

Effect of additional PGF2 α dose on pregnancy rate in Nellore females subjected to FTAI

Efeito de dose adicional de pgf2 α sobre a taxa de gestação de fêmeas bovinas Nellore submetidas a IATF

Jorge Luís Ferreira^{1*}; Matheus Henrique Dias Rodrigues²; Shammara Noleto Santos³; Rodolfo Olinto Rotoli Garcia de Oliveira⁴; Vanessa de Sousa Rodrigues⁵; Dourival Alex Araújo Beserra⁶; José Américo Soares Garcia⁷

Highlights:

Does an additional dose of prostaglandin F2 α improve pregnancy rate?
Prostaglandin F2 α seems to have other effects besides luteolysis.
Prostaglandin F2 α has effects on ovarian dynamics and ovulation.
The sire effect did not have any significance on pregnancy rate.
The additional dose of prostaglandin F2 α provided an increase in cow fertility.

Abstract

This study examined the effect of administering an additional dose of prostaglandin F2 alpha (PGF2 α), in a fixed-time artificial insemination protocol (FTAI), on the fertility of female Nellore cattle. Two experiments were carried out: the first (Experiment I) took place in the state of Tocantins and the second (Experiment II) in the state of Pará, Brazil. In Experiment I (E1), 80 cows were used in three treatments (T1, T2 and T3) in which all received the same FTAI protocol. In T1 (n = 29), the cows received 12.5 mg of Dinoprost on day 9; in T2 (n = 28), they received the additional dose on day 10; and in T3 (n = 23; control group), the animals did not receive the additional PGF2 α dose. Experiment II consisted of 147 bovine females distributed into two treatment groups, namely, T1 - 72 animals receiving the same protocol as T1 of E1; and T2 - 75 animals receiving the same protocol as T3 of E1. Statistical analysis was performed using SAS software, applying the PROC NPARWAY procedure for E1, and means were compared by the Wilcoxon test at the 5% significance level. In Experiment II, the data were subjected to analysis of variance by PROC GLIMMIX and means were compared by the T test at the 5% significance level. The following pregnancy rates were obtained in Experiment I: T1 - 62.06% (18/29); T2 - 57.14% (16/28); and T3 - 52.17% (12/23), with no significant difference observed between treatments. In Experiment II, pregnancy rate in T1 was 66.67% (48/72), whereas in T2 it was 41.33% (31/75), with a significant difference detected (P < 0.05). An additional dose of PGF2 α provides an increase in pregnancy rate in Nellore females.

Key words: Bovine. FTAI. PGF2 α . Pregnancy rate.

¹ Prof. Dr., Universidade Federal do Tocantins, UFT, Câmpus de Araguaína, Araguaína, TO, Brasil. E-mail: jorgeuft@gmail.com

² Discente do Curso de Graduação em Medicina Veterinária, UFT, Câmpus de Araguaína, Araguaína, TO, Brasil. E-mail: mh130499@gmail.com

³ Prof^a M.e, Faculdade de Ciências do Tocantins, FACIT, Câmpus de Araguaína, Araguaína, TO, Brasil. E-mail: mvshammara@outlook.com

⁴ Autônomo, OLIVET Serviços Veterinários, Araguaína, TO, Brasil. E-mail: rodowflex@hotmail.com

⁵ Discente do Curso de Mestrado do Programa de Pós-Graduação em Sanidade Animal e Saúde Pública nos Trópicos, UFT, Câmpus de Araguaína, Araguaína, TO, Brasil. E-mail: vanessarodrigues.uft@gmail.com

⁶ Autônomo, Agropec Produtos Agropecuários, Colinas do Tocantins, TO, Brasil. E-mail: alexcopa_@hotmail.com

⁷ Prof. Dr. Universidade de Brasília, UnB, Faculdade de Agronomia e Veterinária, Campus Darcy Ribeiro, Brasília, DF, Brasil. E-mail: jasgarcia@unb.br

