Conventional fixed-time artificial insemination (FTAI) compared to block FTAI in Nelore cows

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ABSTRACT. Conventional Fixed-Time Artificial Insemination (FTAI) is a biotechnology widely used in beef cattle, however, the technique has shown fertility rates of females almost stabilized. Block FTAI is a technique that aims to maximize these rates, since to obtain good pregnancy results, insemination needs to be performed close to ovulation, this period is defined by the interaction between the inducer and the diameter of the dominant follicle. In this work, the objective was to analyze the pregnancy rate of Nelore cows, comparing the Block FTAI with the conventional FTAI, in two different protocols that are more used in the region, diverging only by the ovulation inducer, being Estradiol Cypionate and o Estradiol benzoate, and amount of handling. Forty multiparous Nellore cows were used. With 10 females in each of the four groups: conventional FTAI with cypionate, FTAI in Block with cypionate, conventional FTAI with benzoate and FTAI in Block with benzoate. FTAI in blocks with cypionate showed a higher pregnancy rate of 70% (P = 0.079), thus, it is a good alternative for producers in the region who did not know this technique.

Keywords: Follicle, Bovine reproduction, Pregnancy rate.

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INTRODUCTION

In recent decades, there has been an intense use of estrus and ovulation synchronization programs, which allow fixed-time artificial insemination (FTAI) and minimize the impacts of estrus detection and postpartum anestrus, characteristic of conventional artificial insemination (AI) programs in Bos indicus females (Meneghetti et al., 2009). After the emergence and dissemination of TAI, there was a 300% increase in the sale of semen between 2000 and 2011 (ASBIA, 2011).

Therefore, the technique stands out as an excellent biotechnology to improve reproductive rates and promote greater economic return for cattle production (Baruselli et al., 2012). However, in the last two decades, the fertility of cows submitted to FTAI protocols seems to have stabilized, with pregnancy rates between 40% and 60% (Meneghetti et al., 2009, Sá Filho et al., 2009).

Several hormonal protocols have been developed with the aim of controlling follicular growth and inducing ovulation, thus allowing the synchronization of estrus and ovulation, enabling the use of fixed-time artificial insemination (FTAI) without the need for estrus detection (Bó et al., 2003).

According to Sá Filho et al. (2010), the diameter of the ovulatory follicle is related to higher concentrations of estradiol, higher probability of ovulation and conception rate. Thus, the high concentration of pre-ovulatory estradiol promoted by the larger diameter of the ovulatory follicle can influence the fertilization of females by promoting changes in the uterine environment, improving sperm transport and favoring conception (Sá Filho et al. 2012).

According to EMBRAPA (2015) FTAI in Blocks has several advantages, such as: it allows cows to be inseminated according to the proximity of ovulation; increased fertility; increase in pregnancy by insemination; it allows evaluating the response of the female before the AI, predicting the fertility of the FTAI in the flock; it makes it possible to diagnose ovarian pathologies and to select or discard animals that did not respond to the synchronization treatment. According to EMBRAPA (2015), evaluating the follicular diameter at the time of FTAI can be an alternative to estimate the maturity and time of ovulation of this follicle and thus adjust the time of inseminations (separating the animals into blocks).

The objective of the study was to analyze the pregnancy rate of Nellore cows, comparing the Block FTAI with the conventional FTAI, in two different protocols and the most used in the region. And so, the study aims to contribute to the producers in the region, showing that the FTAI in Blocks has great advantages.

MATERIALS AND METHODS

In this work, 40 multiparous Nelore cows (Bos indicus) from a collaborating farm of the Instituto Federal Goiano Campus Urutaí, located in the municipality of Luziânia-Go, were synchronized. The project started after being approved by process N° 1293191119 of the Ethics Committee on Animal Use (CEUA) of the Instituto Federal Goiano Campus Urutaí. Practical activities started in 2020, between June and July.

Cows were selected to enter the protocols, having an average Body Score Condition (CEC) of 3.50, according to Ayres (2019) is considered good, and with a postpartum period above 50 days. After selection, the animals were assigned to groups by chance, as they entered the trunk.

Two protocols were used, distinct by the ovulation inducer and number of treatments, one with estradiol cypionate and the other with estradiol benzoate. There were 20 animals submitted to conventional FTAI and 20 animals to FTAI in Blocks. As shown in image 1, we obtained 4 groups, each with 10 cows. Cryopreserved semen from a single bull (Ace, Angus breed) was used, thawed at 37 ºC for 30 seconds.

