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ORIGINAL ARTICLE

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Effect of calving to timed artificial insemination interval on fertility of beef cows

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Abstract

The objectives of this study were to determine the effect of calving to timed artificial insemination (C-TAI) interval on fertility of Bos taurus and Bos indicus cows and to determine the best C-TAI interval to include postpartum cows in TAI programs in each genotype (B. taurus and B. indicus). In experiment 1, a retrospective study was performed, with data from 2,709 TAI in Bos taurus and Bos indicus cows with different C-TAI intervals. There was a positive linear effect of C-TAI interval on the pregnancy probability ($p < .0001$). However, there was no effect or interaction with the genotype. When C-TAI intervals were grouped in classes, pregnancy rate differed in groups ($p < .05$). Receiver operating characteristic (ROC) analysis was performed, and it was observed that 52 days would be the better C-TAI interval to maximize fertility in beef cows. According to the previous result, we further stratified into two C-TAI interval groups (≤ 52 days or > 52 days). Cows with C-TAI longer than 52 days (C-TAI > 52) had greater pregnancy rate (56%) than cows with short C-TAI (46%; $p < .01$). In experiment 2, 18 postpartum beef cows were subjected to TAI protocol at different C-TAI intervals ($n = 6$ cows per group): 40, 60 or 80 days postpartum. Cows with 60 days of C-TAI had a greater preovulatory follicle diameter than cows with 80 days postpartum ($p < .05$). The C-TAI interval did not affect ($p > .05$) the ovulation rate nor the proportion of endometrial polymorphonuclear leucocytes. We conclude that C-TAI interval affects pregnancy rate in both Bos taurus and Bos indicus. We recommend a C-TAI interval longer than 52 days to increase fertility of beef cows included in TAI programs.

KEYWORDS
endometrial cytology, pregnancy rate, reproductive efficiency

1 | INTRODUCTION

The timed artificial insemination (TAI) market has been expanding in the last decade, becoming a reality in agricultural properties. The pregnancy rate after TAI is generally between 50% and 60% of the cows subjected to the protocols (Madureira et al., 2020; Sá Filho et al., 2010; Sales et al., 2012). Several studies have been performed to improve the efficiency of TAI protocols, including modifications of ovulation inducers, the concentration of progesterone present in the vaginal device (Uslenghi et al., 2014), an extra dose of prostaglandin analogue F2alpha (Núñez-Olivera et al., 2019) and the use of equine chorionic gonadotropin (eCG) at different times (Tortorella et al., 2013). These studies have the same partial objective, to change the combination of drugs to increase the pregnancy rate of beef cows submitted to TAI protocols. Nevertheless, the influence of the interval between calving and TAI (C-TAI) has not been well studied in beef cows.

In cases of short intervals between calving and AI, pregnancy rates are negatively affected (Williams & Stanko, 2020). With the expansion of the use of TAI and search for the 12-month of calving interval, females are subjected to insemination from 30 days after calving (Crepaldi et al., 2019), which is a short interval to the reestablishment of the uterine health for establishing a new conception (Silveira et al., 2010).

In dairy cows, the voluntary waiting period is better studied. It is generally recommended to wait 45–60 days (Stangaferro et al., 2019), with a need for more time in primiparous cows (Inchaisri et al., 2011). In some situations, the reestablishment of uterine health occurs later, affecting reproductive efficiency (Ribeiro et al., 2013; Sheldon et al., 2002). In nelore (*B. indicus*) cows raised under tropical conditions, uterine health and fertility improve with increasing intervals between calving and TAI (Pfeifer et al., 2018). However, to the best of our knowledge, no studies determine the ideal interval between parturition and TAI in cows from different genetic groups.

Based on these considerations, our objective was to determine the relationship of the interval between calving and insemination on the reproductive efficiency of *Bos taurus taurus* and *Bos taurus indicus cows* subjected to the TAI protocol, allowing the establishment of the ideal interval between calving and insemination. We hypothesized a difference in the ideal interval between parturition and insemination for *B. taurus* and *B. indicus*.

2 | MATERIALS AND METHODS

2.1 | Experiment 1

This was a retrospective study of 2,709 cows subjected to TAI, with different C-TAI intervals. Data were from cows of different genotypes, 1,638 *Bos taurus* cows and 1,071 *Bos indicus* cows. The animals were located on 10 farms in the south and north of Brazil ranging from 89 to 812 cows from farm (Table 1). The FTAI protocols were performed according to the reproductive management of each farm considering their breeding seasons, and 93% of the cows were inseminated between October and February.

