

## Alternative low doses and routes of administering a prostaglandin F2 $\alpha$ analogue to induce luteolysis in Nelore cows

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The present study was conducted in order to verify the efficacy of lower doses and alternative routes of a prostaglandin F2 $\alpha$  analogue, luproliol (PGF), for the induction of luteolysis and the precipitation of estrus in nonlactating Nelore cows (*Bos taurus indicus*). A conventional dose (15 mg) of PGF was compared to doses lower than the conventional dose, which ranges from 10 to 50%, that were administered intramuscularly (IM), intravulvosubmucosally (IVSM), or in the Bai-hui acupuncture site located within the lumbosacral area. The cows were administered PGF 8 day after estrus in the presence of a corpus luteum, and randomly assigned to the following groups: G1 (positive control), 15 mg, IM (n = 23); G2, 7.5 mg, IM (n = 23); G3, 3.75 mg, IM (n = 24); G4, 7.5 mg, IVSM (n = 25); G5, 3.75 mg, Bai-hui acupoint (n = 24); and G6, 1.5 mg, Bai-hui acupoint (n = 25). The results indicated that 50% of a conventional dose of PGF (7.5 mg) resulted in a complete luteal regression (plasma progesterone <1 ng/ml) at Hour 48, and hastened estrus, regardless of whether or not PGF was administered IM or IVSM. Comparatively, 10 or 25% of the conventional dose, even when administered to the Bai-hui acupoint, resulted in an initial reduction in the concentration of progesterone at Hour 24, followed by an increase observed at Hour 48. In conclusion, 25% of a conventional PGF dose administered via the Bai-hui acupoint proved inadequate to induce a complete luteal regression, whereas 50% of a conventional dose administered IM or IVSM was found to be the minimal dose required to induce effectively a complete luteal regression, and to precipitate the onset of estrus in nonlactating Nelore cows.

**Key words:** Bai-hui acupoint, cows, luproliol, luteolysis, progesterone, prostaglandin

## Introduction

Native prostaglandin F2 $\alpha$  and its analogue (PGF) have frequently been employed for the management of reproduction in domestic animals, since the demonstration of Pharriss and Wyngarden [20], which showed their ability to shorten the lifespan of the corpus luteum (CL) in pseudo-pregnant rats. The manufacture of a variety of forms of PGF has contributed to the broad application of this hormone in studies of bovine reproduction. A demand exists for a method to control reproduction and increase efficiency; for example, to shorten the luteal phase and hasten estrus in heifers and cows.

Several studies have reported on the efficacy of treatment with lower doses of synthetic native PGF, via the intravulvosubmucosal (IVSM) route [2,4,5,11]. The systemic route or mechanism by which PGF reaches the CL after the administration of IVSM has yet to be completely elucidated. However, there is some speculation as to the presence of specific vascular connections that involve the vulva, vagina, uterus, and ovaries, and may enable exogenous PGF to reach the CL [13]. Recently, Gioso *et al.* [11] showed the presence of vascular anastomoses connecting the vagina, cervix, uterine body and veins located at the uterine horns.

The Bai-hui acupuncture point referred to as the “point of 100 meetings” is located in the depression between the spinous processes of the last lumbar and first sacral vertebrae [14,15]. This is an acupuncture point which has been employed in the treatment of specific reproductive disorders, including anestrus, cystic ovaries, cystic CL, silent heat, pseudopregnancy, impotence, penile paralysis, reproductive system inflammation, retained placenta, and uterine prolapse, as well the prevention of abortion or the induction of labor [17,18]. Alvarenga *et al.* [1] reported that native PGF (dinoprost tromethamine), at one-tenth of the conventional recommended dose, was sufficient to induce luteolysis in mares when administered to the Bai-hui acupoint. By way of contrast, the same authors concluded that a similar dose proved ineffective when administered via

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the IM route.

The primary objective of the present study was to conduct a comparison of the efficacy of lower doses and alternative routes of PGF treatment to a conventional dose and route of PGF treatment, with regard to the induction of luteolysis and the precipitation of estrus in nonlactating Nelore cows (*Bos taurus indicus*).