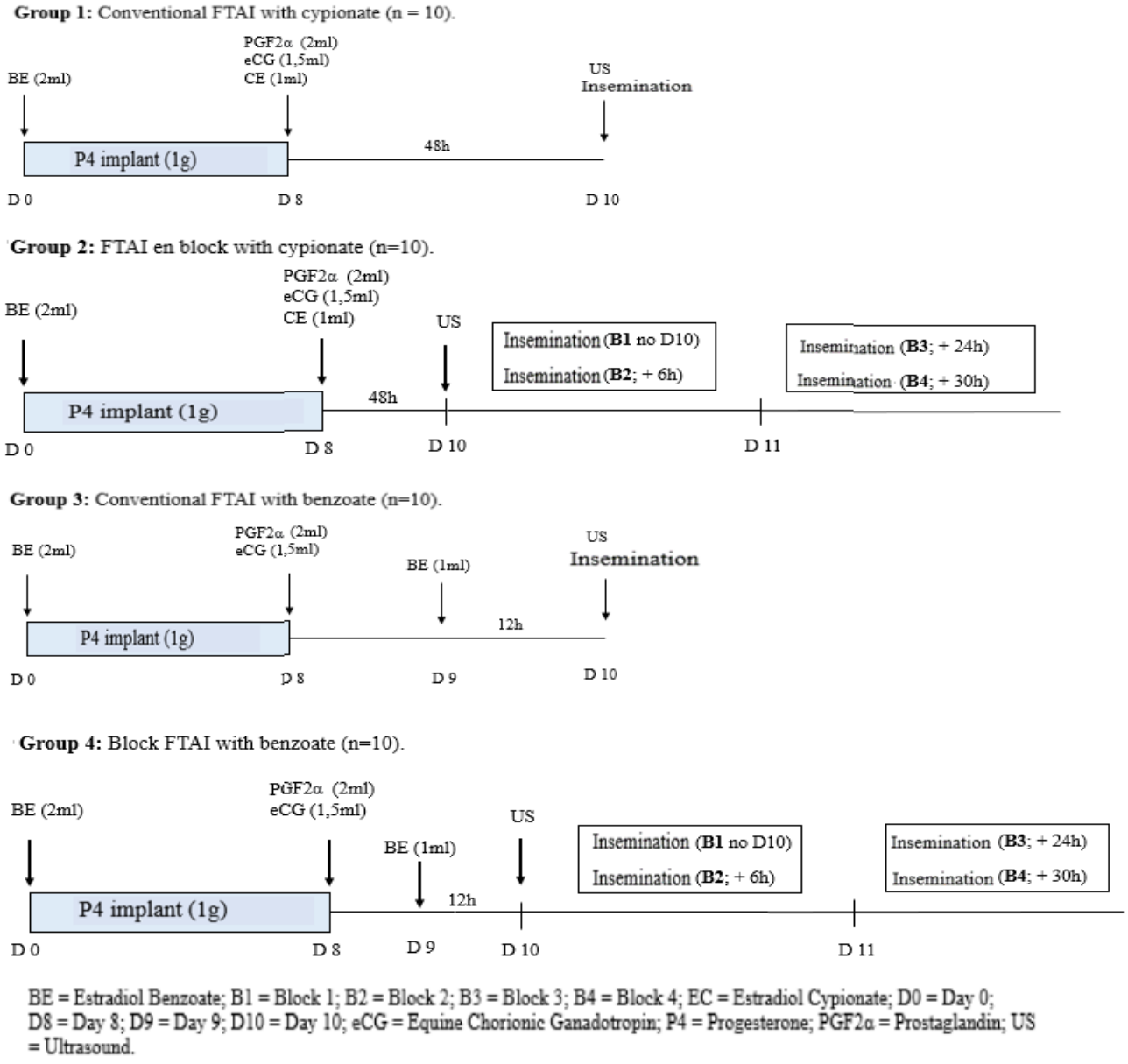

Figure 1: Schematic representation of TAI protocols according to each group.

Protocol 1: Of three managements, on day 0 (D0), all females in this protocol received an intravaginal implant releasing progesterone-P4 (Sincrogest®, Ouro Fino) associated with 2ml of Estradiol Benzoate-BE (Sincrodiol®, Ouro Fino). On day 8 (D8) the implant was removed, the cows received 2ml of prostaglandin-PGF2α (Sincrocio®, Ouro Fino), 1.5ml Equine Chorionic Gonadotropin-eCG (Folligon®, MSD Saúde Animal) and 1ml of Estradiol Cypionate-CE (E.C.P.®, Zoetis).

