

# Strategic re-use of intravaginal progesterone devices increases conception rate in primiparous Nelore COWS

## Reutilização estratégica de dispositivo intravaginal de progesterona aumenta a taxa de concepção em vacas Nelore primíparas

Onadir Geraldo de Jesus Junior<sup>1</sup>; Humberto Luís Del Hoyo Neri<sup>2</sup>; Ana Kelen Felipe Lima<sup>3</sup>; Tânia Vasconcelos Cavalcante<sup>4</sup>; Luciana Rocha Faustino<sup>5</sup>; Cleidson Manoel Gomes da Silva<sup>6\*</sup>; Francisca Elda Ferreira Dias<sup>3</sup>

### Highlights

Conception rate is not affected by the presence of CL at initiation of FTAI protocol.  
P4 devices previously used in cows with CL enhanced the conception rate.  
Conception rate increased by 15.8% on using this alternative strategy.

### Abstract

In the present study, we investigate the effect of the presence or absence of corpus luteum (CL) at the beginning of a fixed-time artificial insemination (FTAI) protocol and to evaluate the impact of one-time use of intravaginal progesterone device (P4 device) in cows with or without CL. A total of 776 primiparous Nelore cows were subjected to FTAI approximately 45 days postpartum. In Experiment 1, 476 cows were divided into two experimental groups: with (CL-present, n=113) or without (CL-absent, n=363) CL, after ultrasound evaluation. On day 0 (D0), all cows received a new P4 device (1.0 g) and 2.0 mg estradiol benzoate (EB). Eight days later (D8), the P4 devices were withdrawn, and prostaglandin (15 mg), estradiol cypionate (0.5 mg), and eCG (300 IU) were administered i.m. All cows were inseminated 48 h after P4 device withdrawal (D10). In

<sup>1</sup> Master in Veterinary Science, Universidade Federal do Norte do Tocantins, UFNT, Araguaína, TO, Brazil. E-mail: onadir.jr@gmail.com

<sup>2</sup> Dr. in Veterinary Science, Biotran Assessoria e Consultoria em Medicina Veterinária, Alfenas, MG, Brazil. E-mail: humbertoluis.neri@gmail.com

<sup>3</sup> Profas Dras, School of Veterinary Medicine and Animal Science, UFNT, Araguaína, TO, Brazil. E-mail: ana.kelen@gmail.com; eldadias@uft.edu.br

<sup>4</sup> Profa Dra, Department of Clinical and Veterinary Surgery, Center of Agricultural Sciences, Universidade Federal do Piauí, UFPI, Teresina, PI, Brazil. E-mail: tania@ufpi.edu.br

<sup>5</sup> Profa Dra, Ministro Reis Velloso Campus, Universidade Federal Delta do Parnaíba, UFDPAr, Parnaíba, PI, Brazil. E-mail: lrfaustino@gmail.com

<sup>6</sup> Prof. Dr., Institute of Studies of the Humid Tropics, Universidade Federal do Sul e Sudeste do Pará, UNIFESSPA, Xinguara, PA, Brazil. E-mail: gomesvet@hotmail.com

\* Author for correspondence

Experiment 2, the cows (n= 300) received (at D0) P4 devices that were previously used once in other cows with (n=109) or without CL (n=191) and 2 mg of EB. The same protocol as that used in Experiment 1 was performed from D8 onwards. In Experiment 1, the overall conception rate after FTAI was 55% (262/476). No difference was found in the conception rate between CL-present and CL-absent cows (52.2 vs. 55.5%). In Experiment 2, the conception rate obtained with P4 devices previously used in cows with CL (58.7%) was greater ( $P<0.05$ ) than that obtained with P4 devices previously used in cows without CL (42.9%). Thus, this strategy resulted in a 15.8% increase in conception rate. In conclusion, the presence or absence of CL at the beginning of the FTAI protocol did not affect the conception rate in cows synchronized with the new P4 device, but the insertion of P4 devices previously used in cows with CL enhanced the conception rates in cows without CL.

