

Postpartum administration of PGF2 α and reproductive performance in timed artificial insemination of beef COWS

Administração pós-parto de PGF2 α e desempenho reprodutivo na inseminação artificial em tempo fixo de vacas de corte

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Highlights

P/AI in multiparous cows treated with PGF2 α tended to be greater than control group.
The proportion of inflammatory cells (PMN) did not differ among studied groups.

Abstract

This study aimed to evaluate the effect of prostaglandins on the proportion of inflammatory cells (PMN) and postpartum pregnancy rates in cows subjected to fixed-time artificial insemination (FTAI) programs. In total, 209 postpartum Nelore cows were used in this study. Females were separated into three groups to receive the following treatments: control group- CTL ($n = 66$), females that were not treated; group 0.5 PG ($n = 68$), cows that received cloprostenol 0.5mg – a PGF2 α analog; and group 1.0 PG ($n = 75$), cows that received 1mg of cloprostenol. All females were subjected to the FTAI protocol at an average of 38.7 ± 7.6 (Mean $\pm$ SD) days postpartum (DPP). Uterine health was assessed using polymorphonuclear cell (PMN) counts. On Day 0 of the FTAI protocol (D0), material was collected using disposable cervical brushes for cytology and analysis of the proportion of inflammatory cells (PMN) recovered from the endometrium in the cervical region. Pregnancy diagnosis was performed by transrectal ultrasonography 30 days after FTAI. Logistic regression was used to analyze the effects of treatment, category, and their interactions on Pregnancy/Artificial Insemination (P/AI). The proportion of PMN cells according to the

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group was analyzed using ANOVA (PROC GLIMMIX; SAS Inst. Inc., Cary, NC, USA), and the means were compared between groups using Tukey's test. A level of 5% was considered significant. The proportion of PMN did not differ among the groups. The overall pregnancy rate was 72.2% (151/209). No effects of group ($P = 0.51$) or category ($P = 0.84$) were detected on the P/AI among the groups. There was a tendency ($P = 0.07$) for a group-category interaction for P/AI. In this regard, the P/AI in multiparous cows treated with 1mg of PGF2 α tended to be higher ($P = 0.08$) than that in the control cows.

Key words: *Bos indicus*. Cloprostenol. Conception. FTAI.

Resumo

Este estudo teve como objetivo avaliar o efeito das prostaglandinas na proporção de células inflamatórias (PMN) e nas taxas de prenhez pós-parto em vacas submetidas a programas de inseminação artificial em tempo fixo (IATF). No total, 209 vacas Nelore pós-parto foram utilizadas neste estudo. As fêmeas foram separadas em três grupos para receber os seguintes tratamentos: grupo controle - CTL ($n = 66$), fêmeas que não foram tratadas; grupo 0,5 PG ($n = 68$), vacas que receberam 0,5mg cloprostenol - um análogo de PGF2 α ; e grupo 1,0 PG ($n = 75$), vacas que receberam 1mg de cloprostenol. Todas as fêmeas foram submetidas ao protocolo de IATF em uma média de $38,7 \pm 7,6$ (média $\pm$ DP) dias pós-parto (DPP). A saúde uterina foi avaliada por meio da contagem de células polimorfonucleares (PMN). No Dia 0 do protocolo FTAI (D0), o material foi coletado usando escovas cervicais descartáveis para citologia e análise da proporção de células inflamatórias (PMN) recuperadas do endométrio na região cervical. O diagnóstico de gestação foi realizado por ultrassonografia transretal 30 dias após a IATF. A regressão logística foi usada para analisar os efeitos do tratamento, categoria e suas interações na Prenhez/Inseminação Artificial (P/IA). A proporção de células PMN de acordo com o grupo foi analisada usando ANOVA (PROC GLIMMIX; SAS Inst. Inc., Cary, NC, EUA), e as médias foram comparadas entre os grupos usando o teste de Tukey. Um nível de 5% foi considerado significativo. A proporção de PMN não diferiu entre os grupos. A taxa geral de prenhez foi de 72,2% (151/209). Nenhum efeito de grupo ($P = 0,51$) ou categoria ($P = 0,84$) foi detectado na P/IA entre os grupos. Houve uma tendência ($P = 0,07$) para uma interação grupo-categoria para P/IA. Nesse sentido, P/AI em vacas multíparas tratadas com 1mg de PGF2 α tendeu a ser maior ($P = 0,08$) do que nas vacas controle.