* Author for correspondence

Resumo

Objetivou-se avaliar o efeito da administração de uma dose adicional de prostaglandina F2 alfa (PGF2 α) em protocolo de inseminação artificial em tempo fixo (IATF) sobre a fertilidade de fêmeas bovinas da raça Nelore. Foram realizados dois experimentos, sendo um (E1) no estado do Tocantins e o segundo (E2) no estado do Pará. No experimento 1 (E1) foram utilizadas 80 fêmeas bovinas distribuídas em três tratamentos (T1, T2 e T3), todos animais receberam o mesmo protocolo de IATF, diferindo no T1 (n=29), que recebeu 12,5 mg de Dinoprost no dia 9, T2 (n=28) recebeu a dose adicional no dia 10 e o T3 (n=23) - Grupo controle, que não recebeu a dose adicional de PGF2 α . O experimento 2 (E2) constituído por 147 fêmeas bovinas distribuídas em dois tratamentos. T1: com 72 animais, recebendo mesmo protocolo do T1 do E1. T2: constituído por 75 animais, com protocolo idêntico ao T3 do E1. A análise estatística foi realizada no programa SAS, sendo no E1 utilizando-se o PROC NPARWAY e as médias comparadas pelo teste de Wilcoxon com nível de significância de 5%. No E2 os dados foram submetidos à análise de variancia pelo PROC GLIMMIX e as médias comparadas pelo teste T com nível de significância de 5%. No experimento 1 a taxa de prenhez no T1 foi de 62,06% (18/29), T2 de 57,14% (16/28) e T3 de 52,17% (12/23), não sendo observada diferença significativa entre os tratamentos. No experimento 2 a taxa de prenhez do T1 foi de 66,67% (48/72), enquanto T2 foi de 41,33% (31/75), verificando-se diferença significativa ($P < 0,05$). A dose adicional de PGF2 α promoveu incremento na taxa de prenhez de fêmeas Nelore.

Palavras-chave: Bovino. IATF. PGF2 α . Taxa de prenhez.

Introduction

The worldwide demand for animal products has called for increased productivity by the Brazilian beef cattle farming industry. This condition has allowed production systems and production indices to be continuously evaluated, especially in view of the low reproductive efficiency of herds, which is one of the main bottlenecks for the maximization of economic return (Magioli, 2017).

The introduction of reproductive biotechnologies in Brazilian herds has considerably increased in recent years. In 2018, the sale of semen rose by 13.7% in comparison with the previous year and fixed-time artificial insemination (FTAI) protocols increased by 16.1%, representing 86% of inseminations performed in Brazil (Baruselli, 2019).

Today, FTAI protocols in beef and dairy cattle are well-established, with observed pregnancy rates between 30 and 65% (Fike et al., 1997; Martinez et al., 2002; Baruselli et al., 2012; Zoetis Brasil, 2019). However, the meat market demands constant reproductive strategies that provide greater economic return and productive efficiency

(Oliveira, 2007). To meet this need, increasingly efficient FTAI protocols are required.

The literature is clear that the use of an additional dose of prostaglandin F2 α (PGF2 α) promotes other effects besides luteolysis. Among these effects are stimulation of ovulation in pre-pubertal heifers (Leonardi et al., 2012) and cycling and/or anestrus beef cows (Randel, Lammoglia, Lewis, Neuendorff, & Guthrie, 1996). Nonetheless, these mechanisms are not fully elucidated, although some hypotheses have been proposed. According to Castro et al. (2018) and Leonardi et al. (2012), PGF2 α can have a local effect on the ovary and another directly in the late growth phase of the dominant follicle and induce ovulation. Furthermore, PGF2 α can act by increasing the sensitivity of the anterior pituitary to the gonadotropin-releasing hormone (GnRH), promoting greater release of luteinizing hormone (LH) and consequent ovulation (Leonardi et al., 2012; Randel et al., 1996; Rodrigues et al., 2017).