The cows were subjected to an estradiol progesterone-based TAI protocol. On day 10 (D-10), they received 2 mg of estradiol benzoate intramuscularly (IM) and an intravaginal progesterone (P4) insert. On day 2 (D-2), females received 0.482 mg of cloprostenol sodium IM, and the intravaginal P4 insert was removed. Estradiol cypionate (1 mg; D-2) or estradiol benzoate (1 mg; D-1) were used as ovulation inducer. Timed AI was performed 48 hr after P4 insert removal, considered as day 0 (D0). The animals were grouped into six classes with intervals of 10 days, forming groups <40, 40 to 49, 50 to 59, 60 to 69, 70 to 79, and ≥ 80 days, to assess the effect of C-TAI interval on pregnancy rate.

Pregnancy diagnosis was performed 30 days after insemination using ultrasonography by a trained technician. Females were considered pregnant when the embryonic vesicle, and embryo were identified.

2.2 | Experiment 2

Eighteen multiparous lactating beef cows (*B. indicus*), 4–8 years with body condition score (BCS) of 2,9 ± 0,06 (0 = lean to 5 = obese; Ayres et al., 2014) with no history of dystocia or reproductive disorders, located on a commercial property in the southwest of Paraná. Cows were raised in an extensive production system with ad libitum offer of pasture (Brachiaria brizantha), water and mineral salt.

Cows were randomly assigned to undergo TAI at 40 (38 ± 1), 60 (58 ± 1), or 80 (78 ± 3) days between calving and insemination. The hormonal protocol started on D-10, using an intravaginal P4 insert with 1 g of P4 (PRIMER®, Tecnopec, São Paulo, Brazil) and 2 mg of estradiol benzoate (RIC-BE®, Tecnopec), administered IM, and on D-2, the intravaginal P4 insert was removed, and endometrial cytology performed using the Cytobrush technique. We gave 1 mg of estradiol cypionate (ECP®, Pfizer, Cravinhos, Brazil) and 0.482 mg of sodium cloprostenol (Sincrocio®, Ouro Fino, São Paulo, Brazil) IM. On D0, 48 hr after the P4 insert removal, AI was performed in all females by a single technician. After insemination,

TABLE 1 Number of cows, calving to timed artificial insemination (C-TAI) interval and pregnancy rate (P/AI) according to each farm enrolled in study

Location/genetic group	Farm	Number of cows	C-TAI interval (mean ± sem)	P/AI (%)
North/B. indicus	1	17	58.4 ± 3.2	41.2
	2	98	47.3 ± 1.3	45.9
	3	89	50.4 ± 1.4	49.4
	4	362	60.8 ± 0.7	54.4
	5	122	57.4 ± 1.4	51.6
	6	197	57.8 ± 0.9	55.8
	7	186	56.4 ± 1.1	52.2
South/B. taurus	8	812	65.3 ± 0.5	53.4
	9	571	69.9 ± 0.6	56.6
	10	255	70.6 ± 0.8	56.5
Overall	-	2,709	63.6 ± 0.3	53.8

Abbreviation: Sem, standard error mean.

the development of the preovulatory follicle was monitored at 12-hr intervals until ovulation or atresia, up to 2 days after insemination. Ovulation was characterized by the absence of the dominant follicle and the presence of the haemorrhagic body in the same ovary. The ovarian structures of the females were measured using a Mindray ultrasonographic device (model DP10Vet) with a transrectal linear transducer at 7.5 MHz.

2.3 | Endometrial cytology

The Cytobrush technique was performed as described by (Kasimanickam et al., 2005). Before collection, the vulva was cleaned with running water and dried with a paper towel, and the brush protected by a cannula and sanitary sheath was introduced through the vagina and cervix until reaching the body of the uterus. Before entering the cervix, the sanitary jacket was withdrawn, and the brush was exposed to the body of the uterus. Samples were collected by rotating the brush clockwise when in contact with the uterine wall. Afterwards, the brush was retracted into the cannula, and the assembly was removed from the female genital tract.