**Key words:** Corpus luteum. FTAI. P4 device. Pregnancy. Zebu.

## Resumo

No presente estudo, investigamos o efeito da presença ou ausência de corpo lúteo (CL) no início de um protocolo de inseminação artificial em tempo fixo (IATF) e avaliamos o impacto do uso único de dispositivo intravaginal de progesterona (dispositivo P4) em vacas com ou sem CL. Um total de 776 vacas Nelore primíparas, aproximadamente 45 dias pós-parto foram submetidas à IATF. No Experimento 1, após avaliação ultrassonográfica, 476 vacas foram divididas em dois grupos experimentais: com (CL-presente, n=113) ou sem (CL-ausente, n=363) CL. No dia 0 (D0), todas as vacas receberam um novo dispositivo de P4 (1,0 g) e 2.0 mg de benzoato de estradiol (BE). Após 8 dias (D8), os dispositivos P4 foram retirados e prostaglandina (15 mg), cipionato de estradiol (0,5 mg) e eCG (300 UI) foram administrados i.m. Todas as vacas foram inseminadas 48 horas após a retirada do dispositivo de P4 (D10). No Experimento 2, as vacas (n= 300) receberam (no D0) um dispositivo de P4 previamente utilizado uma única vez em outras vacas com (n=109) ou sem CL (n=191) e 2 mg de BE. O mesmo protocolo utilizado no Experimento 1 foi realizado a partir do D8. No experimento 1, a taxa geral de concepção após IATF foi de 55% (262/476). Não foi encontrada diferença na taxa de concepção entre as vacas com CL presente e CL ausente (52,2 vs. 55,5%). No Experimento 2, a taxa de concepção obtida com dispositivos P4 previamente utilizados em vacas com CL-presente (58,7%) foi maior ( $P<0,05$ ) quando comparada aos dispositivos P4 previamente utilizados em vacas com CL-ausente (42,9%). Essa estratégia resultou em aumento de 15,8% na taxa de concepção. Em conclusão, a presença ou ausência de CL no início do protocolo de IATF não afetou a taxa de concepção em vacas sincronizadas com dispositivo novo de P4; e a eficácia dos dispositivos de P4 previamente utilizados em vacas com CL é maior durante seu segundo uso em vacas sem CL.

**Palavras-chave:** Corpo lúteo. IATF. Dispositivo de P4. Gestação. Zebu.

## Introduction

Fixed-time artificial insemination (FTAI) programs are widely used for beef cows, and the resulting pregnancy rates vary between 40% and 60% (Bó et al., 2018). Therefore, the identification of factors that influence the response of cows to synchronization is of great importance in increasing reproductive efficiency. According to Vasconcelos et al. (2017), several strategies can be used to increase overall pregnancy rates in FTAI protocols, especially in regions with potential for improvement. For instance, in the Amazon region, the application of FTAI using protocols based on progesterone and estrogens has shown satisfactory results in multiparous beef cows (Faleiro et al., 2019).

Intravaginal progesterone devices (P4 devices) are widely used as components of the FTAI protocol. However, they present a considerable financial impact. Based on this observation, several studies have investigated the possibility of re-using P4 devices as viable and economically attractive alternatives (Pereira et al., 2018; Oliveira et al., 2019). In addition, the re-use of P4 devices can help reduce waste production during assisted animal breeding (Cavaliere et al., 2019). Nellore primiparous cows raised under tropical conditions have been widely studied and found not responding adequately to FTAI protocols. According to Bonato et al. (2021), primiparous cows are the most metabolically challenged Nellore matrices, especially when raised in a pasture system receiving only mineral supplementation.

In this study, two experiments were conducted with primiparous Nellore cows subjected to FTAI. The objective of the first

experiment was to evaluate the effect of the presence or absence of corpus luteum (CL) at the beginning of the FTAI protocol. In the second experiment, we aimed to evaluate the effect of re-using P4 devices that were previously used in cows with or without CL.

## Materials and Methods

All procedures performed in this study were conducted in accordance with the guidelines of the Animal Use and Ethics Committee of the Federal University of Tocantins (UFT; protocol number 231010089/2019-18).

