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Article

Estrus Expression and Its Impact on Fertility in Zebu Cows Undergoing Fixed-Time Artificial Insemination

Artur Azevedo Menezes ^{1,*}, Matheus Augusto Matsumoto dos Santos ¹,
Marcus Vinícius Galvão Loiola ¹, Rodrigo Freitas Bittencourt ¹,
Caio Victor Damasceno Carvalho ¹, Alexandra Soares Rodrigues ²
and Antonio de Lisboa Ribeiro Filho ¹

¹ School of Veterinary Medicine and Zootechnics, Federal University of Bahia (UFBA), Salvador, BA, Brazil
arturmenezes@ufba.br (A.A.M.); matsumoto.matheus@hotmail.com (M.A.M.S.); marcusvinicius@ufba.br
(M.V.G.L.); rfb@ufba.br (R.F.B.); caio.victor@ufba.br (C.V.D.C.); alisboafilho@ufba.br (A.L.R.F.).

² Multidisciplinary Center of the Barra Campus, Federal University of West Bahia (UFOB), Barra, BA, Brazil.
alexandra.rodrigues@ufob.edu.br (A.S.R.).

* Correspondence: arturmenezes@ufba.br

Simple Summary: It is vitally important that scientists are able to describe their work simply and concisely to the public, especially in an open-access on-line journal. The simple summary consists of no more than 200 words in one paragraph and contains a clear statement of the problem addressed, the aims and objectives, pertinent results, conclusions from the study and how they will be valuable to society. This should be written for a lay audience, i.e., no technical terms without explanations. In production systems that combine TAI with natural service, final pregnancy rates at the end of the breeding season depend not only on FTAI conception but also on the fertility of subsequent natural breeding. This study aimed to evaluate the effect on fertility throughout the breeding season in Nellore females subjected to time artificial insemination (TAI). A total of 1053 cows were submitted to a estradiol (E2)/progesterone (P4)-based timed artificial insemination (TAI) protocol. On D10, animals were classified into two experimental groups—With Estrus and Without Estrus—based on the removal of the paint. The overall estrus detection rate (EDR), pregnancy per artificial insemination (P/AI), pregnancy rate from natural mating (P/NM), and final pregnancy rate at the end of the breeding season (P/BS) did not differ among categories. In heifers, estrus expression impacted P/AI ($p < 0,05$). In primiparous cows, no differences were observed for P/AI, P/NM, or P/BS ($p > 0,05$). In multiparous cows the estrus expression influenced P/AI, P/NM, and P/BS ($p < 0,05$).

Abstract: This study aimed to evaluate the effect on fertility throughout the breeding season in Nellore females subjected to time artificial insemination (TAI). A total of 658 multiparous, 138 primiparous, and 257 heifers cows were used. On D0, all females received an intravaginal progesterone-releasing device and E2 benzoate. On D8, the P4 device was removed, and animals received eCG, PGF and E2 cypionate. At this time, all animals were marked with paint between the sacral tuberosity and the tail head. On D10, animals were classified into two experimental groups—With Estrus and Without Estrus—based on the removal of the paint. The overall estrus detection rate (EDR), pregnancy per artificial insemination (P/AI), pregnancy rate from natural mating (P/NM), and final pregnancy rate at the end of the breeding season (P/BS) did not differ among categories. In heifers, estrus expression impacted P/AI ($p < 0,05$). In primiparous cows, no differences were observed for P/AI, P/NM, or P/BS ($p > 0,05$). In multiparous cows the estrus expression influenced P/AI, P/NM, and P/BS ($p < 0,05$). Therefore, estrus detection can be used as a valuable tool to predict FTAI outcomes in heifers and multiparous cows, as well as to forecast overall reproductive performance during the breeding season in multiparous cows.

Keywords: bos taurus indicus; beef cattle; pregnancy; natural mating; breeding season; artificial insemination; fertility

1. Introduction

In modern beef production systems, the use of reproductive biotechnologies is essential to maximize herd productivity and ensure the economic sustainability of cattle operations. Among these technologies, fixed-time artificial insemination (FTAI) is one of the most widely adopted strategies [1].

Within this context, a thorough understanding of the physiological and behavioral mechanisms that enhance fertility in FTAI programs is crucial [2]. Multiple studies have consistently demonstrated that females expressing estrus between progesterone (P4) device removal and insemination exhibit higher pregnancy rates, indicating that estrus expression is a key predictor of reproductive success in cows managed under FTAI protocols [3–7]. Supporting this, Ferraz et al. [8] validated the use of tail paint markers as a simple, affordable, and effective tool for estrus detection in *Bos indicus* (Nelore) females, offering a practical alternative to more expensive and less accessible technologies in beef herds.

