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Luteolysis in *Bos indicus* cows on Days 5 and 7 of estrous cycle with varying doses of $PGF_{2\alpha}$

Marcos Vinicius C. Ferraz Junior [a], Alexandre V. Pires [a,b,*], Marcos Vinicius Biehl [b], Marcelo Henrique Santos [a], Daniel M. Polizel [a], Delci D. Nepomuceno [b], Roberto Sartori [b], João Bosco Barreto Filho [c], José Renato S. Gonçalves [d], Michael L. Day [e]

[a] *Department of Nutrition and Animal Production, Faculty of Veterinary and Animal Science, University of São Paulo, Pirassununga, São Paulo, Brazil*
[b] *Department of Animal Science, College of Agriculture "Luiz de Queiroz" (ESALQ), University of São Paulo, Piracicaba, São Paulo, Brazil*
[c] *Department of Veterinary, Federal University of Lavras, Lavras, Minas Gerais, Brazil*
[d] *Experimental Station Hildegard Georgina Von Pritzelwitz, Fazenda Figueira, Londrina, Paraná, Brazil*
[e] *Department of Animal Sciences, The Ohio State University, Columbus, Ohio, USA*

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ABSTRACT

The aim of this study was to evaluate luteolysis using three doses of $PGF_{2\alpha}$ on Day 5 or Day 7 of the estrous cycle in nonlactating Nellore (*Bos indicus*) cows. Cows (n = 323) were assigned within date of estrus (Day 0 of estrous cycle) to receive 12.5, 25.0, or 50.0 mg of $PGF_{2\alpha}$ on either Day 5 or Day 7 of the estrous cycle in a 3 × 2 factorial arrangement. Blood samples for progesterone (P_4) concentrations were collected at 0, 24, 48, and 72 hours after $PGF_{2\alpha}$ to assess luteolysis (L). Luteolysis was defined on the basis of P_4 concentrations at 72 hours using either less than 0.5 ng/mL (L0.5) or less than 1.0 ng/mL (L1.0) as the cut off. Luteolysis was considered "partial" when P_4 concentration declined within 24 hours after $PGF_{2\alpha}$ but failed to decline further or, in some cases, increased. Incidence of luteolysis was less ($P < 0.01$) on Day 5 than Day 7 of the estrous cycle (17.3 vs. 47.6% and 30.4 vs. 77.2%; for L0.5 and L1.0, respectively). Dose of $PGF_{2\alpha}$ increased ($P < 0.01$) L1.0 (12.5 mg = 38.9%; 25.0 mg = 52.3%; and 50.0 mg = 70.4%). Incidence of partial luteolysis for cows on Day 5 (57.1%) was greater ($P < 0.01$) than that on Day 7 (19.1%) of the estrous cycle and was more prevalent ($P < 0.01$) with lower doses of $PGF_{2\alpha}$ (12.5 mg = 49.1%; 25.0 mg = 37.4%; and 50.0 mg = 27.8%). In conclusion, both days of the estrous cycle and doses of $PGF_{2\alpha}$ influenced the incidence of complete and partial luteolysis in Nellore cows and should be an important consideration when devising estrus synchronization programs in this species.

1. Introduction

Prostaglandin $F_{2\alpha}$ is a hormone that triggers luteolysis in cows, inducing the end of luteal phase [1]. However, despite the presence of similar concentrations of high-affinity $PGF_{2\alpha}$ receptors in the early CL [2,3], luteolysis from exogenous $PGF_{2\alpha}$ does not usually occur until 5 to 6 days after estrus [4–9]. An important aspect of synchronization programs that use GnRH to synchronize emergence of a new follicular wave through induction of ovulation of the dominant follicle is to ensure lysis of the CL 5 to 7 days later in the Ovsynch- and/or CO-Synch–based protocols [10,11]. The mechanisms of action of $PGF_{2\alpha}$ in newly formed CL are not completely understood [6,7,9].

* Corresponding author. Tel.: 55 (19) 3429-4247; fax: 55 (19) 3429-4215.
E-mail address: pires.1@usp.br (A.V. Pires).

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Studies reported that luteolysis occurred in more than 90% in cows that received a single (25 mg) dose of $PGF_{2\alpha}$ after Day 8 of the estrous cycle [11]. In contrast, a single (25 mg) dose of $PGF_{2\alpha}$ in the first 4 days of the cycle did not induce luteolysis [6,7]. However, the incidence of luteolysis induced by a double dose of $PGF_{2\alpha}$ (50 mg) around Day 5 of the estrous cycle is not well established [4,5,7]. In addition, studies have demonstrated that a transient decrease in circulating progesterone (P_4) concentration in cows does not always indicate complete luteolysis when $PGF_{2\alpha}$ is administered around Day 5 of the estrous cycle [5,6]. This response is referred to as partial luteolysis and is characterized as a decrease in P_4 concentration during the first 24 hours after $PGF_{2\alpha}$, but after that, no further decline or, in some cases, a rebound in systemic concentrations occur [5,6,12].

Ensuring complete luteolysis and total decrease in circulating P_4 at the time of artificial insemination (AI) is essential for optimizing results in fixed timed AI (FTAI) protocols [13]. To induce complete luteolysis in recently formed CL, several strategies have been studied, including the use of a greater dose of $PGF_{2\alpha}$ and/or multiple doses of $PGF_{2\alpha}$ given at varying intervals [6,7].

In recent years, incidence of luteolysis during the first days of the estrous cycle has been investigated in multiple experiments [6,7]. All these studies have used *Bos taurus* cows as the animal model. The objective of the present study was to investigate the efficacy of differing $PGF_{2\alpha}$ doses to induce luteolysis on Day 5 or Day 7 of the estrous cycle in Nellore cows.

