, but heifers were monitored daily by transrectal ultrasonography as in the other two groups. Ultrasonographic examinations were conducted daily from the beginning of the experiment to ovulation (all groups) or, in the absence of ovulation, up to 5 days after the PGF injection (PG and PPG groups), or until detection of a dominant follicle > 8 mm in diameter of the subsequent follicular wave in the control group. In heifers that ovulated, three ultrasonographic examinations were performed (7, 10, and 14 days after ovulation) to determine CL diameter.

2.2. Definitions

Heifers were examined daily by transrectal ultrasonography with a Honda HS-1500V (Toyohashi, Aichi, Japan) B-mode scanner with a 7.5 MHz linear-array transducer. At each examination, the diameter and location of all follicles > 4 mm in diameter were recorded. The day of wave emergence, defined as the day when the dominant follicle of a wave was first recorded (4 to 5 mm in diameter) with a concurrent increase in 4 mm follicles, was determined retrospectively and designated as Day 0. Ovulation was defined as the disappearance of a previously identified follicle > 8 mm in diameter [10]. On average, heifers were examined ultrasonographically for 8.6 ± 0.5 days to detect Day 5 of the follicular wave, coinciding with the period that CIDRs were kept in the vagina in PPG group.

2.3. Statistical analyses

Proportions with dichotomus outcomes, such as ovulation (yes/no) and the presence of CL at 7, 10, and

14 days after ovulation, were evaluated by chi-square analysis or by Fisher's Exact test (if the "expected value" was < 5). Single-point measures (e.g., maximum diameter of the ovulatory follicle, growth rate of the ovulatory follicle, and day of ovulation) were compared among groups by one-way ANOVA. Follicle diameters over time, pre- and post-PGF treatment were compared among groups by the MIXED procedure, using SAS 9.0 (SAS Institute, Inc., Cary, NC, USA) for repeated measures to evaluate the main effects of treatment, time, and their interactions [11]. Differences among groups were considered statistically significant when probability (P value) was less than or equal to 0.05. When the main effects or interactions were different, a least square difference test was used to determine differences among means or treatment effects at each time.

3. Results

The percentage of heifers that ovulated within 5 days of the PGF treatment did not differ between the two treatment groups, but was higher than in the control group (P < 0.001). Ovulation occurred in 78.6% (11/14) of heifers in the PG group and 83.3% (10/12) of heifers in the PPG group (P > 0.05). One out of 14 (7.1%) heifers in the control group ovulated 96 h after Day 5 of follicular wave. Ovulations occurred 69.6 ±

5.9 h and 93.8 ± 5.7 h after PGF treatment in the PPG and PG groups, respectively (P = 0.13).

Ovarian follicular growth profiles are summarized (Fig. 1). The duration of the growth phase of the ovulatory follicle (from wave emergence to ovulation) and growth rate did not differ among groups (P = 0.13 and P = 0.57, respectively). Considering all groups, the maximum diameter of the dominant follicle was larger in heifers that ovulated (12.5 ± 0.22 mm) than in heifers that did not ovulate (10.9 ± 0.35 mm; P < 0.05). No differences in the diameters of ovulatory follicles were detected between PPG and PG groups. Also, there was no difference in the growth rate of ovulatory follicles (1.1 ± 0.05 mm per day) and anovulatory follicles (1.0 ± 0.05 mm per day).

Only one heifer in the control group ovulated, but 7 days later a CL was not present. There was no difference in the percentage of heifers with a detectable CL 7 days after ovulation between PPG (10/12; 83.3%) and PG (11/14; 78.6%) groups (P > 0.05). At 10 and 14 days after ovulation, the proportion of heifers with a CL was also similar between PPG (8/12; 66.7%) and PG (7/14; 50.0%) groups (P > 0.05).

4. Discussion

The results of this study supported the hypothesis that treatment with PGF during the late growing phase
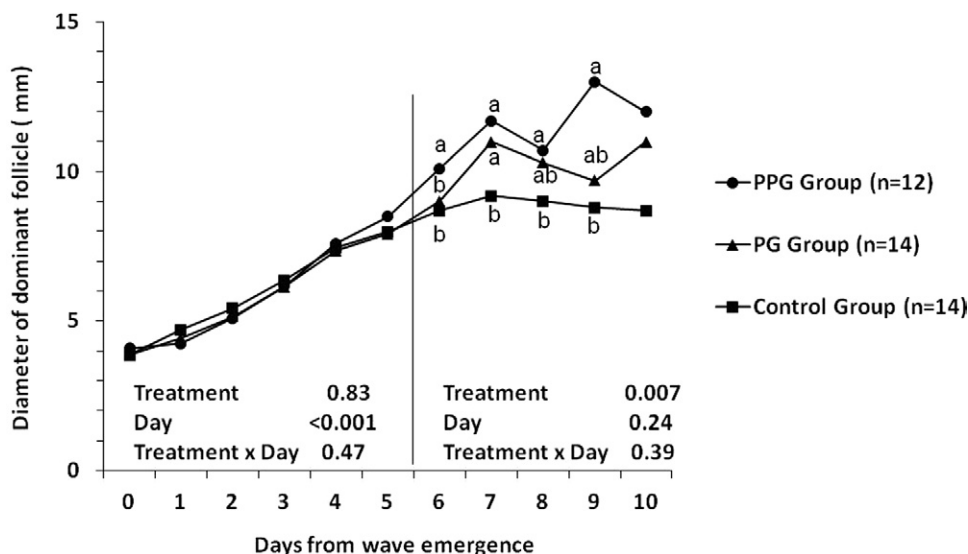