In a study with three different experiments, Pfeifer et al. (2014) demonstrated that PGF2 α can be used to induce and synchronize ovulation in cattle subjected to FTAI. In the different experiments,

the authors reported similar pregnancy rates in comparison with estradiol esters such as estradiol cypionate and estradiol benzoate.

In view of the above-described scenario, this study was developed to examine the impact of using an additional dose of PGF₂α, during the FTAI protocol, on the pregnancy rate of beef cows reared in the northern region of Brazil.

Material and Methods

The study was divided into two experiments, which were carried out in different herds and states and at different times, with further details given below. Experiment I (E1) was developed in the state of Tocantins and Experiment II in the state of Pará. Both experiments were approved by the Animal Experimentation Ethics Committee of the Federal University of Tocantins (approval no. 23101.006723/2018-17).

Experiment I

This study was developed on a commercial beef cattle farm located in the rural area of the municipality of Piraquê, state of Tocantins, Brazil (06°46'25" S, 48° 17'49" W, 184 m above sea level), from April to August 2018. The climate of the region, according to the Köppen classification, is a tropical wet-dry (AW) type, with an average annual precipitation of 1,680 mm.

The experiment involved 80 multiparous Nelore cows with an average age of 52 months (± 7.98), average weight of 380 kg (± 36.93) and average body condition score (BCS) of 3, according to a scale of 1 to 5 points (Meneghetti & Vasconcelos, 2008), and fit for reproduction.

The animals were distributed at random into three treatments, based on age and average weight, aiming at homogenizing the experimental groups. Treatment 1 (T1) consisted of 29 animals; Treatment 2 (T2), 28 animals; and Treatment 3 (T3, control

group), 23 animals. At first, in the formulation of the experimental design, the total number of animals was 84 (T1 = 30; T2 = 30; T3 = 24). However, due to management conditions and problems with loss of vaginal implants, adjustments were made in the number of animals per group and the above-mentioned criteria were established.

To provide more uniform and equal management and environmental conditions to the animals, a pre-experimental diet consisting of protein-mineral supplementation was provided to all animals for 40 days prior to the experiment in an extensive grazing regime with free access to high-quality water and mineral salt.

After this period, the FTAI protocol began, with all cows being subjected to similar procedures, as described next:

Day 0 (D0) - the animals received an intravaginal implant containing 1.9 g progesterone (P4) (CIDR[®], Zoetis, Brazil) for first use, followed by intramuscular (IM) administration of 2 mg estradiol benzoate (EB) (Gonadiol[®], Zoetis, Brazil).

D7 - IM administration of 12.5 mg Dinoprost (PGF₂α, Lutalyse[®], Zoetis, Brazil).

D9 - removal of the P4 implant and IM administration of 0.6 mg estradiol cypionate (EC) (ECP[®], Zoetis, Brazil), 300 IU equine chorionic gonadotropin (eCG, Novormon[®], Zoetis, Brazil) and 12.5 mg Dinoprost (Lutalyse[®], Zoetis, Brazil).

D11 - 48 h after the P4 implant was removed, FTAI was performed.

The protocol employed in T1 was as described previously. Treatment 2 (T2) differed in that the management actions performed on D7, D9 and D11 were carried out on D8, D10 and D12, respectively. In T3, a protocol similar to that of T1 was applied, except that 2.5 mL of placebo solution were used in place of PGF₂α on D9 (Figure 1). All inseminations were conducted with semen from the same straw from one bull and applied by a single inseminator.