### *Location and animals*

Two experiments were conducted on a commercial farm located in Piçarra, PA, Brazil (06°26'S, 48°52'W) from December 2019 to April 2020. A total of 776 Nellore primiparous cows (*Bos indicus*) with an initial body condition score of  $2.75 \pm 0.54$  (range 1–5) was used in this study. All animals were kept under the same conditions and reared in pastures of *Brachiaria* sp under ad libitum access to water and mineral supplementation. Approximately 45 days postpartum, which was established based on the birth date of the calf, all cows were subjected to FTAI.

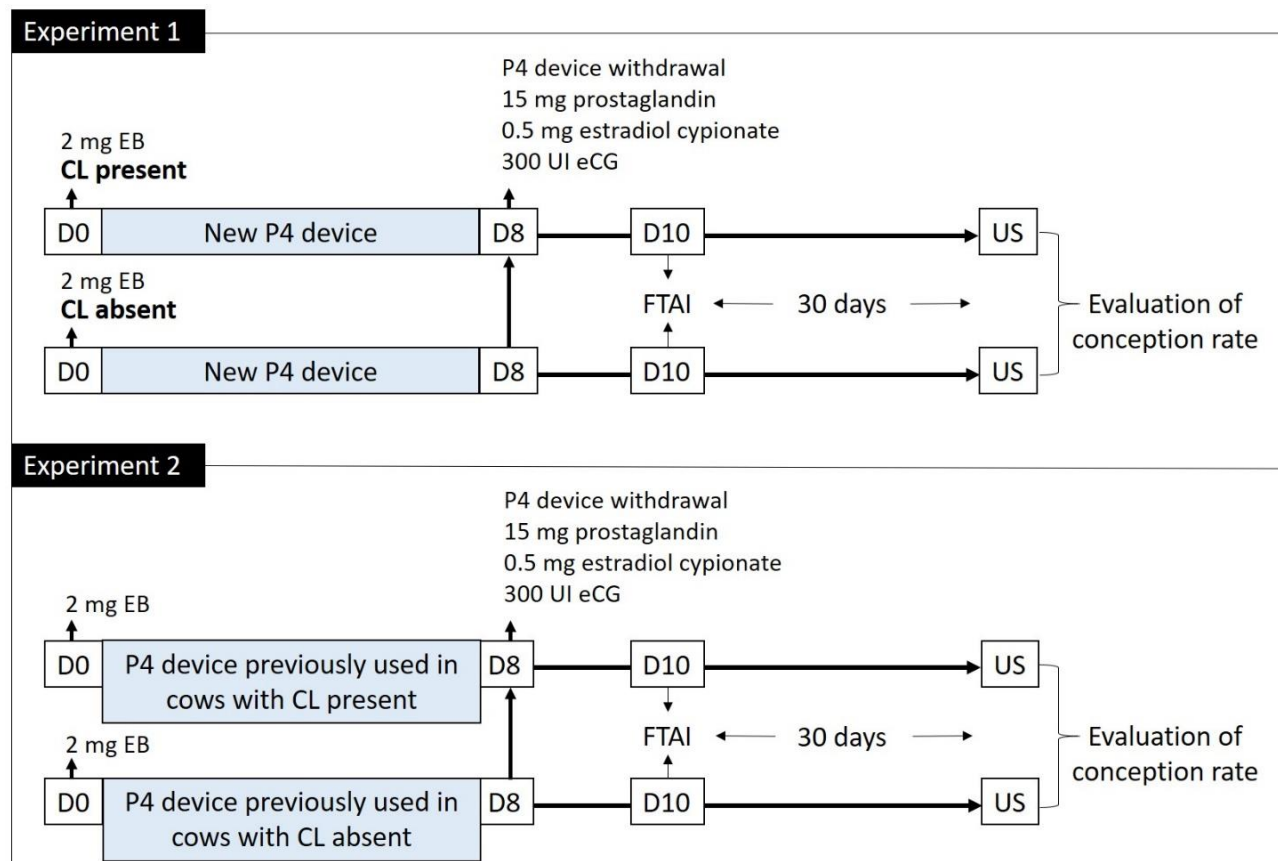
### *Experimental design and hormonal treatments*