The slides for cytology examination were prepared by rolling the brush and after drying in the air, they were stained using the Panoptic technique. Each slide was examined by optical microscopy (1,000 times magnification in an immersion objective) to count 200 cells, establishing the number of polymorphonuclear leucocytes (PMN). Two adequately trained technicians performed the count. Slides in which there was a difference greater than 10% between the two evaluators were recounted. The result was calculated as the arithmetic mean of the two evaluators and transformed into percentages.

2.4 | Statistical analysis

The effect of C-TAI interval, genotype (Bos indicus or Bos taurus) and their interaction on pregnancy rate were analysed using

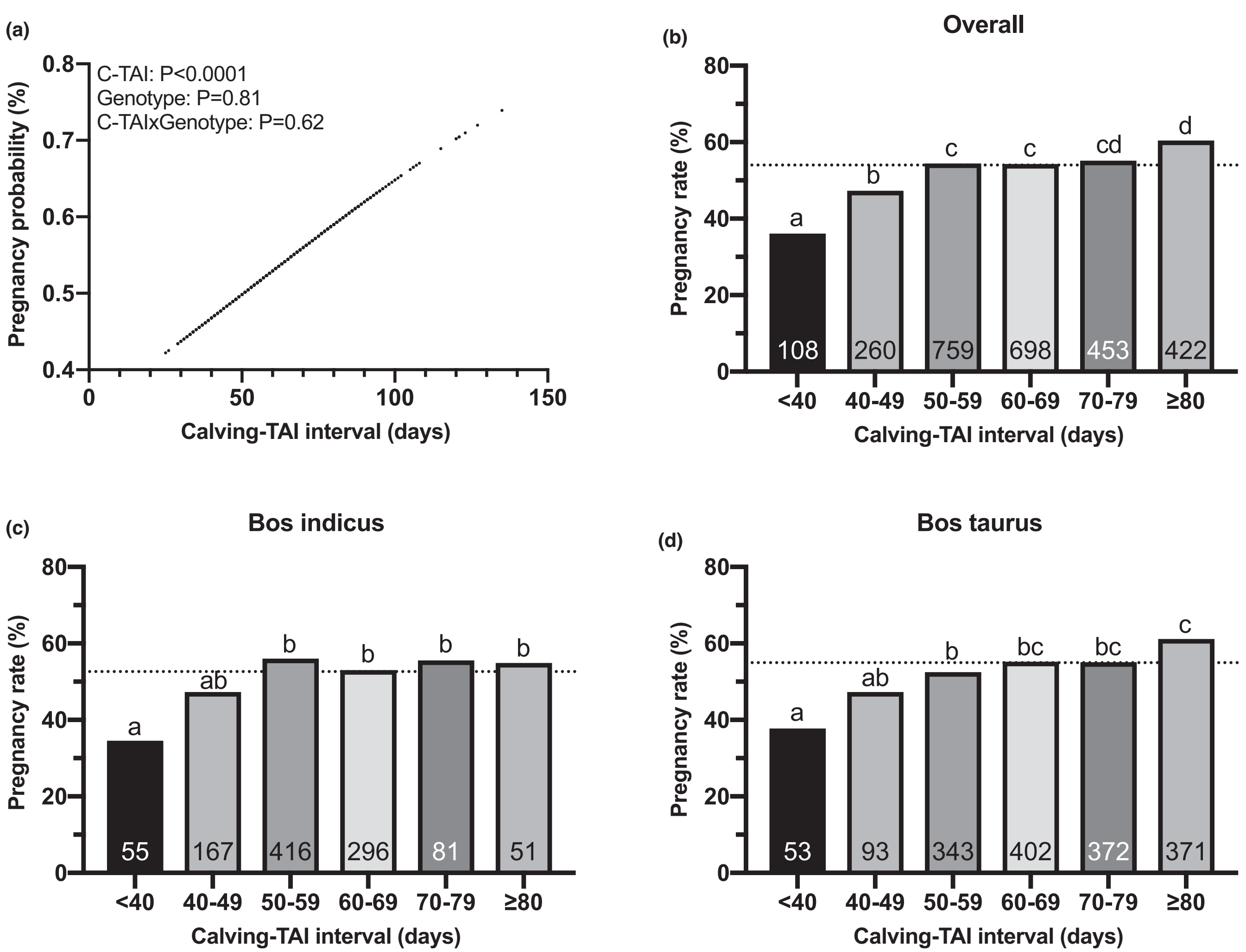

FIGURE 1 Pregnancy probability (a) and pregnancy rate of overall (b), Bos indicus (c) or Bos taurus (d) cows subjected to TAI protocol with different calving to timed artificial insemination intervals (C-TAI). Different letters indicate a significant difference between the C-TAI interval classes ($p < .05$). The dotted line indicates the overall population mean

logistic regression. The receiver operating characteristic (ROC) curve was used to determine the cut-off point for the pregnancy rate, that is the C-TAI interval that determines better pregnancy rates, with larger value of sensitivity—(1 - specificity). The odds ratio to cow become pregnant was calculated in 10 days range of C-TAI interval.