Palavras-chave: *Bos indicus*. Cloprostenol. Concepção. IATF.

Introduction

Uterine involution and the resumption of postpartum cyclicity in beef cattle are essential for establishing satisfactory reproductive indices, both economically and productively. In this sense, the uterine muscle

layer plays an important role in parturition and puerperium, expelling the conceptus and fetal membranes and considerably reducing uterine diameter a few days postpartum (DPP). Myometrial contraction is triggered by PGF2 α , released 48 hours before parturition. Thus, when there is low production and/

or endogenous release of PGF2 α , the puerperium period is extended and occurs more slowly (Bencharif et al., 2000; Forde et al., 2011).

Consequently, delayed uterine involution causes a delay in the return to cyclicity and increases the presence of inflammation and infection in the uterus. In addition, it promotes loss of quality in the follicular microenvironment, hindering oocyte development and subsequent ovulation. The presence of inflammatory secretions makes fertilization and implantation of the embryo in the uterus difficult if the latter is formed (Cheong et al., 2016). Typically, female cattle (*Bos taurus indicus*) under goes complete uterine involution and reduction in the presence of inflammatory cells around 30 days postpartum, presenting better conception rates when inseminated after 35 days of parturition (Andrade et al., 2021; Araújo et al., 2022).

Therefore, strategies that ensure the establishment of a normal puerperium will allow for the return of cyclicity, favoring the herd's reproductive efficiency. In a similar study, Salasel and Mokhtari (2011) applied PGF2 α at 20 DPP in dairy cows. They demonstrated improvements in several reproductive indices, emphasizing an increase in the conception rate at first service and an increase in the pregnancy rate at 150 days. Recently, Carbonari et al. (2024) concluded that treatments with PGF2 α helped in a more efficient uterine involution and a faster ovarian recovery.

In this context, Abdel-Khalek et al. (2013) demonstrated that the application of PGF2 α in the period 6–12 hours after calving

in dairy cows resulted in a decrease in uterine diameter and an earlier reduction in cervical length compared to the control group. It was also possible to observe an increase in the conception rate at 120 days after calving in the group treated with PGF2 α ($P < 0.05$) (Abdel-Khalek et al., 2013). Other researchers also demonstrated the positive effect of PGF2 α as an adjuvant to the uterine recovery process and consequent improvement of reproductive indices (Patel et al., 2015; Abou-Aiana et al., 2019; Solanki et al., 2019).

However, most studies were developed with crossbred beef and dairy cows, and few studies have applied synthetic analogs of PGF2 α to female *Bos t. indicus*. However, there has not yet been a consensus on a dose or timing of administering the PGF2 α postpartum which can be used to provide greater success in the reproductive efficiency of the herd and reduce the presence of inflammation in these females.

Therefore, the objective of this study was to analyze the effect of the application of a synthetic analog of PGF2 α , administered ten days prior to the start of hormonal synchronization for fixed-time artificial insemination (FTAI) in Nelore females, to study the reduction of the inflammatory response and increase in the pregnancy rate in these cattle.

Material and Methods

This study was conducted in accordance with the Animal Experimentation Ethics Committee of the State University of Londrina, Federal Law 11,794, on October 8, 2008, CEUA-013/2019.

Experimental design

The experiment was conducted on a commercial farm located at 21° 15' 21" S, 52° 02' 13" O, Brazil. A total of 209 postpartum Nelore cows, aged 4–12 years, were included in this study. All cows were housed in an extensive pasture system with mineral supplementation and water available *ad libitum*.