2. Materials and methods

The experiment was performed in the Experimental Station Agrozootécnica Hildegard Georgina Von Pritzelwitz (23°34′25″ S; 50°58′17″ W), in Londrina, Paraná, Brazil, which belongs to the Fundação de Estudos Agrários Luiz de Queiroz.

Nonlactating, cyclic Nellore cows (n = 323) with no uterine or ovarian abnormalities (based on a transrectal scan of the reproductive tract by ultrasonography) were used. Groups of approximately 80 cows at random stages of their estrous cycle received 25.0 mg of $PGF_{2\alpha}$ (dinoprost tromethamine–Lutalyse, Zoetis, São Paulo, SP, Brazil). Estrus detection was performed twice daily for 5 days; aided by vasectomized bulls fitted with chin-ball markers.

Cows detected in estrus were assigned, on the day of estrus, to receive one of three doses of $PGF_{2\alpha}$ on either Day 5 or Day 7 of the ensuing estrous cycle. Doses were 12.5, 25.0, or 50.0 mg of $PGF_{2\alpha}$ given on Day 5 or Day 7 of the estrous cycle. The 3 × 2 factorial arrangement resulted in six treatments: Day 5/12.5$PGF_{2\alpha}$ (n = 54), Day 5/25.0$PGF_{2\alpha}$ (n = 54), Day 5/50.0$PGF_{2\alpha}$ (n = 53), Day 7/12.5$PGF_{2\alpha}$ (n = 54), Day 7/25.0$PGF_{2\alpha}$ (n = 53), and Day 7/50.0$PGF_{2\alpha}$ (n = 55).

Ovulation was confirmed by detection of CL by ultrasonography (US) on Day 5 or Day 7, and CL diameter was recorded when $PGF_{2\alpha}$ was given (0 hours) and at 96 hours to assess morphologic luteolysis. Estrus detection was performed twice daily, aided by the use of vasectomized bulls fitted with chin-ball markers for a period of 120 hours.

Blood samples (10 mL) were collected via a coccygeal vessel at 0, 24, 48, and 72 hours after $PGF_{2\alpha}$ administration. Blood samples were centrifuged for 15 minutes at 1800 × *g*. Serum was frozen at −20 °C for further analysis. Progesterone concentrations were measured by chemiluminescent assay using commercial IMMULITE 1000 kits (Siemens Healthcare Diagnostics, Deerfield, IL, USA). All the analyses were performed in the Nutrition and Animal Reproduction Laboratory–LZT/ESALQ/USP. The coefficients of variation were 2.7% and 3.6% for low and high adjuster, respectively.

Luteolysis was determined to have occurred when P_4 concentrations were less than 1.0 ng/mL (L1.0) or less than 0.5 ng/mL (L0.5) 72 hours after $PGF_{2\alpha}$. When P_4 concentrations declined during the first 24 hours after treatment but did not decline further, or in some cases increased, this was classified as partial luteolysis. If P_4 concentration did not decrease during the 24 hours after $PGF_{2\alpha}$, this was classified as failure of luteolysis.

A randomized experimental design in a 3 × 2 factorial arrangement (day of estrous cycle × $PGF_{2\alpha}$ dose) was used. The variables were analyzed after running normality (Shapiro–Wilk) and homogeneity of variance (Welch) tests. Variables with non-normal distribution were submitted to either logarithmic ($\log_{10}$ [x + 1]) or quadratic (SQRT [x + 1/2]) transformation before analysis. Variables such as CL diameter at 0 and 96 hours after $PGF_{2\alpha}$ administrated and the interval to estrus were analyzed using the MIXED procedure. The same procedure with repeated measures over time analyses was used to evaluate P_4 concentrations at 0, 24, 48, and 72 hours after $PGF_{2\alpha}$ in each day of the estrous cycle (Day 5 and Day 7), separately, according to $PGF_{2\alpha}$ dose. Three analyses of P_4 concentrations in each day of the estrous cycle (Day 5 and Day 7) were performed. The first analysis considered all cows, the second, only cows with complete luteolysis, and the third considering only cows with partial luteolysis. The GLIMMIX procedure was used for variables with binomial distribution, such as L0.5, L1.0, partial luteolysis rate, no luteolysis, and incidence of estrus.

The effect of relative drop in circulating P_4 concentration (100 − [P_4 concentration at 24 hours after $PGF_{2\alpha}$ × 100]/P_4 concentration at 0 hour at $PGF_{2\alpha}$) on estrous behavior was analyzed by logistic regression (LIFETEST procedure) comparing $PGF_{2\alpha}$ doses or days of the estrous cycle when $PGF_{2\alpha}$ treatments were employed. The oddsratio statement was used to compare difference among doses of $PGF_{2\alpha}$. Logistic regression curves were done using the coefficient generated by Interactive Data Analysis of SAS (logit = intercept + slop × [drop in P_4]). Probability curve was made by Y = (EXP [logit]/1 + EXP [logit]) × 100. Significant differences were considered when $P < 0.05$. All procedures used were from SAS 9.3 statistical software.

3. Results

There was no interaction between $PGF_{2\alpha}$ doses and day of the estrous cycle in any of the analyzed variables. Dose of $PGF_{2\alpha}$ did not alter the time of estrus after $PGF_{2\alpha}$ (70.2 ± 1.76 hours), and 50.4% of the cows that exhibited estrus showed estrus within 72 hours after $PGF_{2\alpha}$ (Fig. 1). However, the mean time of estrus was later ($P < 0.01$) in cows treated on Day 5 than those on Day 7 (76.0 ± 3.12 vs. 66.0 ± 2.29 hours, respectively). Cows treated with $PGF_{2\alpha}$ on Day 7 were more likely to exhibit estrus ($P < 0.01$) than