The effect of C-TAI interval groups on binomial variables (ovulation and pregnancy rate) was also analysed by logistic regression followed by contrasts to determine the differences between classes. The effect of C-TAI interval groups on continuous variables (follicular diameter, BCS and PMN) was determined using analysis of variance followed by Student's *t* as a post hoc test.

All continuous variables were tested for normality using the Shapiro–Wilk test. Analyses were performed using the JMP statistical package (SAS Institute Inc., Cary, NC), and the accepted significance level was $p \le .05$.

3 | RESULTS

3.1 | Experiment 1

There was a linear effect of C-TAI interval on the pregnancy probability ($p < .0001$; Figure 1a). However, there was no effect or interaction with the genotype (*B. taurus* or B. *indicus*; Table 2). There was no effect of farm on pregnancy rate ($p > .05$; Table 1). The odds ratio for a 10-day increase in C-TAI was 1.1312; that is a 10-day increase in C-TAI increased the probability of a cow becoming pregnant by 13%.

TABLE 2 Estimates, standard error and *p*-values of fixed effects of statistical model

Effect	Estimate	Std. error	*p*-value
Intercept	−0.62	0.2	.0019
C-TAI interval	0.012	0.003	.0001
Genotype	0.010[a]	0.043[a]	.81
C-TAI × Genotype	0.002[b]	0.003[b]	.61

[a]Estimate and std. error related to indicus genotype.
[b]Estimate and std. error related to (C-TAI interval - 63.7) × Genotype[indicus].

The pregnancy rate differed in groups with different classes of C-TAI, regardless of genotype ($p < .05$; Figure 1). ROC analysis was performed to determine the C-TAI in which the female would be more likely to become pregnant in the TAI, and we calculated an interval from 52 days after parturition (area under the curve [AUC] = 0.55; Figure 2a). When females were stratified into two C-TAI interval groups, according to previous result (≤ 52 days or > 52 days), cow with C-TAI longer than 52 days (C-TAI > 52) had higher pregnancy rate (56%) than cows with short C-TAI (46%; $p < .01$; Figure 2b).

3.2 | Experiment 2

The diameter of the preovulatory follicle was affected by C-TAI interval (Figure 3a; $p < .05$). Cows with a 60-day C-TAI interval had larger preovulatory follicle than 80-day-old cows. C-TAI did not influence the ovulation rate ($p > .05$), which was 50% (3/6) in all groups. There was no difference in BCS between animals of different C-TAI ($p > .05$; Figure 3b). The increase in C-TAI did not reduce the number of neutrophils on endometrial cytology ($p > .05$; Figure 3C).

4 | DISCUSSION

We evaluated the effect of C-TAI interval and its interaction with genetic groups on pregnancy rate of cows submitted to TAI protocol. Our hypothesis that there is an effect or interaction of the genetic group with C-TAI interval on probability of pregnancy was refuted. However, the effect of C-TAI interval on the pregnancy rate was highly significant, regardless of genetic group. Several studies aimed to increase the efficiency of TAI protocols, especially with changes in the hormonal protocol (Madureira et al., 2020; Núñez-Olivera et al., 2019; Tortorella et al., 2013; Uslenghi et al., 2014). However, these studies did not consider the interval between calving and initiation of the protocol, which in the present study we demonstrate has a significant effect on the efficiency of the TAI. We determined