The experimental design and hormonal protocol used for the FTAI are shown in Figure 1. In Experiment 1, all cows (n=476) were examined by ultrasonography

to determine the presence or absence of CL just before initiating any treatments. The cows were then divided into two experimental groups: with (CL-present, n=113) and without (CL-absent, n=363) CL. On day 0 (D0), all cows were synchronized by inserting a new P4 device containing 1.0 g of progesterone (Primer®, Agener-TecnoPec, São Paulo, Brazil) and 2.0 mg of estradiol benzoate (EB; Zoetis, São Paulo, Brazil), i.m. On D8, the P4 devices were withdrawn, and 15.0 mg prostaglandin (Estron®, Agener-TecnoPec, São Paulo, Brazil), 0.5 mg estradiol cypionate (CroniCip®, Biogenesis Bagó, Curitiba, Brazil), and 300 IU equine chorionic gonadotropin (eCG; Novormon®, Zoetis Saúde Animal, São Paulo, Brazil) were administered i.m. On D10, 48 h after P4 device withdrawal, all the cows were subjected to FTAI. All P4 devices used in Experiment 1 were identified, sanitized as described by Pereira et al. (2018), and stored separately for future use in Experiment 2.

In Experiment 2, only cows without CL were used (n=300). On D0, the cows received P4 devices that were previously used once in other cows with (n=109) or without CL (n=191) accompanied by 2.0 mg of EB. The procedures from D8 were the same as those performed in Experiment 1.

All inseminations were performed by the same technician, who had no knowledge of the experimental design. In both experiments, the semen of a Nellore bull frozen in straws from the same ejaculate was used homogeneously (Host from São Lucas 000NE00688; Nellore; Genex Brazil, São Carlos, SP, Brazil). Pregnancy was diagnosed based on ultrasound examination (Sonoescape, model A5S, Guangdon, China) 30 days after the FTAI protocol. The presence of an embryonic vesicle with a viable embryo was used as an indicator of pregnancy. Conception rate was defined as the number of pregnant cows divided by the total number of cows subjected to the FTAI protocol  $\times 100$ .



**Figure 1.** Reproductive management of Nelore primiparous cows submitted to fixed-time artificial insemination (FTAI) programme.

CL, corpus luteum, D, day; EB, estradiol benzoate; P4, progesterone; eCG, equine chorionic gonadotropin; US, ultrasound. Experiment 1: For synchronization of estrus, the cows (n=476) with CL present or CL absent received (at D0) a new P4 device for eight days. Experiment 2: Cows (n= 300) received (at D0) P4 device previously used once in females with (n=109) or without CL (n=191). Therefore, all P4 devices used in experiment 2 were previously used in experiment 1.

### Statistical analysis

The data were analyzed using a statistical program (BioEstat, version 5.0). The difference in conception rate was evaluated via the chi-square statistical test. Statistical significance was set at  $P < 0.05$ .

### Results and Discussion

The conception rates of cows synchronized with a new P4 device in Experiment 1 are shown in Table 1. The overall conception rate after FTAI was 55.0% (262/476). This agrees with the results of previous studies that reported pregnancy rates between 40.0% and 60.0% in beef

cows subjected to the FTAI protocol (Bó et al., 2018), including those conducted in the

Amazon region (Pereira et al., 2018; Faleiro et al., 2019).

**Table 1**  
**Conception rate in Nellore primiparous cows with or without CL on D0 of the FTAI protocol, using new P4 device**

Cows (n=476)	Non-pregnant (%)	Pregnant (%)	Overall conception rate
CL present	54/113 (47.8)	59/113 (52.2)	262/476 (55%)
CL absent	160/363 (44.1)	203/363 (55.9)	

No significant effect of the presence or absence of CL on D0 of the FTAI protocol was observed on conception rate (52.2 vs. 55.5%), as seen in Table 1. This lack of difference may be attributed to the administration of eCG at the time of P4 device withdrawal in both the groups tested. According to Núñez-Olivera et al. (2014), the addition of eCG to progesterone- and estradiol-based treatment for FTAI protocols improves the ovulation rate and luteal function in beef cows. However, this effect is more evident in beef cows with more pronounced anestrous condition (Baruselli et al., 2004). The application of eCG can be used to improve the conception rates of cows in postpartum anestrus or cows without CL at the beginning of the FTAI protocol, especially in primiparous cows (Sales et al., 2016; Bottino et al., 2021).