Evidence indicates that females exhibiting estrus prior to FTAI tend to ovulate larger dominant follicles and have higher circulating estradiol (E2) concentrations [6,9–11]. Elevated E2 levels are directly associated with enhanced follicular vascularization, which facilitates the delivery of critical metabolites required for follicular growth, maturation, and subsequent ovulation [12]. Consequently, the physiological benefits of estrus expression occur not only during the peri-ovulatory period but also extend into the luteal phase. Together, these effects contribute to an endocrine and uterine environment that favors embryo development and pregnancy establishment following FTAI [4].

Nevertheless, in production systems that combine FTAI with natural service, final pregnancy rates at the end of the breeding season depend not only on FTAI conception but also on the fertility of subsequent natural breedings [2]. Despite its importance, there is a notable lack of studies investigating the mechanisms that influence both FTAI success and fertility over the entire breeding season in females that fail to conceive at insemination.

Therefore, considering the potential of estrus expression as a predictive tool for conception and the lack of studies evaluating its role not only at the time of FTAI but also longitudinally throughout the breeding season, the objective of this study was to characterize estrus expression and assess its impact on fertility dynamics during the breeding season across different categories of Zebu females.

2. Materials and Methods

2.1. Study Location and Period

The study was conducted on a commercial beef cattle farm located in Baixa Grande, Bahia, Brazil. Cows were managed on *Cenchrus ciliaries* pasture with free access to mineral supplementation and water.

2.2. Animals

A total of 1,053 Nelore (*Bos indicus*) females were enrolled in the study, stratified into three categories: 658 multiparous cows (8.9 ± 0.18 years old; BCS = 2.8 ± 0.01; 67.2 ± 1.05 days postpartum), 138 primiparous cows (3.3 ± 0.04 years old; BCS = 2.7 ± 0.02; 73.7 ± 2.32 days postpartum), and 257 cyclic heifers (2.4 ± 0.02 years old; BCS = 2.9 ± 0.02). Body condition score (BCS) was assessed on a scale from 1 to 5 [13]. The experimental procedures were approved by the Institutional Animal Care and Use Committee (CEUA – EMEVZ/UFBA, protocol no. 24/2022).

2.3. Estrus Synchronization Protocol

All females were subjected to the following timed artificial insemination (TAI) protocol. On Day 0 (D0), animals received a 0.5 g intravaginal progesterone (P4) device (PRIMER Monodose®, Agener União, São Paulo, Brazil) combined with an intramuscular (IM) injection of 2 mg estradiol benzoate (Estrogin®, Biofarm, São Paulo, Brazil). On Day 8 (D8), the P4 devices were removed, followed by

IM administration of 520 μg PGF 2α (Estron®, Agener União, São Paulo, Brazil), 1 mg estradiol cypionate (Cipiotec®, Agener União, São Paulo, Brazil), and 300 IU equine chorionic gonadotropin (eCG; Sincro eCG®, Ourofino, São Paulo, Brazil). At this time, animals were marked between the sacral tuberosity and the tail head using a paint stick (RAIDEX®, GmbH, Dettingen/Erms, Germany) to assess estrus expression.

On Day 10 (D10), females were classified into two groups based on estrus expression, determined by the degree of paint removal: (1) No Estrus (NO ESTRUS)—paint fully intact or partially removed, and (2) Estrus (ESTRUS)—paint completely removed. Immediately following estrus classification, all females underwent TAI performed by the same technician using semen from three Nelore bulls.

2.4. Natural Service Exposure

On Day 20 (D20), all females were exposed to Nelore bulls at a bull-to-cow ratio of 1:30 for natural service until the end of the breeding season (Day 130). All bulls passed a breeding soundness evaluation prior to the breeding season, in accordance with the minimum standards set by the Brazilian College of Animal Reproduction (CBRA, 2013).

2.5. Pregnancy Diagnosis

Pregnancy following TAI (P/AI) was diagnosed via transrectal ultrasonography (Mindray, DP2200VET, São Paulo, Brazil) on Day 40 (D40). Pregnancies resulting from natural service (P/NS) and the final pregnancy rate for the entire breeding season (P/BS) were assessed on Day 130 (D130). Pregnancies were classified according to fetal age to distinguish between conceptions from TAI and natural service.

2.6. Pregnancy Loss

Pregnancy loss was defined as females that were diagnosed pregnant at D40 (P/AI) but were found non-pregnant at the final pregnancy check on D130.

2.7. Assessment of Treatment Homogeneity

To assess the homogeneity of treatments among categories, body condition score (BCS) and days postpartum (DPP; interval from calving to TAI) were evaluated and compared.

2.8. Statistical Analysis

Statistical analyses were conducted using R software (stats package, 2023). The Shapiro-Wilk test ($P < 0.05$) indicated that BCS and DPP were not normally distributed. Therefore, BCS comparisons among categories were performed using the Kruskal-Wallis test, and DPP comparisons between groups were performed using the Mann-Whitney U test ($P < 0.05$). Data are presented as means \pm standard error.