## Materials and Methods

A commercial herd of nonlactating Nelore cows with regular estrous cycles and body condition scores in a range of from 3 to 4 [23], in an age range of 4 to 10 years, were assessed in this study during the spring, in the southern hemisphere in Parana State, Brazil. The cows were kept in pasture (*Brachiaria brizantha*) with free access to water and mineralized salt.

A total of 500 cows were initially observed, and the first 144 cows evidencing natural estrus were randomly distributed into 6 PGF-treatment groups. A synthetic PGF (Luprostiol; Intervet, Brazil) was then administered 8 days after the detection of estrus (Hour 0 = immediately prior to treatment) in the presence of a CL detected via transrectal ultrasonography (5 MHz linear array; Pie Medical, Netherlands). The PGF-treatment groups were as follows: Group 1 (G1, positive control), 15 mg intramuscularly (IM, conventional dose;  $n = 23$ ); Group 2 (G2), 7.5 mg IM (50% of conventional dose;  $n = 23$ ); Group 3 (G3), 3.75 mg IM (25% of conventional dose;  $n = 24$ ); Group 4 (G4), 7.5 mg intravulvosubmucosally (IVSM, 50% of conventional dose;  $n = 25$ ). Bai-hui acupuncture in the lumbosacral area was used as an injection route in group 5 (G5) 3.75 mg (25% of conventional dose;  $n = 24$ ) and Group 6 (G6) 1.5 mg (10% of conventional dose;  $n = 25$ ), and in these groups the PGF was diluted to 5 ml with saline solution. The intramuscular injections were either administered in the semimembranosus or semitendinosus muscles, and the injections at the Bai-hui acupoint were conducted to a depth of approximately 3 cm within the lumbosacral space.

The efficacy of the PGF treatments for the induction of complete luteal regression was determined via a reduction in

the circulating concentrations of progesterone ( $<1$  ng/ml) 48 h post-treatment. Estrus detection was conducted in all groups using a teaser bull (one bull for 30 cows) at a frequency of 3 times a day for 6 days after treatment with PGF.

Blood samples were collected from all cows via jugular venipuncture immediately prior to PGF treatment at Hour 0, and after treatment at Hours 24 and 48, and stored in heparinized tubes. The samples were then centrifuged and plasma was stored at  $-20^{\circ}\text{C}$  until the progesterone assays were conducted using a commercial radioimmunoassay kit (Diagnostic Products, USA). The assay sensitivity was 0.08 ng/ml and the intra- and inter-assay coefficients of variation were 4.3 and 8.5%, respectively.

Differences in plasma progesterone concentrations among treatment groups within hours (group versus group) and within the treatment groups among hours (hour versus hour) were determined via Proc mixed from SAS using fixed effects of group, hour (repeated measures), and group versus hour interaction. The means were compared by T- tests, with 0.05 set as the significance level. The proportion of cows evidencing luteolysis (progesterone  $<1$  ng/ml), estrus behavior, and the ratio of estrus to luteolysis at 48 h post- treatment were compared among groups via Fisher's Exact Test.

## Results

All PGF-treatment regimens resulted in sharp reductions in progesterone concentrations at Hour 24; however, only Groups 1, 2, and 4 evidenced concentrations of  $<1$  ng/ml at Hour 48 (Table 1).

Both 15 mg (G1) and 7.5 mg (G2 and G4) of PGF resulted in a higher ( $p < 0.05$ ) proportion of cows evidencing luteolysis at Hour 48 and estrus behavior over 6 days of post-treatment as compared with 3.75 mg (G3 and G5) and 1.5 mg (G6), regardless of the administration route utilized (Table 2). The ratio of the number of cows in estrus to luteolysis did not differ among the groups.