Cows were randomly distributed among the following groups: the control group - CTL ($n = 66$) received an application of saline solution (0.9% NaCl); group 0.5 PG ($n = 68$) in which females received 0.5mg of sodium cloprostenol (Ciosin® CL, MSD Saúde Animal, São Paulo, Brazil); and group 1.0 PG ($n = 75$) which received 1.0mg of sodium cloprostenol (Ciosin® CL, MSD Saúde Animal, São Paulo, Brazil). PGF2 α analog and saline solution applications were performed ten days before hormonal treatment for FTAI via intramuscular injection.

The uterine health of the females was assessed indirectly by counting the polymorphonuclear defense cells (PMN) recovered from the endometrium in the cervical region. Samples were collected at the beginning of the FTAI protocol (D0). Cytological brushes (Kolplast, São Paulo, Brazil) attached to the shaft of a semen applicator and protected with a sanitary sheath were used as described by Kasimanickam et al. (2005).

After cleaning the vulvar region, the applicator was inserted into the vaginal canal of the female up to the cervix. Upon entering the cervix, the cytological brush was exposed and rotated 360° clockwise. Immediately after collecting the material, the cytological brush

was gently rolled onto a slide for microscopic analysis (Nikon, Tokyo, Japan). Subsequently, the slides were stained with a commercial kit (Quick Panoptic®, Laborclin, Pinhais, Brazil) and analyzed under a microscope. Each slide was examined using a 400x objective lens. Two hundred polymorphonuclear or epithelial cells were counted, excluding erythrocytes, to calculate the proportion of inflammatory cells in each sample.

Hormonal treatment for FTAI was performed equally for all groups and was initiated at 38.7 ± 7.6 (Mean $\pm$ SD) DPP. On D0, an intravaginal device containing 1.2g of progesterone (P4; Fertilcare® implant 1200, MSD Saúde Animal, São Paulo, Brazil) was inserted, associated with the application of 2mg of estradiol benzoate (BE; Fertilcare® synchronization, MSD Saúde Animal, São Paulo, Brazil) intramuscularly. The device was removed on day 8 (D8). Then the following were administered intramuscularly: 1mg of sodium cloprostenol (Ciosin® CL, MSD Saúde Animal, São Paulo, Brazil), 1mg of estradiol cypionate (CE; Fertilcare® ovulation, MSD Saúde Animal, São Paulo, Brazil) and 300 IU of equine chorionic gonadotropin (eCG; Folligon®, MSD Saúde Animal, São Paulo, Brazil).

All cows were subjected to FTAI 48 hours after removal of the intravaginal progesterone device on day ten (D10). The FTAI procedures were carried out by a single technician using conventional semen straws from three bulls of proven fertility, which were equally distributed among the treatment groups. Pregnancy diagnosis was performed by transrectal ultrasonography (SonoScape® A5V Ultrasound) with a linear probe (5.0 MHz) 30 days after FTAI. The experimental design conducted in this work is illustrated in Figure 1.

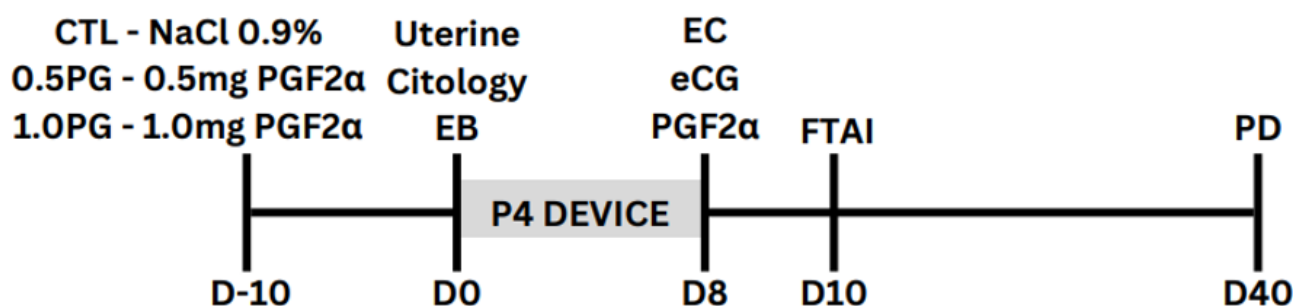


Figure 1. Experimental design.