Binary outcomes—including pregnancy rate after TAI (P/AI), after natural service (P/NS), and at the end of the breeding season (P/BS)—were analyzed using binary logistic regression models with a binomial distribution and a logit link function.

The analysis followed a completely randomized design. The models for pregnancy outcomes included the fixed effects of estrus expression, animal category, and body condition score. However, BCS was removed from some models due to missing data and because the majority of animals with recorded BCS had scores between 2 and 3.

For each response variable, three models were evaluated by stepwise elimination of non-significant variables. The best-fitting model for each response variable was selected based on the lowest corrected Akaike Information Criterion (AICc).

Once the best-fitting models for P/AI, P/NS, and P/BS were selected, the impact of estrus expression was analyzed within each animal category (heifers, primiparous, and multiparous cows).

3. Results

Heifers (BCS = 2.9 ± 0.02) exhibited a significantly higher body condition score (BCS) compared to both multiparous (BCS = 2.8 ± 0.01) and primiparous cows (BCS = 2.7 ± 0.02) ($P \leq 0.01$). Furthermore, multiparous cows had a higher BCS ($P \leq 0.01$) than primiparous cows. Regarding days postpartum (DPP), primiparous cows (DPP = 73.7 ± 2.32) showed a significantly longer postpartum interval compared to multiparous cows (DPP = 67.2 ± 1.05) ($P \leq 0.01$). No significant differences ($P > 0.05$) were observed among reproductive categories for estrus detection rate (EDR), pregnancy rate following timed artificial insemination (P/AI), pregnancy rate following natural service (P/NS), or final pregnancy rate at the end of the breeding season (P/BS) (Table 1).

Table 1. Estrus detection rate (EDR), pregnancy rate to timed artificial insemination (P/AI), pregnancy rate to natural service (P/NS), and final pregnancy rate (P/BS) in Nelore females classified by reproductive category.

CATEGORY	TDE	P/AI	P/NM	P/BS
	% (n/n)	% (n/n)	% (n/n)	% (n/n)
Heifers	72.76 (187/257)	65.36 (168/257)	66.29 (59/89)	88.33 (227/257)
Primiparous	61.59 (85/138)	62.31 (86/138)	61.54 (32/52)	84.05 (116/138) ¹
Multiparous	65.95 (434/658)	60.79 (400/658)	70.15 (181/258)	83.89 (552/658) ²
TOTAL	67.04 (706/1053)	62.11 (654/1053)	68.17 (272/399)	84.99 (895/1053) ³

¹ Pregnancy loss of 1.7% (2/118). ² Pregnancy loss of 4.9% (29/581). ³ Pregnancy loss of 3.3% (31/926).

Regarding the influence of estrus expression on heifer fertility throughout the breeding season, estrus expression had a positive effect on pregnancy rate to timed artificial insemination (P/AI). However, no significant differences were observed for pregnancy rate following natural service (P/NS) or for the final pregnancy rate at the end of the breeding season (P/BS) between heifers that expressed estrus and those that did not (Table 2).

Table 2. Pregnancy rate to timed artificial insemination (P/AI), pregnancy rate following natural service (P/NS), and final pregnancy rate at the end of the breeding season (P/BS) in heifers classified according to estrus expression status.

	EXPERIMENTAL GROUPS		
	WITH ESTRUS	WITHOUT ESTRUS	P-value
P/AI, % (n/n)	71.65 (134/187)	48.57 (34/70)	0.001
P/NM, % (n/n)	64.15 (34/53)	69.44 (25/36)	0.22
P/BS, % (n/n)	89.84 (168/187)	84.28 (59/70)	0.68

Regarding the effect of estrus expression on fertility in primiparous cows, no significant differences were observed between experimental groups for pregnancy rate to timed artificial insemination (P/AI), pregnancy rate following natural mating (P/NM), or final pregnancy rate at the end of the breeding season (P/BS), as presented in Table 3.

Table 3. Pregnancy rates to timed artificial insemination (P/AI), pregnancy rates following natural mating (P/NM), and final pregnancy rates at the end of the breeding season (P/BS) in primiparous cows, stratified by estrus expression status.

	EXPERIMENTAL GROUPS		
	WITH ESTRUS	WITHOUT ESTRUS	P-value
P/AI, % (n/n)	62.35 (53/85)	62.26 (33/53)	0.99
P/NM, % (n/n)	59.37 (19/32)	65.00 (13/20)	0.68
P/BS, % (n/n)	83.53 (71/85) ¹	84.90 (45/53) ²	0.83

¹ Pregnancy loss of 1.38% (1/72). ² Pregnancy loss of 2.17% (1/46).