To evaluate the impact of previously used P4 devices, only CL-absent cows were used in Experiment 2. The conception rate obtained by re-using P4 devices previously used in cows with CL (58.7%) was higher ( $P < 0.05$ ) than that observed using devices from cows without CL (42.9%), as shown in Table 2. The initial hypothesis was that P4

devices previously used in cows with CL would increase conception rate during their second use. This hypothesis was accepted based on the observation of 15.8% increase in the conception rate on using P4 devices with one-time use history in cows with CL. These findings indicate that the status of ovarian cyclicity in cows that used the P4 device for the first time influenced the results of its second use. It is possible that the P4 devices previously used in cows with CL have a higher remnant P4 concentration. Although we did not evaluate the residual concentration of P4 in the devices, a previous study had demonstrated that P4 devices release less P4 when used in animals with CL, resulting in the availability of more residual P4 concentration during the subsequent uses (Neri et al., 2015). Low residual P4 may be insufficient to ensure the positive effects of this hormone on pre-ovulatory follicle development and post-ovulatory fertility (Pursley & Martins, 2011), particularly if the P4 device is re-used in anestrous postpartum animals. Therefore, an increase in P4 prior to timed artificial insemination can substantially improve the fertility of lactating primiparous Nellore cows.



**Table 2****Conception rate in Nellore primiparous cows using P4 device previously used for 8 days**

Cows (n=300)	P4 device previously used in CL present cows	P4 device previously used in CL absent cows	Conception rate increased
Pregnant (%)	64/109 (58.7) <sup>a</sup>	82/191 (42.9) <sup>b</sup>	15.8%
Non-pregnant (%)	45/109 (41.3) <sup>b</sup>	109/191 (57.1) <sup>a</sup>	

<sup>a,b</sup> Different letters within the same row represent significant differences (P<0.05).

The results of the study are interesting as they can positively impact the cost of implementing FTAI protocols, which is a serious disadvantage in the beef industry. Thus, the strategy developed in the present study can maintain an adequate conception rate while realizing cost reduction of FTAI program. In addition, re-use of P4 devices helps reduce waste production in assisted animal breeding (Cavaliere et al., 2019).

## Conclusion

This study demonstrated that the presence or absence of CL at the beginning of the FTAI protocol did not affect the conception rate in cows synchronized with new P4 devices and that the re-use of P4 devices with one-time use in cows with CL greatly improved the conception rates of cows without CL. Therefore, the strategic re-use of P4 devices at the farm level could be an additional tool for improving reproductive efficiency in zebu primiparous cows.

## Acknowledgement

We thank the support from the Tocantins Research Support Foundation-FAPT and the National Program for Academic

Cooperation in the Amazon-PROCAD/Amazônia-UFT/UFRA/UECE from the Coordination for the Improvement of Higher Education Personnel-CAPES, Brazil. We would like to thank Editage (www.editage.com) for English language editing.

## Declaration of Competing Interests

The authors declare that they have no competing interests.

## References

- Baruselli, P. S., Reis, E. L., Marques, M. O., Nasser, L. F., & Bó, G. A. (2004). The use of hormonal treatments to improve reproductive performance of anestrus beef cattle in tropical climates. *Animal Reproduction Science*, 82-83, 479-486. doi: 10.1016/j.anireprosci.2004.04.025
- Bó, G. A., Huguenine, E., Mata, J. J., Núñez-Olivera, R., Baruselli, P. S., & Menchaca, A. (2018). Programs for fixed-time artificial insemination in South American beef cattle. *Animal Reproduction*, 15(1), 952-962. doi: 10.21451/1984-3143-AR2018-0025