With regard to the number of cows that evidenced or did not evidence estrus over the 6 day post-PGF period, plasma progesterone concentrations combined over all treatment

**Table 1.** Plasma progesterone concentrations immediately prior to treatment with a prostaglandin F2 $\alpha$  analogue, luprostiol (unit: ng/ml)

Group (dose/route)	Hour 0*	Hour 24 <sup>†</sup>	Hour 48 <sup>‡</sup>
15 mg/IM	5.1 $\pm$ 1.5 <sup>ACa</sup>	0.8 $\pm$ 0.5 <sup>Ab</sup>	0.5 $\pm$ 0.5 <sup>Ab</sup>
7.5 mg/IM	6.2 $\pm$ 1.5 <sup>ABa</sup>	1.4 $\pm$ 0.6 <sup>ABb</sup>	0.8 $\pm$ 1.0 <sup>Ab</sup>
3.75 mg/IM	6.7 $\pm$ 2.0 <sup>Ba</sup>	2.3 $\pm$ 1.4 <sup>Bb</sup>	3.1 $\pm$ 2.1 <sup>Bb</sup>
7.5 mg/IVSM	6.5 $\pm$ 1.7 <sup>Ba</sup>	1.2 $\pm$ 0.5 <sup>Ab</sup>	0.9 $\pm$ 0.7 <sup>Ab</sup>
3.75 mg/Bai-hui acupoint	4.2 $\pm$ 1.3 <sup>Ca</sup>	1.5 $\pm$ 0.8 <sup>ABb</sup>	3.4 $\pm$ 2.0 <sup>Bc</sup>
1.5 mg/Bai-hui acupoint	6.1 $\pm$ 1.4 <sup>ABa</sup>	2.0 $\pm$ 0.8 <sup>ABb</sup>	3.1 $\pm$ 1.2 <sup>Bc</sup>

Different uppercase characters in a column and lowercase characters in a row are different ( $p < 0.05$ ). IM; intramuscular injection. IVSM; intravulvosubmucosal injection. All Values are mean  $\pm$  SD. \*Hour 0 means 8 day after pretreatment estrus detection. <sup>†</sup>Hour 24 and 48 means at 24 and 48 h post-treatment, respectively.

**Table 2.** Proportion of cows evidencing luteolysis at Hour 48 and estrus behavior detected during the 6 days after PGF treatment at Hour 0, and the estrus to luteolysis ratio

Group (dose/route)	Luteolysis (%)	Estrus behavior (%)	Estrus/Luteolysis (%)
15 mg/IM	20/23 (86.9) <sup>A</sup>	14/23 (60.9) <sup>A</sup>	14/20 (70.0) <sup>A</sup>
7.5 mg/IM	16/23 (69.6) <sup>A</sup>	12/23 (52.2) <sup>A</sup>	12/16 (75.0) <sup>A</sup>
3.75 mg/IM	5/24 (20.8) <sup>B</sup>	2/24 (8.3) <sup>B</sup>	2/5 (40.0) <sup>A</sup>
7.5 mg/IVSM	19/25 (76.0) <sup>A</sup>	9/25 (36.0) <sup>A</sup>	9/19 (47.4) <sup>A</sup>
3.75 mg/ Bai-hui acupoint	5/24 (20.8) <sup>B</sup>	2/24 (8.3) <sup>B</sup>	2/5 (40.0) <sup>A</sup>
1.5 mg/ Bai-hui acupoint	1/25 (4.0) <sup>B</sup>	0/25 (0.0) <sup>B</sup>	0/1 (0.0) <sup>A</sup>

Different characters in a column are different ( $p < 0.05$ ). IM; intramuscular injection, IVSM; intravulvosubmucosal injection.

**Table 3.** Plasma progesterone concentrations combined across PGF-treatment groups immediately prior to PGF treatment at Hour 0 and after treatment at Hours 24 and 48 in relation to the estrus behavior (unit: ng/ml)

Estrus behavior	Hour 0	Hour 24	Hour 48
Detected (n = 39)	5.70 $\pm$ 1.68 <sup>A</sup>	0.89 $\pm$ 0.52 <sup>A</sup>	0.37 $\pm$ 0.38 <sup>A</sup>
Not detected (n = 105)	5.88 $\pm$ 1.83 <sup>A</sup>	1.78 $\pm$ 1.01 <sup>B</sup>	2.57 $\pm$ 1.86 <sup>B</sup>

Different characters in a column are different ( $p < 0.05$ ). All values are mean  $\pm$  SD.

groups did not differ immediately prior to PGF treatment at Hour 0, but were lower ( $p < 0.05$ ) at hours 24 and 48 in cows exhibiting estrus (Table 3).