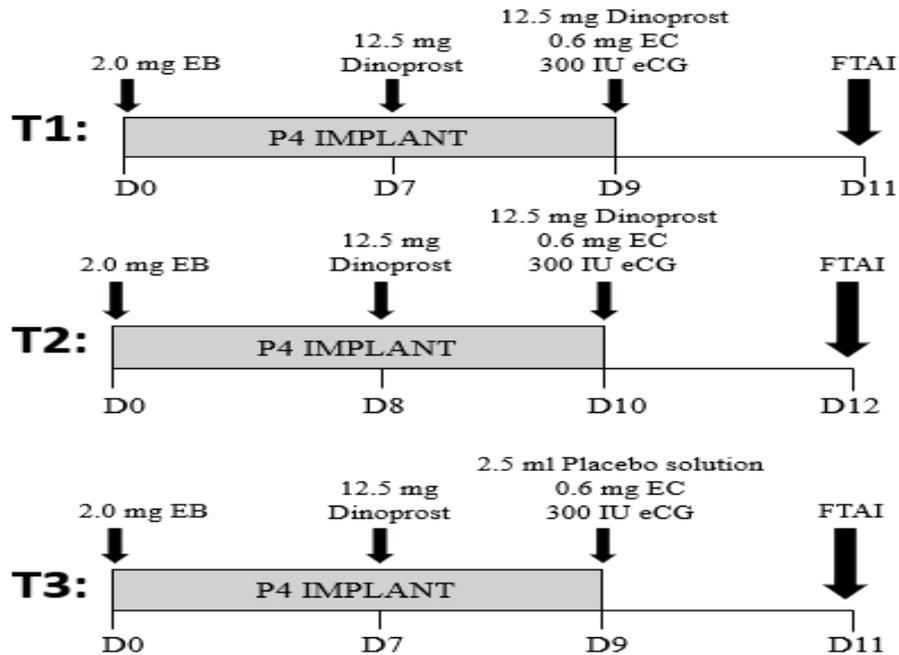


Figure 1. Schedule of treatments used in Experiment I.

Experiment II

A second experiment (Experiment II) was carried out on a farm located in the rural area of the municipality of Altamira, state of Pará, Brazil (03°12'12" S; 52°12'23" W), from September to November 2018. The climate of the region, according to the Köppen classification, is equatorial (Aw and Am type), with an average annual precipitation of 1,844 mm.

The experiment involved 147 multiparous Nellore cows with an average age of 108 months (± 34.74), average body weight of 394 kg (± 42.38) and an average BCS of 3, based on a scale of 1 to 5 points (Meneghetti & Vasconcelos, 2008). The animals were randomly distributed into two treatments so that the experimental groups were as homogeneous as possible in terms of age, weight and BCS. Each treatment consisted of 72 (T1) and 75 (T2) animals.

All animals were fed a pre-experimental diet consisting of mineral supplementation in an extensive grazing system with free access to water,

which began 35 days before the start of the FTAI protocol. The purpose of this diet was to provide the same conditions for the animals as well as homogenize the experimental groups and remove the seasonal effect.

After this period, all females received a FTAI protocol in which T1 was identical to T1 in E1 and T2 to T3 in E1. All inseminations were carried out with semen from two bulls, by a single inseminator.

Pregnancy diagnosis

In both experiments (I and II), pregnancy diagnosis was performed at 40 days after FTAI, using an ultrasound machine (MINDRAY DP-2200 VET, with a 10-Mhz linear transducer).

Statistical analysis

In Experiment I, the data were tabulated in Microsoft Excel® spreadsheets and analyzed using the NPARWAY procedure of Statistical Analysis

System [SAS] (2002). Means were compared by the Wilcoxon test at the 5% significance level.

In Experiment II, the data were also tabulated in spreadsheets and analyzed using Statistical Analysis System [SAS] (2002). After normality of residuals was checked, the data were subjected to analysis of variance by the GLIMMIXED procedure of Statistical Analysis System [SAS] (2002), using the effects of treatments (and control), sire and treatment \times sire interaction as sources of variation. In the model, the sire effect was considered fixed, whereas the effects of treatments were random.

Means were compared using the t-test at the 5% significance level.

Results and Discussion

In Experiment I, the overall pregnancy rate was 57.50% (46/80), with 62.06% (18/29) in T1, followed by 57.14% (16/28) in T2 and 52.17% (12/23) in T3 (control) (Table 1). As seen in the statistical analysis of the data, there was no significant difference between the treatments ($P = 0.54$).