Protocol 2: Of four managements, on D0 all females in this protocol received a progesterone-releasing intravaginal implant, and 2ml of EB. On D8, he removed the implant, administered 2ml of PGF2α, and 1.5ml of eCG. And on D9 (day 9) he administered 1ml of EB. The commercial nomenclature of the drugs was the same as in protocol 1.

All drugs in the protocols were administered intramuscularly. And the handlings carried out at the same time, from 11:00 to 12:00. On day 10 of the protocols (D10), all cows were evaluated by transrectal ultrasonography, aiming at measuring the preovulatory follicular diameter (FPO). According to this assessment, the animals in the Block FTAI groups were classified into four blocks to be inseminated at different times, ranging from the immediate moment after the ultrasound examination (D10) until the afternoon of D11.

Cows with more than 15mm of follicular diameter were selected for block 1, and inseminated right after the ultrasound examination; animals measuring 13-14.9 mm were in block 2 and inseminated 6 hours after the ultrasound examination; cows with 10-12.9mm were transferred to block 3 and inseminated after 24h; matrices with 8-10mm remained in block 4 and inseminated after 30h; females below 8mm follicular diameter are not inseminated.

On D10, insemination was performed for group 1, (No. of animals: 22, 155, 17, 88, 279, 149, 203, 116, 90, 176), for group 3 (No. 231, 53, 170, 246, 202, 227,141,189, 285, 169) and the 1st Cypionate Block (N° 210), from 11:00 am to 12:00 pm. On the same day, from 5 pm to 6 pm, he inseminated the 2nd Block of Cypionate (N° 158, 233, 92, 68, 211) and the 2nd Block of Benzoate (N° 288, 250, 206, 235, 187, 347).

On D11 (day 11), the 3rd Cypionate Block (N°260, 283, 232, 106) and the 3rd Benzoate Block (N° 126) were inseminated, both from 11:00 am to 12:00 pm. The insemination of the 4th Benzoate block (No., 237 and 143) took place from 5:00 pm to 5:30 pm, it is worth mentioning that all inseminations in this experiment were performed only by the property's veterinarian. After 45 days of D0, the pregnancy diagnosis was carried out in all 40 cows, using a linear transducer with a frequency of 6.0MHz. The pregnancy diagnosis was considered positive in the presence of the embryonic vesicle with a viable embryo (heartbeat).

Due to the distribution characteristic of the data, they were submitted to deviance analysis (ANODEV) and subsequently submitted to the analysis of generalized linear models (GLM), the data were tested to determine their adequacy to the models (Package Hnp) (Moral et al., 2017). The Pregnancy variable was adapted to a model, which is the binomial model. It was then subjected to multiple comparisons of means (Multcomp Package) (Hothorn et al., 2008).

Follicular size data (mm) were submitted to analysis of variance (ANOVA), being tested for assumptions of residual normality using the Shapiro-Wilk test and homogeneity of variance using the Bartlett test. The assumptions of the ANOVA were accepted, then performing multiple comparisons between the means using Tukey's HSD test according to the variance coefficient (ExpDes Package) (Ferreira et al, 2018).

All analyzes were performed in the R statistical computing environment version 4.2.1 (R Core Team, 2022).

RESULTS AND DISCUSSIONS

The pregnancy rate (%) was calculated by dividing the total number of pregnant cows by the total number of inseminated cows. The Fixed-Time Block Artificial Insemination methodology using Estradiol Cypionate as inducer showed the highest pregnancy rate (%) (P = 0.079), followed by conventional methods with both inducers (P = 0.214) and the lowest pregnancy rate was observed in the block methodology using Estradiol Benzoate as inducer (P = 0215) (Table 1).

Table 1. Pregnancy rate (%) of Nelore females submitted to conventional and en bloc fixed-time artificial insemination (FTAI) with ovulation induction with Estradiol Cypionate (CE) or Estradiol Benzoate (BE).

FTAI	Pregnancy Rate (%)	P value
Block + CE	70 a	0,079
Conventional+ BE	60 ab	0,214
Conventional + CE	60 ab	0,214
Block + BE	50 b	0,215
Deviance	48,66	

*Means followed by the same lowercase letter in the column do not differ from each other by Tukey's HSD test at 0.10 significance.