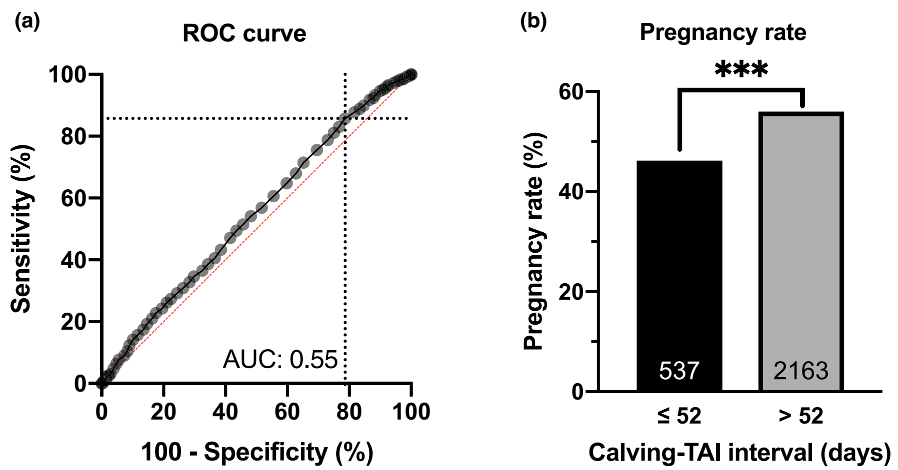

FIGURE 2 A: Receiver operating characteristic (ROC) curve to achieve a pregnant cow on basis of calving-TAI interval. The dotted line is at larger sensitivity—(1 - specificity) value that correspond 52 days of calving-TAI interval. B: Pregnancy rate in cows with an interval between calving and TAI equal to or less than 52 days or cows with an interval greater than 52 days. Asterisks (***) indicate a highly significant difference between groups ($p < .001$). AUC: Area under the curve

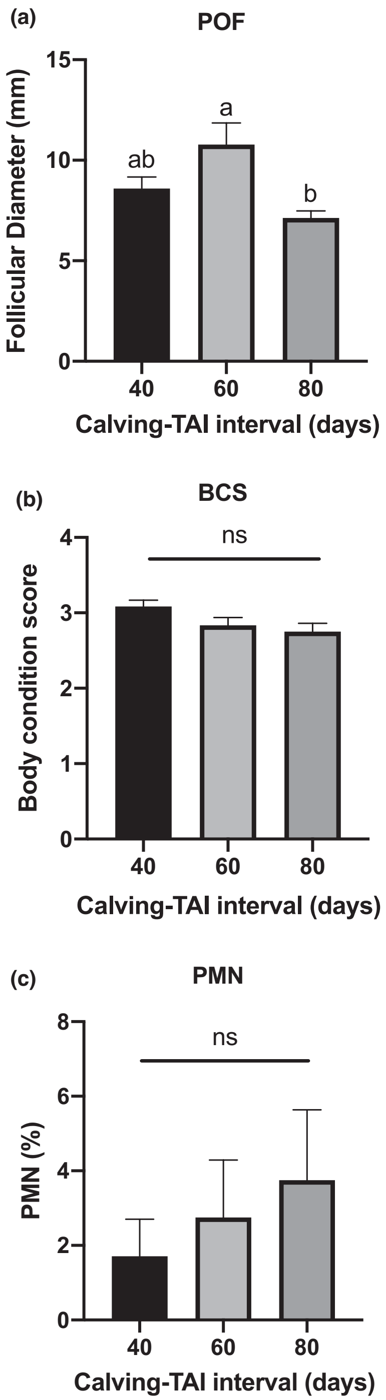

FIGURE 3 Diameter of the preovulatory follicle (POF) (a) body condition score (BCS) (b) and percentage of polymorphonuclear leucocytes (PMN) found in endometrial cytology using the Cytobrush technique (c) in cows with different intervals between calving and TAI. Different letters indicate a significant difference between groups ($p < .05$). NS, Not significant difference ($p \geq .05$)

that TAI from 52 days after calving presents higher pregnancy rate, both in *B. indicus* and *B. taurus*.

We used a large number of animals, and the pregnancy rate was 54%. Most studies with TAI report a pregnancy rate of approximately 50% (Cedeño et al., 2021; Madureira et al., 2020; Pfeifer et al., 2017; Sales et al., 2012). Allocating animals into groups, we found that at 50–59 days of C-TAI interval, cows had higher pregnancy rates, similar results to those found in crossbred cows with C-TAI from 50 days onwards (Williams & Stanko, 2020).