In multiparous females, those that exhibited estrus demonstrated significantly higher pregnancy rates to timed artificial insemination (P/AI), pregnancy rates following natural mating (P/NM), and final pregnancy rates at the end of the breeding season (P/BS) compared to females that did not exhibit estrus.

Table 4. Pregnancy rates to timed artificial insemination (P/AI), pregnancy rates following natural mating (P/NM), and final pregnancy rates at the end of the breeding season (P/BS) in multiparous females, stratified by estrus expression.

	EXPERIMENTAL GROUPS		
	WITH ESTRUS	WITHOUT ESTRUS	WITH ESTRUS
P/AI, % (n/n)	68.20 (296/434) ^a	46.43 (104/224) ^b	0.001
P/NM, % (n/n)	78.98 (109/138) ^a	60.00 (72/120) ^b	0.004
P/BS, % (n/n)	88.94 (386/434) ^{a1}	74.11 (166/224) ^{b2}	0.001

¹ Pregnancy loss of 4.69% (19/405). ² Pregnancy loss of 5.58% (10/176).

4. Discussion

Concerns regarding reproductive strategies that adequately meet the physiological needs of each reproductive category have been reported since the 1970s. In this context, Carvalho et al. [14], working with Nelore females, observed that primiparous cows exhibit a more severe negative energy balance (NEB) postpartum compared to other categories, which is justified by their higher energy demand [13,15]. Consequently, the lower body condition score (BCS) observed in primiparous cows relative to other categories may be explained by a more severe NEB during the postpartum period [14,16].

No differences were observed in the dominant follicle diameter (DFD) among categories ($P > 0.05$). Similarly, Bottino et al. [17], in their study with beef females, reported an overall DFD of 62.8%, with 64.5% and 61.8% for primiparous and multiparous categories, respectively ($P = 0.26$). However, Silva et al. [18] found that multiparous Nelore cows exhibited a higher DFD than primiparous cows.

Regarding fertility at fixed-time artificial insemination (FTAI) across categories, Marques et al. [19] and Silva et al. [18] reported no difference in pregnancy per insemination (P/AI) among Nelore females of different reproductive categories. In contrast, Simões et al [20] observed a higher P/AI in multiparous cows compared to primiparous cows, attributing this to the more severe NEB experienced by primiparous cows postpartum, which negatively impacts their fertility.

Concerning fertility at rebreeding and at the end of the breeding season, the present study's results corroborate those of Marques et al. [19], who found no differences in fertility between primiparous and multiparous *Bos indicus* cows at season end. Similarly, Andrade et al. [21], in a study involving Zebu females of different reproductive categories, reported pregnancy rates of 70.2% (593/844), 82.4% (526/638), and 84.2% (508/603) for heifers, primiparous, and multiparous cows, respectively.

To the authors' knowledge, this study is the first to evaluate the impact of estrus expression not only on FTAI fertility but also on subsequent estrous cycles throughout the breeding season in females that fail to conceive at AI. Regarding the positive effect of estrus expression on P/AI in heifers, similar results have been reported in the literature [3,22]. Elevated estradiol (E2) concentrations near insemination are associated with increased fertility in cows [7]. Thus, the positive association between conception probability and estrus expression is related to higher E2 concentrations in females exhibiting estrus [9].

E2 influences follicular cells preparing for ovulation and luteinization; it is critical for the resumption of oocyte meiosis; it facilitates sperm transport and adjusts uterine pH, enhancing sperm viability. Consequently, these factors favor successful fertilization [9,10]. Moreover, elevated preovulatory E2 concentrations correlate with increased uterine area [23]. Beef heifers exposed to prolonged and higher E2 concentrations during proestrus exhibit differences in uterine receptivity-related receptor expression compared to heifers exposed to lower E2 exposure periods [24].

Despite the benefit of estrus on heifer fertility at FTAI, no increases were observed in pregnancy rates at rebreeding (P/NM) or at season end (P/BS). In this context, Perry et al. [9], studying beef heifers, observed that 92% of animals already had a corpus luteum (CL) at the start of the experiment. Thus, the lack of estrus influence on P/NM and P/BS in heifers in the present study may be explained by the animals being cyclic at study initiation, with both experimental groups remaining cyclic throughout the breeding season.

In primiparous cows, no statistical differences were observed between experimental groups for P/AI ($P = 1.0$). Contrasting with this study, Abel et al. [25], working with beef primiparous cows, evaluated the effect of different durations of progesterone exposure on FTAI fertility and found that females expressing estrus, regardless of protocol, had higher P/AI.

It is important to highlight that in Abel et al.'s study, animals were treated with equine chorionic gonadotropin (eCG) concurrently with proestrus onset—that is, at progesterone device removal and PGF2 α administration. Numerous studies describe eCG's capacity to stimulate follicular development, increase ovulation rate, enhance CL size and steroidogenic capacity, and raise plasma progesterone during the subsequent luteal phase. This strategy yields more pronounced results in cases of inadequate LH modulation, such as postpartum anestrous beef cows [17,27].