## Discussion

In the positive control group, a single conventional luteolytic dose of PGF (15 mg) administered 8 days after pre-treatment estrus detection induced complete luteolysis in 87% of the cows, as had been expected [19]. The proportion of cows in estrus during the 6 days post-PGF (61%) was within a previously reported range between 40% [3,6,7,8,12,16,21] and 70% [19] for *Bos taurus indicus* after treatment.

In the nonconventional PGF-treatment groups, 50% of a conventional dose of PGF administered via the IM or IVSM route induced the complete regression of the CL, based on a significant reduction in the circulating concentrations of progesterone at 48 h post-treatment, regardless of the employed route of treatment. These results are comparable to those reported by others who have used 50% of a conventional dose of a different PGF analogue (cloprostenol, 250  $\mu$ g) administered intramuscularly to induce luteolysis in cattle [2,11] and 25% (cloprostenol, 125  $\mu$ g) administered intravulvosubmucosally to the water buffalo (*Bubalus bubalis*) [4]. Thus, the present results concur and substantiate the previous results, that a nonconventional exogenous dose of PGF (50% of recommended) administered in a nonconventional manner (intravulvosubmucosally) can effectively reach the CL, and can induce luteal regression and hasten a return to estrus in these animals.

Luteolysis was induced, although not to a significant degree, in 4% of the cows administered 10% of a conventional dose of PGF via the Bai-hui acupoint, and in 21% of the cows administered 25% PGF. The observed dose-response

relationship indicates that the administration of PGF via a relevant acupuncture site can reach the CL, but that the lack of luteolysis observed in a majority of the cows (plasma progesterone  $>1$  ng/ml) was likely attributable, at least in part, to insufficient PGF dosage. In this regard, circulating concentrations of progesterone were observed to decrease in all groups at 24 h post-treatment; however, at 48 h post-treatment, concentrations increased from those observed at 24 h in cows treated with a 10% dose of PGF administered via the Bai-hui acupoint, and with 25% PGF administered both intramuscularly and at the Bai-hui acupoint. The observed rebound in progesterone concentrations was most likely attributable to the reduced number of cows that evidenced estrus behavior in the respective groups. Thus, the administration of PGF at 25% or less of the recommended dose, regardless of route, effectively reached the CL, but ineffectively induced complete luteal regression, as was reflected by the partial and transitory alterations detected in plasma progesterone concentrations. The present results are consistent with the findings of previous studies that partial or full recovery of luteal function can occur as the result of sub-luteolytic doses of PGF [22].

In mares, 10% of a conventional dose of native PGF (0.5 mg) given at the Bai-hui acupoint induced luteolysis but not when 25% of a conventional dose (1.25 mg) was given intramuscularly [1]. In contrast, luteolysis was not induced in mares when 10% of a conventional dose of native PGF (0.5 mg) was given at the Bai-hui acupoint [18]. However, 10% of a conventional dose of a PGF analogue (cloprostenol, 25  $\mu$ g) was effective via the Bai-hui acupoint and the same luteolytic response was observed when the same dose was given intramuscularly. The basis for the different results among the present and previous studies is not known; however, they do suggest that caution is necessary when

interpreting the results in response to various regimens of PGF treatment within and among species since there may be dramatic differences in the rate of absorption, distribution and metabolism of PGF, especially considering the diverse local and systemic transport routes of PGF, from the uterus to the ovary in cattle and horses, respectably [9,10,18].

In conclusion, 10% or 25% of a conventional dose of PGF administered via the Bai-hui acupoint or 25% IM proved inadequate to induce complete luteal regression, whereas 50% of a conventional dose administered IM or IVSM was the minimal dose required to induced efficiently and effectively a complete luteal regression and hastened the onset of estrus in non-lactating Nelore cows.

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