- Bonato, D. V., Carrer, L. F., Santos, E. S., Figueira, M. R., Cerezetti, M. B., Morotti, F., & Seneda, M. M. (2021). Estrus expression and pregnancy rates in heifers primiparous and multiparous Nelore cows subjected to timed artificial insemination with strategic use of gonadotropin-releasing hormone. *Semina: Ciências Agrárias*, 42(6), 3825-3836. doi: 10.5433/1679-0359.
- Bottino, M. P., Simoes, L. M. S., Silva, L. A. C. L., Giroto, R. W., Scandiuzzi, L. A., Jr., Massoneto, J. P. M., Baruselli, P. S., Souza, J. C., & Sales, J. N. S. (2021). Effects of eCG and FSH in timed artificial insemination treatment regimens on estrous expression and pregnancy rates in primiparous and multiparous *Bos indicus* cows. *Animal Reproduction Science*, 228(14), 106751. doi: 10.1016/j.anireprosci.2021.106751
- Cavaliere, F. L. B., Burali, P. H. B., Morotti, F., Seneda, M. M., Andreazzi, M. A., & Colombo, A. H. B. (2019). Effects of a reusable progesterone device on conception rates and estrus cycle re-synchronization in Nelore cows. *Semina: Ciências Agrárias*, 40(6), 3501-3510. doi: 10.5433/1679-0359.2019v40n6 Supl3p3501
- Faleiro, N. S., Neves, K. A. L., Pereira, L. L., Silva, C. M. G., Vale, W. G., & Minervino, A. H. H. (2019). Effect of oestrous resynchronization on the reproductive efficiency of zebu cows. *Reproduction in Domestic Animals*, 54(7), 1050-1053. doi: 10.1111/rda.13468
- Neri, H. L., Palhao, M. P., Costa, D. S., Viana, J. H. M., & Fernandes, C. A. C. (2015). Endogenous progesterone concentrations affect progesterone release from intravaginal devices used for oestrous synchronization in cattle. *Reproduction in Domestic Animals*, 50(4), 692-695. doi: 10.1111/rda.12550
- Núñez-Olivera, R., Castro, T. de, García-Pintos, C., Bó, G., Piaggio, J., & Menchaca, A. (2014). Ovulatory response and luteal function after eCG administration at the end of a progesterone and estradiol' based treatment in postpartum anestrous beef cattle. *Animal Reproduction Science*, 146(3-4), 111-116. doi: 10.1016/j.anireprosci.2014.02.017
- Oliveira, D. A. M., Kozicki, L. E., Gaievski, F. R., Pedrosa, V. B., Weiss, R. R., Segui, M. S., & Bergstein-Galan, T. G. (2019). Resynchronization of ovulation with new and reused intravaginal progesterone-releasing devices without previous pregnancy diagnosis in *Bos taurus indicus* cows subjected to timed-artificial insemination. *Reproduction in Domestic Animals*, 54(5), 779-785. doi: 10.1111/rda.13427
- Pereira, L. L., Ferreira, A. P., Vale, W. G., Serique, L. R., Neves, K. A. L., Morini, A. C., Monteiro, B. M., & Minervino, A. H. H. (2018). Effect of body condition score and reuse of progesterone-releasing intravaginal devices on conception rate following timed artificial insemination in Nelore cows. *Reproduction in Domestic Animals*, 53(3), 624-628. doi: 10.1111/rda.13150
- Pursley, J. R., & Martins, J. P. (2011). Impact of circulating concentrations of progesterone and antral age of the ovulatory follicle on fertility of high-producing lactating dairy cows. *Reproduction Fertility and Development*, 24(1), 267-271. doi: 10.1071/RD11917



Sales, J. N. S., Bottino, M. P., Silva, L. A. C. L., Giroto, R. W., Massoneto, J. P. M., Souza, J. C., & Baruselli, P. S. (2016). Effects of eCG are more pronounced in primiparous than multiparous *Bos indicus* cows submitted to a timed artificial insemination protocol. *Theriogenology*, 86(9), 2290-2295. doi: 10.1016/j.theriogenology.2016.07.023

Vasconcelos, J. L. M., Carvalho, R., Peres, R. F. G., Rodrigues, A. D. P., Claro, I., Jr., Meneghetti, M., Aono, F. H., Costa, W. M., Lopes, C. N., Cooke, R. F., & Pohler, K. G. (2017). Reproductive programs for beef cattle: incorporating management and reproductive techniques for better fertility. *Animal Reproduction*, 4(3), 547-557. doi: 10.21451/1984-3143-AR998