Therefore, the results obtained in the primiparous category may result from the gonadotropic support for final dominant follicle growth promoted by eCG treatment, compensating for the low LH pulsatility in animals not expressing estrus and thereby reducing differences in pregnancy rates after FTAI between experimental groups.

No impact of estrus expression was observed on rebreeding or final season fertility in primiparous cows ($P > 0.05$). Alves et al. [28], working with Zebu primiparous cows subjected to different FTAI protocols, observed that only 23.6% (86/364) presented a CL at the start of the resynchronization protocol, indicating that even after FTAI and ovulation, these animals may return to anestrus.

Thus, the absence of differences in post-FTAI fertility in primiparous cows may be due to their return to anestrus regardless of estrus expression. Consequently, resynchronization protocols may serve as important tools to improve fertility during reproductive programs in beef cattle [2].

In this experiment, estrus positively impacted P/AI in multiparous cows ($P < 0.05$). Studies show that females displaying estrous behavior prior to AI had larger ovulatory follicles and higher E2 concentrations [4,10]. Alves et al. [28], studying multiparous cows undergoing FTAI, observed larger preovulatory follicle diameters and greater fertility in animals that expressed estrus. Similar studies also demonstrated estrus expression influences luteal size and serum progesterone concentrations post-ovulation, suggesting a correlation between estrus expression and luteal morphofunctional parameters [6,29].

Thus, some effects of estrus expression manifest peri-ovulation, while others occur during the luteal phase [7,30]. Experiments by Cooke et al. [4] confirmed that the combination of these effects promotes optimal conditions for maternal recognition of pregnancy.

Regarding increased fertility at rebreeding and at the end of the breeding season in females expressing estrus, to the authors' knowledge, this study is the first to highlight the positive impact of estrus expression not only at FTAI but also in subsequent cycles throughout the reproductive season in multiparous cows failing to conceive at AI.

One advantage of ovulation synchronization protocols is inducing cyclicity in postpartum anestrous cows [7]. However, Alves et al. [28], studying Nelore females, observed that 50% of multiparous cows failing to conceive post-FTAI returned to anestrus. Therefore, understanding mechanisms maintaining cyclicity post-FTAI is critical.

Fertility in cows relates to E2 concentrations during proestrus and progesterone levels during diestrus[31]. Bridges et al. [30], studying beef females, found that animals with E2 concentrations <10 pg/mL had a higher incidence of short estrous cycles in the subsequent cycle compared to cows with preovulatory E2 peaks ≥ 10 pg/mL.

In this context, Mesquista et al. [32], studying Nelore cows undergoing ovulation synchronization, indicated that animals with larger follicle diameters and greater periovulatory E2 production had reduced expression of OXTR and PI3 genes, which are closely linked to luteolysis. Thus, cows with shortened proestrus may experience increased short cycles due to reduced E2 exposure prior to ovulation [33].

Endometrial E2 receptor expression is proportional to circulating E2 concentrations and inversely proportional to circulating progesterone levels, being higher during proestrus and estrus than during the luteal phase [34]. Moreover, elevated E2 during proestrus reduces endometrial oxytocin receptor concentrations and PGF2 α secretion following oxytocin challenge [35].

Hence, E2 appears to regulate the expression of genes involved in extracellular matrix remodeling, glycoprotein secretion, and temporal differentiation of uterine biology [36]. Therefore, the preovulatory endocrine environment likely modulates endometrial gene expression and, consequently, uterine microenvironment receptivity to the conceptus during early gestation, influencing embryonic survival (Cooke et al., 2019).

5. Conclusions

In conclusion, estrus expression during the fixed-time artificial insemination (FTAI) protocol positively influenced fertility in both heifers and multiparous cows. In heifers, estrus expression was associated with an increased pregnancy rate per insemination (P/AI), while in multiparous cows, enhanced fertility was observed not only at FTAI but also in subsequent estrous cycles.

Therefore, estrus detection can serve as a valuable tool for identifying animals with a higher probability of conception and may also function as a reliable predictor of overall reproductive outcomes in multiparous cows throughout the breeding season.