The overall mean follicular size, measured on D10 was 12.37 mm. The mean follicular size of the four groups, measured on D10, when compared, did not show statistical significance (P = 0.755) (Table 2). Thus, no group obtained advantages at the beginning of the experiment in terms of follicle size.

Table 2. Follicular size (mm) of Nelore females submitted to conventional TAI and en bloc with ovulation induction with Estradiol Cypionate (CE) and Estradiol Benzoate (BE).

FTAI	Follicular size (mm)
Block + CE	12,85 a
Conventional + BE	12,26 a
Conventional + CE	11,91 a
Block + BE	12,47 a
CV (%)	15,99
P value	0,755

*Means followed by the same lowercase letter in the column do not differ from each other by Tukey's HSD test at 0.10 significance.

These results denote the efficiency of combining the block method with Estradiol Cypionate, since even with equal follicular sizes on D10, it showed a higher pregnancy rate. Because, if at the time of insemination the female does not have a follicular size equal to or greater than 15 mm, the block method allows these follicles to grow more, since the insemination is postponed for a few hours depending on the size, a time that favors the growth.

According to Embrapa (2015), this new technique increases the pregnancy rate of cows submitted to conventional FTAI, the block FTAI was designed to take advantage of the maximum reproductive potential of beef females submitted to a protocol of FTAI.

The study by Pfeifer et al., 2015, shows that cows with larger follicles ovulate earlier than cows with smaller follicles, even when the ovulation inducer was applied at the same time in both females. The presence of a larger diameter follicle at the time of insemination is an indicator of better ovarian response and conception rate in Bos indicus females submitted to FTAI programs (Ribeiro Filho, 2013). According to Pfeifer et al., 2015, the moment of ovulation is defined by the interaction between the inducer and the diameter of the dominant follicle.

An important finding, which may be related to these results, is the fact that females that ovulated small follicles had a reduced concentration of estradiol at the time of ovulation compared to females that ovulated larger diameter follicles or when ovulation occurred spontaneously. (Vasconselos et al., 2001). Similar data were also observed by Sá Filho et al. (2009), who found a linear proportion between ovulatory follicle diameter and estradiol concentration in Nellore cows. Likewise, Atkins et al. (2010) found a positive and significant correlation between estradiol concentration and ovulatory follicle diameter.

Thus, the production of a viable embryo includes the ovulation of a competent oocyte, an appropriate production of P4 by the corpus luteum and an adequate uterine environment (Perry et al., 2007). Thus, sufficient estradiol secretion is required to promote a suitable uterine environment, preovulatory LH secretion and changes in granulosa cells to synthesize progesterone after luteinization (Meneghetti et al., 2009).

According to Vasconselos et al. (2001), the ovulation of smaller diameter follicles may represent the formation of a smaller corpus luteum and, consequently, a low capacity to produce progesterone (P4) and insufficient embryonic development, thus promoting a reduction in fertility.

Second, Sá Filho et al. (2011), measuring the diameter of the ovulatory follicle at the time of FTAI is an important strategy to predict high fertility in zebu females submitted to a FTAI protocol and allow the direction of matings, for example, when using sexed semen or of high economic value.

In all, the BE groups had a lower conception rate when compared to the CE groups, however, studies by França et al, 2015, show that replacing BE with a treatment with CE as an ovulation inducer does not seem to compromise reproductive indices in progesterone-based FTAI protocols, representing an alternative to simplify synchronization programs in Holstein-Gy crossbred females.

For Perry et al. (2005), the ovulation of follicles with diameters smaller than 11mm results in an increase in the percentage of embryonic loss and fetal mortality in beef cows synchronized with the Ovsynch protocol. According to these authors, the highest percentage of embryonic losses may be due to inadequate oocyte development at the time of ovulation of follicles with a smaller diameter.

CONCLUSION

The gestational rate of multiparous Nelore cows *(Bos indicus),* in the estradiol cypionate group in the FTAI in blocks, was higher, denoting efficiency when separating the animals in blocks according to follicular size, with the objective of waiting for them to grow more, because follicular size is related to a higher pregnancy rate. In this work, the cypionate hormone stood out over the benzoate, in which the FTAI in blocks obtained better performance. In this way, the effectiveness of FTAI in Blocks with estradiol cypionate was elucidated, providing a better cost-benefit ratio for producers in the region, as they were not yet aware of this biotechnology.

CONFLICT OF INTEREST DECLARATION

The author(s) declare(s) no potential conflict of interest in connection with the research, authorship, and/or publication of this article.

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