Table 1
Conception rate of cows in Experiment I

Treatment	N of animals	Conception rate
T1	29	62.06% (18/29)
T2	28	57.14% (16/28)
T3	23	52.17% (12/23)

T1: additional PGF2 α dose on D9; T2: additional PGF2 α dose on D10; T3: control group. No significant difference ($P > 0.05$).

In Experiment II, the total pregnancy rate of the herd was 53.74% (79/147), and the conception percentage in the group treated with an additional PGF2 α dose (T1) was 66.67% (48/72), versus 41.33% (31/75) in control group (Table 2), with

significant differences detected between the treatments ($P < 0.05$). No significant effects of sire or sire \times treatment interaction were detected on pregnancy rate ($P > 0.05$).

Table 2
Conception rate of cows in Experiment II

Treatment	N of animals	Conception rate
T1	72	66.67% (48/72) a
T2	75	41.33% (31/75) b

T1: group treated with an additional PGF2 α dose; T2: group treated without an additional PGF2 α dose (Control). Different letters denote a significant difference ($P < 0.05$).

The total pregnancy rates in the two experiments ($T = 57.5\%$; $T2 = 53.74\%$) were within the national average obtained in FTAI protocols, which range from 30 to 65% (Fike et al., 1997; Martinez et al., 2002; Baruselli et al., 2012).

In both experiments (I and II), considering only the absolute values of each group, a trend of absolute contribution of the additional dose of prostaglandin to pregnancy rate was observed in relation to the control groups. Although a statistically significant

difference was only observed between the groups of Experiment II, it should be stressed that, in absolute terms, the treatments with an additional prostaglandin dose provided higher means than the untreated groups.

Peterson et al. (2011) showed that the second dose of PGF2 α tended to improve conception rate in beef cows, although no significant differences were observed ($P>0.05$). Similarly, in a study with dairy cows, Ribeiro et al. (2012) determined the importance of using a double dose of PGF2 α to promote an ideal endocrine profile in the pre-ovulatory period in FTAI protocols, increasing estrus expression and follicular development.

In Experiment II, the treatment with a second dose of PGF2 α resulted in an absolute increase of 25.34% (from 41.33 to 66.67%), providing 61.31% (25.34/41.33) more pregnancies with two PGF2 α doses as compared with control group, based on the methodology proposed by Pereira et al. (2015) and Wiltbank et al. (2015).

Likewise, in an experiment with Holstein cows, Wiltbank et al. (2015) found a 10% increase in pregnancy rate in animals that received two doses of PGF2 α , with significantly higher values seen in multiparous cows. Ribeiro et al. (2012), who worked with dairy cows, and Kasimanickam, Day, Rudolph, Hall and Whittier (2009), who evaluated beef cows, also observed a beneficial effect of the administration of two doses of PGF2 α on pregnancy rate.

The data from Experiment II also reveal that prostaglandin seems to have an effect not only on luteolysis, because even in unsuitable environmental conditions and with low-quality pasture, the obtained rates surpassed the national average, which is around 51.6% for zebu cattle (Zoetis Brasil, 2019). It is worth noting that the experiments were carried out at a time when the average temperature variation in both locations is around 32.6 °C and the pastures already had lower quality; and that even

under adverse conditions it was possible to observe the trend of the additional-dose protocol favoring a higher pregnancy rate.

Pfeifer et al. (2014) proposed an explanation, establishing the hypothesis that prostaglandin also has an effect on the pulsatile release of LH due to a greater responsiveness to GnRH and/or a direct effect on the dominant follicle, since this hormone induces ovulations in a synchronized manner. Corroborating these findings, the present results were potentially associated with an indirect effect of prostaglandin, which acted to induce luteolysis, reduced progesterone and subsequently increased pulsatile LH. Thus, prostaglandin could have an effect on ovulation.