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Data Availability Statement: The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Torres-Júnior, J. R. S.; Penteado, L.; Sales, J. N. S.; Sá Filho, M. F.; Ayres, H.; & Baruselli, P. S. (). A comparison of two different esters of estradiol for the induction of ovulation in an estradiol plus progestin-based timed artificial insemination protocol for suckled *Bos indicus* beef cows. *Ani. Repro. Sci.* **2014**, *151*(1–2), 9–14. <https://doi.org/10.1016/j.anireprosci.2014.09.019>
2. Baruselli, P. S.; Ferreira, R. M.; Sá Filho, M. F.; Bó, G. A. Review: Using artificial insemination v. natural service in beef herds. *Animal*, **2018**, *12*, 45–52. <https://doi.org/10.1017/S175173111800054X>
3. Colazo, M. G.; Whittaker, P.; Macmillan, K.; Bignell, D.; Boender, G.; de Carvalho Guimaraes, R.; Mapletoft, R. J. Evaluation of a modified GnRH-based timed-AI protocol associated with estrus detection in beef heifers inseminated with sex-selected or conventional semen. *Theriogenology*, **2018**, *118*, 90–95. <https://doi.org/10.1016/j.theriogenology.2018.05.037>

4. Cooke, R. F.; Pohler, K. G.; Vasconcelos, J. L. M.; Cerri, R. L. A. Estrous expression during a fixed-time artificial insemination protocol enhances development and interferon-tau messenger RNA expression in conceptuses from *Bos indicus* beef cows. *Animal*, **2019**, *13*(11), 2569–2575. <https://doi.org/10.1017/S1751731119000636>
5. Fernandes, V. S.; Velho, G. dos S.; Santos, M. F. O.; Evangelista, K. A.; Gasperin, B. G.; Pfeifer, L. F. M.; Ferreira, R.; Gonçalves, P. B. D.; Gastal, G. D. A.; Dalto, A. G. C.; Rovani, M. T. Does GnRH treatment at TAI regardless of estrus occurrence increase pregnancy rate in crossbred *Bos taurus* suckled cows? *Ani. Repro.* **2024**, *21*(1). <https://doi.org/10.1590/1984-3143-ar2023-0123>
6. Nogueira, E.; Silva, M. R.; Silva, J. C. B.; Abreu, U. P. G.; Anache, N. A.; Silva, K. C.; Cardoso, C. J. T.; Sutovsky, P.; Rodrigues, W. B. artificial insemination plus heat I: Effect of estrus expression scores on pregnancy of cows subjected to progesterone-estradiol-based protocols. *Animal*, **2019**, *13*(10), 2305–2312. <https://doi.org/10.1017/S1751731119000442>
7. Pereira, M. H. C.; Cappelozza, B. I.; Kloster, A. K. M.; Mamedes, M.; Vasconcelos, J. L. M. Evaluation of pregnancy per artificial insemination of dairy animals detected in estrus during the synchronization protocol and assigned to conventional or sexed semen. *Anim. Reprod. Sci.* **2023**, *257*. <https://doi.org/10.1016/j.anireprosci.2023.107324>
8. Ferraz, P. A.; Loiola, M. V. G.; Rodrigues, A. S.; Lima, M. C. C.; de Bittencourt, T. C. B. dos S. C.; Ribeiro Filho, A. de L. The effect of the intensity of estrus expression on the follicular diameter and fertility of nellore cows managed under a FTAI program. *Cie. Ani. Brasi.* **2017**, *18*(1). <https://doi.org/10.1590/1089-6891v18e-37643>
9. Perry, G. A.; Smith, M. F.; Roberts, A. J.; MacNeil, M. D.; Geary, T. W. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. *J. Animal Science*, **2007**, *85*(3), 684–689. <https://doi.org/10.2527/jas.2006-519>
10. Perry, G. A.; Swanson, O. L.; Larimore, E. L.; Perry, B. L.; Djira, G. D.; Cushman, R. A. Relationship of follicle size and concentrations of estradiol among cows exhibiting or not exhibiting estrus during a fixed-time AI protocol. *Dom. Ani. Endo.* **2014**, *48*(1), 15–20. <https://doi.org/10.1016/j.domaniend.2014.02.001>
11. Pfeifer, L. F. M.; Moreira, E. M.; da Silva, G. M.; de Souza, V. L.; Nunes, V. R. R.; Andrade, J. de S.; Neves, P. M. A.; Ferreira, R. (). Effect of estradiol cypionate on estrus expression and pregnancy in timed artificially inseminated beef cows. *Livest. Sci* **2020**, *231*. <https://doi.org/10.1016/j.livsci.2019.103886>
12. Pugliesi, G.; Rezende, R. G.; de, Silva, J. C. B.; Lopes, E.; Nishimura, T. K.; Baruselli, P. S.; Madureira, E. H.; Binelli, M. Uso da ultrassonografia Doppler em programas de IATF e TETF em bovinos. *Rev. Brasi. de Repro. Ani.* **2017**, *41*(1), 140–150. [http://cbra.org.br/portal/downloads/publicacoes/rbra/v41/n1/p140-150%20\(RB662\).pdf](http://cbra.org.br/portal/downloads/publicacoes/rbra/v41/n1/p140-150%20(RB662).pdf)
13. Ayres, H.; Ferreira, R. M.; Torres-Júnior, J. R. S.; Demétrio, C. G. B.; de Lima, C. G.; Baruselli, P. S. Validation of body condition score as a predictor of subcutaneous fat in Nelore (*Bos indicus*) cows. *Livest. Sci.* **2009**, *123*(2–3), 175–179. <https://doi.org/10.1016/J.LIVSCI.2008.11.004>
14. Carvalho, R. S.; Cooke, R. F.; Cappelozza, B. I.; Peres, R. F. G.; Pohler, K. G.; Vasconcelos, J. L. M. Influence of body condition score and its change after parturition on pregnancy rates to fixed-timed artificial insemination in *Bos indicus* beef cows. *Anim. Reprod. Sci.* **2022**, *243*. <https://doi.org/10.1016/j.anireprosci.2022.107028>
15. Cooke, R. F.; Pohler, K. G.; Vasconcelos, J. L. M.; Cerri, R. L. A. Estrous expression during a fixed-time artificial insemination protocol enhances development and interferon-tau messenger RNA expression in conceptuses from *Bos indicus* beef cows. *Animal* **2019**, *13*(11), 2569–2575. <https://doi.org/10.1017/S1751731119000636>
16. Vedovatto, M.; Leccioli, R. B.; Lima, E. de A.; Rocha, R. F. A. T.; Coelho, R. N.; Moriel, P.; da Silva, L. G.; Ferreira, L. C. L.; da Silva, A. F.; Alves, W. V. R.; de Oliveira, D. M., & Franco, G. L. Impacts of body condition score at beginning of fixed-timed AI protocol and subsequent energy balance on ovarian structures, estrus expression, pregnancy rate and embryo size of *Bos indicus* beef cows. *Livestock Science*, **2022**, *256*. <https://doi.org/10.1016/j.livsci.2022.104823>