Fig. 1. Comparison of pattern of the dominant follicle growth pre- and post- prostaglandin $F_{2\alpha}$ (PGF) treatment in prepubertal heifers treated with PGF with (PPG) or without (PG) previous exposure to progesterone, and untreated controls. The letters "a" and "b" refer to the days when there were differences among groups (P < 0.05). Exogenous PGF was given on Day 5 of the wave in PG and PPG groups only.

of the dominant follicle of a wave can result in ovulation by a luteolysis-independent mechanism. To the best of our knowledge, this is the first report providing data that PGF per se can induce ovulation in prepubertal heifers (i.e., in the absence of a CL). Previous studies have provided data on the effect of progesterone treatment on first ovulation of prepubertal heifers [12,13]. However, results of an earlier study documented that PGF and progesterone may act synergistically in inducing ovulation in prepubertal heifers [9]. Progesterone may have reduced the negative feedback of estradiol on the hypothalamus by reducing the number of estradiol receptors [14], whereas PGF may have increased the responsiveness of the pituitary to GnRH [7]. Therefore, one might expect a higher ovulation rate in the PPG group, because of the synergistic effects of progesterone plus PGF [15]. However, there was no difference in the proportion of heifers that ovulated, whether or not progesterone was added to the PGF treatment protocol. Based on the present results, we inferred that PGF may have potential applications for induction of ovulation in fixed-time AI (FTAI) programs, especially in GnRH-based protocols. Prostaglandin could enhance the response to GnRH, or even replace GnRH, thus reducing the costs of FTAI protocols, particularly in countries where the use of estradiol is prohibited [16,17].

Several studies have been done attempting to understand how PGF acts to induce ovulation [7,18–20]. Although prepubertal heifers ovulated 94 h after PGF treatment in the present study, an increased frequency of LH release occurred 6 h after treatment with a PGF analogue in anestrous cows [7]. Apparently, PGF increases pituitary responsiveness to GnRH, thereby enhancing the release of LH [7] in a process leading to ovulation. In contrast, in a more recent study, PGF inhibited LH secretion in rat pituitary cells [18]. These considerations imply the need for more comprehensive studies using PGF as a promoter of ovulation to confirm its use in FTAI programs.

Besides the central effect described previously, PGF seems to play a local role in the ovary. Prostaglandin secreted by the preovulatory follicle was closely linked with the ovulatory process [20]. Prostaglandins (PGE_2 and $PGF_{2\alpha}$) were produced by granulosa cells [19], and acted directly on the preovulatory follicle. Considering this experimental evidence, ovulation induced by PGF could be an associated effect of sensitizing GnRH receptors in the pituitary [7] or by a direct action of PGF on the target cells in preovulatory follicles. Although prostaglandin in bovine periovulatory follicles would appear to be essential for ovulation, the actions of prostaglandins on bovine preovulatory follicles are still being studied [8].

In the current study, dominant follicles of the ovulatory wave were larger than those of nonovulatory waves, as described [9]. However, the effect of progesterone on the diameter of dominant follicle and rate of follicular growth [3,9,21] were not observed.

Surprisingly, the CL lifespan observed in heifers treated with PGF indicates that ovulation was not associated with a short luteal lifespan, as would be expected when progesterone is not used. Several authors have reported that the CL formed following the first ovulation was smaller and of shorter lifespan than those of subsequent or physiological estrous cycles [12,22]. Although no difference in the CL lifespan over a period of three ultrasonographic evaluations (7, 10, and 14 days) after ovulation was observed, one must take into account that ultrasonographic examinations to evaluate the CL could not reflect its health and/or its progesterone production [23]. Unfortunately, it was not possible to assess the length of the subsequent estrous cycle following the induction of ovulation with PGF or if these ovulations and the prevailing uterine environment would lead to successful establishment of a pregnancy. It has been reported that the lack of exposure of the endometrium to progesterone can promote premature release of PGF, which is thought to be caused by inadequate expression of progesterone receptors and consequent unsynchronized expression of estrogen receptor-α and oxytocin receptor in the endometrium [24,25]. Although progesterone seems to be pivotal in orchestrating the luteolytic cascade by regulating the expression of progesterone receptors, estrogen receptor-α, and oxytocin receptors [26], based on the present results, we inferred that PGF per se can cause ovulation without any detrimental effects on CL formation. However, further studies are necessary to confirm whether prostaglandin-induced ovulation results in formation of a CL with a normal lifespan in prepubertal heifers.

In conclusion, PGF treatment was associated with ovulation in prepubertal heifers, regardless of whether exogenous progesterone was used as a pretreatment, and the hypothesis that PGF will induce ovulation by a luteolysis-independent mechanism was supported.

Acknowledgments

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