In the current study, the application of an additional dose of PGF2 α provided an increase in fertility when compared with the group that received only one dose, suggesting that it may have other roles, e.g. in ovulation and in the uterus of the animals. This confirms the conclusions of Pereira et al. (2015), who proposed that PGF2 α may have some uterine action to increase conception rate in anestrus cows ovulating small follicles.

Young, Anderson and Plenderleith (1984) had reported similar results, in which cows with basal amounts of P4 (indicating absence of corpus luteum) treated with PGF2 α exhibited better conception rates (70%) than cows that did not receive PGF2 α (40%), demonstrating that this result is not a luteolytic effect.

Conclusions

The use of an additional dose of PGF2 α has a significant effect on fertility rate in Nelore cows under FTAI protocols, increasing it. However, more in-depth studies are necessary to elucidate other effects of PGF2 α on the reproductive cycle of bovine females reared in a tropical climate.

References

- Baruselli, P. S. (2019). *Avaliação de mercado de IATF no Brasil*. Boletim Eletrônico do Departamento de Reprodução Animal/FMVZ/USP. Recuperado de <http://vra.fmvz.usp.br/boletim-eletronico-vra/>
- Baruselli, P. S., Sá, M. F., F^o., Ferreira, R. M., Sales, J. N., Gimenes, L. U., Vieira, L. M.,... Bó, G. A. (2012). Manipulation of follicle development to ensure optimal oocyte quality and conception rates in cattle. *Reproduction in Domestic Animals*, 47(4), 134-141. doi: 10.1111/j.1439-0531.2012.02067x
- Castro, N. A., Moreira, E. M., Andrade, J., Neves, P. M. A., Lemos, I. C., Souza, V. L.,... Pfeifer, L. F. M. (2018). Perfil de liberação de LH circulante após tratamento com prostaglandina F₂alfa em vacas ovariectomizadas. *Anais da Reunião Anual da Sociedade Brasileira de Tecnologia de Embriões*, Florianópolis, SC, Brasil, 32.
- Fike, K. E., Day, M. L., Inskeep, E. K., Kinder, J. E., Lewis, P. E., Short, R. E., & Hafs, H. D. (1997). Estrus and luteal function in suckled beef cows that were anestrous when treated with an intravaginal device containing progesterone with or without a subsequent injection of estradiol benzoate. *Journal of Animal Science*, 75(8), 2009-2015. doi: 10.2527/1997.7582009x
- Kasimanickam, R., Day, M. L., Rudolph, J. S., Hall, J. B., & Whittier, W. D. (2009). Two doses of prostaglandin improve pregnancy rates to timed-AI in a 5-day progesterone-based synchronization protocol in beef cows. *Theriogenology*, 71(5), 762-767. doi: 10.1016/j.theriogenology.2008.09.049
- Leonardi, C. E., Pfeifer, L. F., Rubin, M. I., Singh, J., Mapletoft, R. J. A., Pessoa, G. A., Silva, C. A. (2012). Prostaglandin F₂α promotes ovulation in prepubertal heifers. *Theriogenology*, 78(7), 1578-1582. doi: 10.1016/j.theriogenology.2012.06.030
- Magioli, C. A. (2017) Considerações sobre possíveis irregularidades em produtos de origem animal. *Vigilância Sanitária em Debate: Sociedade, Ciência & Tecnologia*, 5(4), 2-8. doi: 10.22239/2317-269x.00972
- Martinez, M. F., Kastelic, J. P., Adams, G. P., Cook, B., Olson, W. O., & Mapletoft, R. J. (2002). The use of progestins in regimens for fixed-time artificial insemination in beef cattle. *Theriogenology*, 57(3), 1049-1059. doi: 10.1016/S0093-691X(01)00682-3