17. Bottino, M. P.; Simões, L. M. S.; Silva, L. A. C. L.; Giroto, R. W.; Scanduzzi, L. A.; Massoneto, J. P. M.; Baruselli, P. S.; Souza, J. C.; Sales, J. N. S. Effects of eCG and FSH in timed artificial insemination treatment regimens on estrus expression and pregnancy rates in primiparous and multiparous *Bos indicus* cows. *Anim. Reprod. Sci.* **2021**, *228*. <https://doi.org/10.1016/j.anireprosci.2021.106751>
18. Silva, S. A.; Mondadori, R. G.; Noleto, G. S.; Barbosa, I. P.; Gonçalves, R. L.; Gasperin, B. G.; Rovani, M. T.; Paz, E. F.; Gomes, L. S.; Pfeifer, L. F. M. GnRH34 with or without estradiol cypionate in timed AI in *Bos indicus* beef cows. *Theriogenology*, **2023**, *209*, 134–140. <https://doi.org/10.1016/j.theriogenology.2023.06.026>
19. Marques, M. de O.; Morotti, F.; da Silva, C. B.; Júnior, M. R.; da Silva, R. C. P.; Baruselli, P. S.; Seneda, M. M. Influence of category-heifers, primiparous and multiparous lactating cows-in a large-scale resynchronization fixed-time artificial insemination program. *J. of Vet. Sci.* **2015**, *16*(3), 367–371. <https://doi.org/10.4142/jvs.2015.16.3.367>
20. Simões, L. M. S.; Orlandi, R. E.; Massoneto, J. P. M.; Scanduzzi, L. A.; Freitas, B. G.; Bastos, M. R.; Souza, J. C.; Sales, J. N. S. Exposure to progesterone previous to the protocol of ovulation synchronization increases the follicular diameter and the fertility of suckled *Bos indicus* cows. *Theriogenology*, **2018**, *116*, 28–33. <https://doi.org/10.1016/j.theriogenology.2018.04.031>
21. Andrade, J. P. N.; Monteiro, P. J. L.; Prata, A. B.; Robl, A. J.; Neto, J.; Lippe, B.; Ribeiro, H. S.; Hartman, D.; Sartori, R.; Wiltbank, M. C. Optimizing ReBreed21 II: Fertility and reproductive efficiency in different parities during a shortened breeding season in beef cattle. *Theriogenology*. **2024**, *224*, 41–49. <https://doi.org/10.1016/j.theriogenology.2024.04.019>
22. Rich, J. J.; Northrop, E. J.; Larimore, E. L.; Perry, G. A. Influence of GnRH supplementation at CIDR removal on estrus expression and interval to estrus in beef cattle. *Theriogenology*, **2018**, *119*, 76–79. <https://doi.org/10.1016/j.theriogenology.2018.06.029>
23. Motta, J. C. L.; Madureira, G.; Silva, L. O.; Alves, R. L. O. R.; Silvestri, M.; Drum, J. N.; Consentini, C. E. C.; Prata, A. B.; Pohler, K. G.; Wiltbank, M. C.; Sartori, R. Interactions of circulating estradiol and progesterone on changes in endometrial area and pituitary responsiveness to GnRH. *Bio. Reprod.* **2020**, *103*(3), 643–653. <https://doi.org/10.1093/biolre/iaaa065>
24. Bridges, G. A.; Mussard, M. L.; Pate, J. L.; Ott, T. L.; Hansen, T. R.; Day, M. L. Impact of preovulatory estradiol concentrations on conceptus development and uterine gene expression. *Anim. Reprod. Sci.* **2012**, *133*(1–2), 16–26. <https://doi.org/10.1016/j.anireprosci.2012.06.013>
25. Abel, J. M.; Bishop, B. E.; Thomas, J. M.; Ellersieck, M. R.; Pooch, S. E.; Smith, M. F.; Patterson, D. J. Comparing strategies to synchronize estrus before fixed-time artificial insemination in primiparous 2-year-old beef cows. *Theriogenology*. **2017**, *87*, 306–315. <https://doi.org/10.1016/j.theriogenology.2016.09.010>
26. 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. Effects of eCG are more pronounced in primiparous than multiparous *Bos indicus* cows submitted to a timed artificial insemination protocol. *Theriogenology*, **2016**, *86*(9), 2290–2295. <https://doi.org/10.1016/j.theriogenology.2016.07.023>
27. Alves, R. L. O. R.; Silva, M. A.; Consentini, C. E. C.; Silva, L. O.; Folchini, N. P.; Oliva, A. L.; Prata, A. B.; Gonçalves, J. R. S.; Wiltbank, M. C.; Sartori, R. Hormonal combinations aiming to improve reproductive outcomes of *Bos indicus* cows submitted to estradiol/progesterone-based timed AI protocols. *Theriogenology*. **2021**, *169*, 89–99. <https://doi.org/10.1016/j.theriogenology.2021.04.007>
28. Pereira, M. H. C.; Wiltbank, M. C.; Vasconcelos, J. L. M. Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. *J. Dairy Sci.*, **2016**, *99*(3), 2237–2247. <https://doi.org/10.3168/jds.2015-9903>
29. Bridges, G. A.; Mussard, M. L.; Burke, C. R.; Day, M. L. Influence of the length of proestrus on fertility and endocrine function in female cattle. *Anim. Reprod. Sci.* **2010**, *117* (3–4), 208–215. <https://doi.org/10.1016/J.ANIREPROSCI.2009.05.002>
30. Sales, A.; Cappellozza, B. I.; Vilela, E.; Claro Júnior, I.; Sá Filho, O. G.; Vasconcelos, J. L. M. Effects of estradiol cypionate dosage and body condition on reproductive performance of Nellore cattle synchronized for timed-artificial insemination. *Theriogenology*, **2023**, *210*, 207–213. <https://doi.org/10.1016/j.theriogenology.2023.07.032>