To achieve a 12-month calving interval, the TAI pregnancy rates must be optimized. We identified the minimum interval between C-TAI with satisfactory pregnancy results. Using 10-day TAI protocols, cows calved with 42 days or more would be subject to entering the hormonal manipulation protocol; therefore, they will be inseminated 10 days later, from 52 days of calving. Pfeifer et al. (2018), using *B. indicus* cows, suggested starting the TAI protocol from 35 DPP because cows in a tropical climate show a rapid reduction in neutrophils after calving. The animals inseminated after 52 days postpartum showed a pregnancy 9% higher than those inseminated before. However, the AUC was 0.55 that means pregnancy based on C-TAI can be correctly predicted in only 55% of the animals, which is low for a binomial variable. Therefore, C-TAI > 52 days can be used to recommend greater reproductive efficiency but, as expected, it cannot be used to predict the pregnancy rate in a given population.

The preovulatory follicle diameter was larger in 60 days postpartum cows than cows 40 or 80 days after parturition. The decrease in preovulatory follicle size in 80 days C-TAI cows can be related to the high demand for milk by the calf, as they are lactating cows. Throughout development, calves increase the demand for milk (Sapkota et al., 2020). With greater demand for milk production by the mammary gland, the female enters into a negative energy balance that can be alleviated with better nutritional support (Ayres et al., 2014). This fact can be corroborated by the frequent decrease in body condition observed in animals after 60 days postpartum (Szura et al., 2020).

The influence of follicular size on ovulatory rate, corpus luteum size, progesterone concentration and pregnancy rate in TAI protocols is well defined (Pugliesi et al., 2016; Velho et al., 2022). However, in previous studies the calving-TAI interval was not considered. Therefore, the authors expected an increase in follicular size with increasing parturition-TAI interval until reaching a plateau, to support pregnancy rate observed. However, follicular size alone can not explain the pregnancy rate. Furthermore, the authors can speculate that in beef cows under extensive conditions, as is the case of the present work, there is a reduction in body condition after 60 postpartum. Therefore, the decrease in follicular size may be related to the nutritional effect. However, we can not test this hypothesis with the available data. Based on these considerations, the authors understand that the pregnancy rate improves with the increase in parturition-TAI interval until reaching a plateau as a result of improved follicular growth and uterine condition. However, sucking cows that started cyclicity at 60 days postpartum, even if the follicular growth rate decreases in some animals because of fat mobilization (depending on the nutritional management of each farm) does not compromise TAI pregnancy rate.

At the time of calving, bacterial contamination of the endometrium and physical trauma leading to infection of the reproductive tract can occur (Sheldon et al., 2006). Subclinical endometritis is defined as the presence of 10% neutrophils in the endometrium (Kasimanickam et al., 2004) and the absence of clinical secretions (Sheldon et al., 2006). In dairy cattle, the presence of *Escherichia coli* was associated with lower concentrations of progesterone and prolonged luteal phase (Sheldon et al., 2009). Furthermore, the increase in neutrophils decreased the probability of postpartum conception and increased the service rate per conception in dairy cattle (Ahmadi et al., 2016). In the present study, regardless of the C-TAI, the animals presented low percentages of neutrophils in the endometrium; there was an absence of uterine pathologies, demonstrating the ability of cows to re-establish uterine health 40 days after parturition. Taken together, our findings suggest that the greater reproductive efficiency observed in beef cows with more than 52 days postpartum is more closely associated with cyclicity than the uterine environment.

The present study was performed with a representative number of cows from different farms in Brazil's north and south regions and different genotypes. Therefore, we can safely indicate the best C-TAI, allowing for planning the best time to perform TAI protocols. Using these findings, farmers and field technicians should wait for at least 52 days of C-TAI interval to optimize conception rate at TAI.

5 | CONCLUSION

The interval between calving and TAI influences the pregnancy rate, but without an effect or interaction with the genotype (*B. taurus taurus* or *B. taurus indicus*). Furthermore, the minimum interval from calving to the TAI should be 52 days to achieve a higher pregnancy rate.

AUTHOR'S CONTRIBUTIONS

All authors participated in the proposal and experimental design. JTF performed the experiments and wrote the manuscript. RF analysed the data and revised the manuscript. All authors reviewed and agreed with the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ETHICS COMMITTEE APPROVAL

The Animal Experimentation Ethics Committee of the State University of Santa Catarina evaluated and approved all procedures involving animals—CEUA n° 8637091020.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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