- Meneghetti, M., & Vasconcelos, J. L. M. (2008). Mês de parição, condição corporal e resposta a protocolos de inseminação artificial em tempo fixo em vacas de corte primíparas. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 60(4), 786-793. doi: 10.1590/S0102-09352008000400002
- Oliveira, D. J. C. (2007). Inseminação artificial em tempo fixo: uma biotecnologia a serviço do empresário rural. *Pesquisa & Tecnologia*, 4(1), p.1-8. Recuperado de http://www.infobibos.com/Artigos/2007_4/inseminacao/index.htm
- Pereira, M. H., Wiltbank, M. C., Barbosa, L. F., Costa, W. M., Jr., Carvalho, M. A., & Vasconcelos, J. L. (2015). Effect of adding a gonadotropin releasing- hormone treatment at the beginning and second prostaglandin F₂α treatment at the end of an estradiol-based protocol for timed artificial insemination in lactating dairy cows during cool or hot seasons of the year. *Journal of Dairy Science*, 98(2), 947-959. doi: 10.3168/jds.2014-8523
- Peterson, C., Alkar, A., Smith, S., Kerr, S., Hall, J. B., Moore, D., & Kasimanickam, R. (2011). Effects of one versus two doses of prostaglandin F₂α on AI pregnancy rates in a 5-day, progesterone-based, CO-Synch protocol in crossbred beef heifers. *Theriogenology*, 75(8), 1536-1542. doi: 10.1016/j.theriogenology.2010.12.017
- Pfeifer, L. F. M., Leonardi, C. E. P., Castro, N. A., Viana, J. H. M., Siqueira, L. G. B., Castilho, E. M.,... Rubin, M. I. B. (2014). The use of PGF₂α as ovulatory stimulus for timed artificial insemination in cattle. *Theriogenology*, 81(5), 689-695. doi: 10.1016/j.theriogenology.2013.11.016
- Randel, R. D., Lammoglia, M. A., Lewis, A. W., Neuendorff, D. A., & Guthrie, M. J. (1996). Exogenous PGF(2) alpha enhanced GnRH-induced LH release in postpartum cows. *Theriogenology*, 45(3), 643-654. doi: 10.1016/0093-691X(95)00410-a
- Ribeiro, E. S., Bisinotto, R. S., Favoreto, M. G., Martins, L. T., Cerri, R. L., Silvestre, F. T.,... Santos, J. E. (2012). Fertility in dairy cows following presynchronization and administering twice the luteolytic dose of prostaglandin F₂α as one or two injections in the 5-day timed artificial insemination protocol. *Theriogenology*, 78(2), 273-284. doi: 10.1016/j.theriogenology.2012.01.012
- Rodrigues, W. B., Jara, J. P., Borges, J. C., Oliveira, L. O. F., Abreu, U. P. H., Silva, K. C.,... Nogueira, E. (2017). Ação da prostaglandina F₂α como indutor de ovulação em vacas de corte submetidas a protocolos de IATF. *Anais do Colégio Brasileiro de Reprodução Animal*, Santos, SP, Brasil, 22.

- SAS Institute Inc. Statistical Analysis System user's guide. Version 9.0 ed. Cary: SAS Institute, USA, 2002.
- Wiltbank, M. C., Baez, G. M., Cochrane, F., Barletta, R. V., Trayford, C. R., & Joseph, R. T. (2015). Effect of a second treatment prostaglandin F2 during the Ovsynch protocol on luteolysis and pregnancy in dairy cows. *Journal of Dairy Science*, 98(12), 8644-8654. doi: 10.3168/jds.2015-9353
- Young, I. M., Anderson, D. B., & Plenderleith, R. W. J. (1984). Increased conception rate in dairy cows after early postpartum administration of PGF2 α . *The Veterinary Record*, 115(17), 429-431. doi: 10.1136/vr.115.17.429
- Zoetis Brasil (2019). *Gerar: Benchmarking Iatf 2019*. São Paulo: Zoetis. (Informativo Técnico). Recuperado de https://www.grupogerar.agr.br/wp-content/themes/gerar_theme/file/benchmarking-gerar-corte-2019.pdf