31. Mesquita, F. S.; Pugliesi, G.; Scolari, S. C.; França, M. R.; Ramos, R. S.; Oliveira, M.; Papa, P. C.; Bressan, F. F.; Meirelles, F. v.; Silva, L. A.; Nogueira, G. P.; Membrive, C. M. B.; Binelli, M. Manipulation of the periovulatory sex steroidal milieu affects endometrial but not luteal gene expression in early diestrus Nelore cows. *Theriogenology*, **2014**, *81*(6), 861–869. <https://doi.org/10.1016/j.theriogenology.2013.12.022>
32. Rantala, M. H.; Mutikainen, M.; Schuler, G.; Katila, T.; Taponen, J. Endometrial expression of progesterone, estrogen, and oxytocin receptors and of 20 α -hydroxysteroid dehydrogenase and cyclooxygenase II 2 and 5 days after ovulation in induced short and normal estrous cycles in dairy cows. *Theriogenology*, **2014**, *81*(9), 1181–1188. <https://doi.org/10.1016/j.theriogenology.2014.01.024>
33. Martins, J. P. N.; Wang, D.; Mu, N.; Rossi, G. F.; Martini, A. P.; Martins, V. R.; Pursley, J. R. Level of circulating concentrations of progesterone during ovulatory follicle development affects timing of pregnancy loss in lactating dairy cows. *J. Dairy Sci.* **2018**, *101*(11), 10505–10525. <https://doi.org/10.3168/jds.2018-14410>
34. Robinson, R. S.; Mann, G. E.; Lamming, G. E.; Wathes, D. C. Expression of oxytocin, oestrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows. *Reproduction*, **2001**, *122*(6), 965–979.
35. Shimizu, T.; Krebs, S.; Bauersachs, S.; Blum, H.; Wolf, E.; Miyamoto, A. Actions and interactions of progesterone and estrogen on transcriptome profiles of the bovine endometrium. *Physiological Genomics*, **2010**, *42*, 290–300. <https://doi.org/10.1152/physiolgenomics.00107.2010>.

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