Abbreviations: CTL - Control group; 0.5 PG – 0.5mg PGF2 α ; 1.0 PG – 1mg PGF2 α ; EB - 2mg Estradiol benzoate; P4 device - 1.2g progesterone intravaginal device; EC - 1mg Estradiol Cipionate; eCG - 300 IU equine chorionic gonadotropin; FTAI - Fixed-time artificial insemination; PD - Pregnancy diagnosis by ultrasonography.

Statistical analysis

In this study, P/AI was analyzed using logistic regression. The initial variables included in the model were the treatment, sire, BCS, and category (primiparous vs. multiparous). The sire and BCS variables had no significant effect on P/AI and were excluded from the model. Finally, logistic regression was used to analyze the effects of treatment, category, and their interaction on P/AIs. The proportion of PMN cells in each group was analyzed using ANOVA (PROC GLIMMIX; SAS Inst. Inc., Cary, NC, USA), and the means were compared between groups using Tukey's test. A significance level of 5% was considered significant.

Results

The overall pregnancy rate was 72.2% (151/209), which demonstrated the efficiency of the technique. The pregnancies per AI according to the group (0.5 PG, 1.0 PG and control) are shown in the Table 1). No effects of group ($P = 0.51$) or category ($P = 0.84$) were detected on the P/AI among the groups. There was a tendency ($P = 0.07$) for a group-category interaction for P/AI. In this regard, the P/AI in multiparous cows treated with 1mg PG tended to be greater ($P = 0.08$) than that in control cows (Figure 2). The incidence of PMN did not differ between the groups (Table 1).

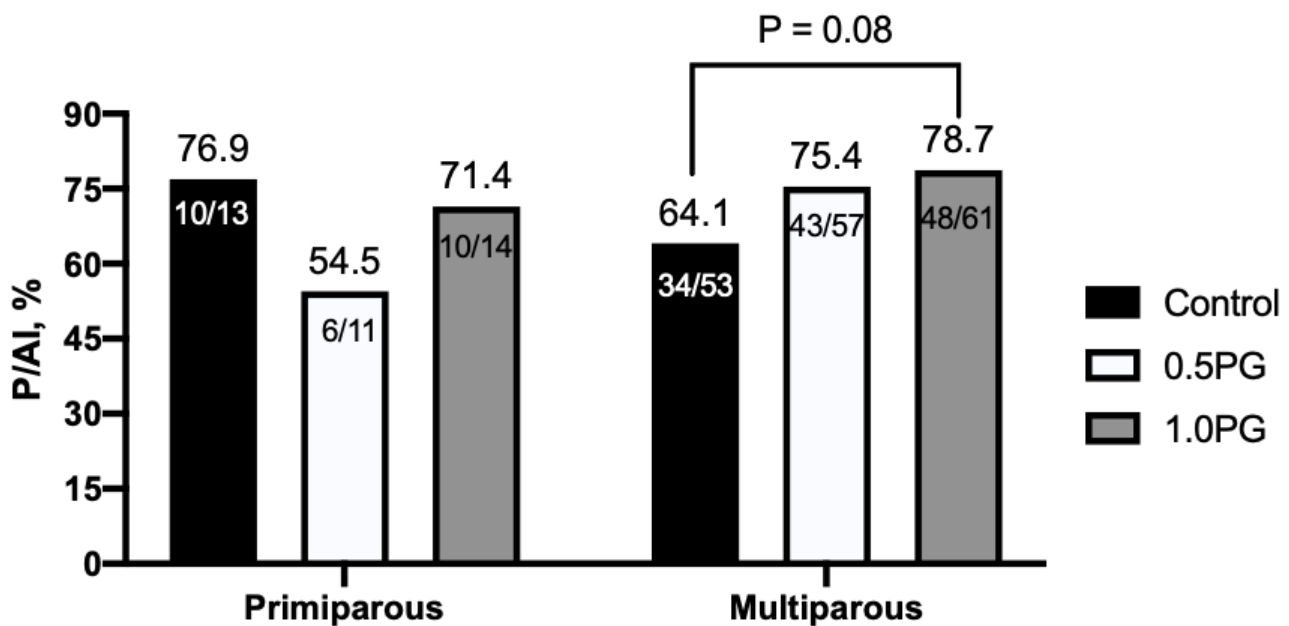


Figure 2. Pregnancy rate after FTAI in cows from control (CTL), 0.5 PG, and 1.0 PG groups according to parity. Values for $P < 0.05$ are identified as statistically significant.

Abbreviations: P/AI – Pregnancy/ Artificial Insemination; CTL – Control group; 0.5 PG – 0.5mg PGF2 α ; 1.0 PG – 1mg PGF2 α .

Table 1

Pregnancy rate and inflammatory cells rate according to the experimental group

	CTL	0.5 PG	1.0 PG	P-value
P/AI (%)	66.7 (44/66)	72.1 (49/68)	77.3 (58/75)	0.51
PMN Cells (%)	4.4 $\pm$ 1.1	4.8 $\pm$ 1.4	6.8 $\pm$ 1.3	0.23

Abbreviations: P/AI – Pregnancy/ Artificial Insemination; PMN – Polymorphonuclear; CTL – Control group; 0.5 PG – 0.5mg PGF2 α ; 1.0 PG – 1mg PGF2 α .

Discussion

Our study demonstrated that the practical application of 1 g of synthetic analog of PGF2 α , ten days before FTAI treatment in multiparous Nelore females with an average of 38.7 ± 7.6 (Mean $\pm$ SD) DPP, tended to be a higher pregnancy rate than control cows (77.3% vs. 66.7%, respectively; $P = 0.08$, Figure 2).

It is worth highlighting that the overall average pregnancy rate of the cattle population in this study, 72.2% (151/209), is well above the Brazilian average, 50% (Baruselli et al., 2017). Therefore, the results obtained using PGF2 α may have been masked. The high average pregnancy rate present in the farm used in this study could be attributed to the intense investments in nutrition and genetic improvement of the cattle over the years. It is, therefore, a model setup in terms of productive and reproductive efficiency.

Regarding the use of PGF2 α , Fernandes et al. (2012) found that in crossbred beef cows ($n = 434$), with the application performed between three and five DPP, the resumption of reproductive activities in these females was anticipated by more than ten days, reducing the interval between calving. The possible explanation for this divergence related to your results may be related to the DPP in which the PGF2 α application was performed. Other studies with dairy cattle used PGF2 α in the early postpartum period, generally administered immediately or up to 20 days after birth (Salasel & Mokhtari, 2011; Solanki et al., 2019). These studies demonstrated its positive effect on uterine involution, reduced uterine bacterial contamination, increased

conception, and shorter intervals between births, among other benefits (Salasel & Mokhtari, 2011; Solanki et al., 2019).

It is also crucial to emphasize that, since this is a commercial farm, this study tested the application of PGF2 α at a favorable time to handle the Nelore cow and her calf safely. In beef cattle, especially the Nelore breed, it is necessary to physically restrain the animal to ensure the correct application of drugs without causing wear and tear to the animal and to ensure safety for the worker and the well-being of the animals. This type of management is typically conducted in containment pens. With newborn calves, it becomes difficult to manage these females on a large scale, making it impossible to apply PGF2 α like that used with dairy cattle, whether immediately after calving or a few days later.

This study was presented with an unusual challenge. The excellent condition of the herd probably interfered with the comparison between the groups. The general conception rate after single-timed artificial insemination was high at 72.2% (151/209). However, there was a tendency ($P = 0.07$) for a group-category interaction for P/AI. In this regard, the P/AI in multiparous cows treated with 1 mg of PG tended to be higher ($P = 0.08$) than that in the control cows. Thus, it is possible to hypothesize that this approach can be repeated in herds under regular conditions.

Conclusions

Considering the tendency of $P = 0.07$, we believe there are advantages for multiparous cows treated with 1 mg of a

synthetic analog of PGF2 α applied ten days before FTAI and 30 to 40 days postpartum. The unusually excellent conception rate of all animals, including the control group, possibly minimized the positive effect of PGF2 α in improving the health of the uterus.

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