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Reproductive Performance of Bovine Females Subjected to Fixed-Time Artificial Insemination (FTAI)


Desempenho Reprodutivo de Fêmeas Bovinas Submetidas a Inseminação Artificial em Tempo Fixo (IATF)


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Marlon Nardi Molon: Universidade Federal da Fronteira Sul, campus Realeza. PR, Brasil. 

Monica Bertoncelli: Universidade Federal da Fronteira Sul, campus Realeza. PR, Brasil. 

Thais Vasconcelos de Almeida Alves: Universidade Federal da Fronteira Sul, campus Realeza. PR, Brasil. 

Gabriel Luis Werner Kerkhoff: Instituto Federal Farroupilha, campus Frederico Westphalen. RS, Brasil. 

Rangel Fernandes Pacheco: Instituto Federal Farroupilha, campus Frederico Westphalen. RS Brasil. 

Jonatas Cattelan: Universidade Federal da Fronteira Sul, campus Realeza. PR, Brasil. E-mail: jonatas.cattelan@uffs.edu.br 

Abstract

This study aimed to evaluate the performance of Nelore female cattle submitted to the fixed-time artificial insemination (FTAI) protocol. The females were separated into the following categories: heifers, primiparous or multiparous, and by their physiological stage: lactating or non-lactating. All females were subjected to the same hormonal protocol. The data obtained were subjected to logistic regression, and the variables were tested for multicollinearity. The results were interpreted using the odds ratio test. There was a difference in weight between heifers and multiparous cows, with primiparous showing an intermediate result. The pregnancy rate, similar among the categories, was 45.0%, 41.3%, and 50.3% for heifers, primiparous and multiparous, respectively. There was a difference in body condition score for lactating and non-lactating females of 2.81 and 3.16 points, with no difference in pregnancy rates (48.9% and 46.6%). Concerning gonadotropin-releasing hormone (GnRH) use, the pregnancy rate was 34.8%, 32.3% and 43.3% for heifers, primiparous and multiparous, and 32.3% and 38.6% for non-lactating and lactating cows. Every 12 months beyond the average age, the chance of pregnancy increased by 24.1%, and each 0.25-point raise in body condition score increased the probability of pregnancy by 24.5%. The category and occurrence of lactation did not affect the frequency of GnRH use and reproductive indexes in females submitted to FTAI. The increase in body score and age improves the reproductive performance

of female cattle.

Keywords: Nelore Females. Reproductive Indexes. Hormonal Protocol. Reproduction.

Resumo

O trabalho teve como objetivo avaliar o desempenho de fêmeas bovinas submetidas ao protocolo de inseminação artificial em tempo fixo (IATF). As fêmeas foram separadas nas categorias novilhas, primíparas ou múltiparas; e estágio fisiológico lactantes ou não lactantes. Todas as fêmeas foram submetidas ao mesmo protocolo hormonal. Os dados obtidos foram submetidos a regressão logística e as variáveis testadas quanto a presença de multicolinearidade. Os resultados foram interpretados com auxílio de teste de razão de chances. Houve diferença no peso entre novilhas e múltiparas, com comportamento intermediário para as primíparas. A taxa de gestação, similar entre as categorias, foi de 45,0%, 41,3%, e 50,3% para novilhas, primíparas e múltiparas. Houve diferença entre fêmeas lactantes e não lactantes no escore corporal, de 2,81 e 3,16 pontos, sem diferir nas taxas de gestação, que foram 48,9% e 46,6%. Para o uso de hormônio liberador de gonadotrofina (GnRH), a taxa de gestação foi de 34,8%, 32,3% e 43,3% para novilhas, primíparas e múltiparas, e para estágio fisiológico a taxa de gestação foi de 32,3% para não lactantes e 38,6% para lactantes. A cada 12 meses, além da idade média, a chance de prenhez aumentou em 24,1%, e cada aumento em 0,25 pontos no escore corporal elevou em 24,5% a chance de prenhez. A categoria e a ocorrência da lactação não afetam a frequência de uso de GnRH e os índices reprodutivos em fêmeas submetidas à IATF. O incremento no escore corporal e o aumento da idade melhoram o desempenho reprodutivo de fêmeas bovinas.

Palavras-chave: Fêmeas Nelore. Índices Reprodutivos. Protocolo Hormonal. Reprodução.

1 Introduction

Brazil is one of the world's leading cattle producers, with a herd of over 235 million animals (IBGE, 2023). However, the productivity indices of Brazilian livestock farming are still low, reflected in a low utilization rate, mainly influenced by poor reproductive performance. The breeding season coincides with the late winter months, characterized by low temperatures in the southern states of Brazil. During this period, forage availability is still limited in most farms, leading to low reproductive efficiency in the early months of the breeding season.

The low forage supply hinders or delays the return to cyclicity and the females' development, primarily observed through the low body condition score (BCS) in breeding females (Santos *et al.*, 2009). BCS is related to follicular growth and, consequently, fertility (Grecellé *et al.*, 2006). In addition to BCS, factors such as female age, the presence of a calf at foot, calving interval, and the use of hormonal protocols can influence the cattle females' reproductive performance.

According to Bertan, Binelli, and Madureira (2006), the reproductive performance of cattle herds is a critical factor with direct implications for the beef farms profitability. Therefore, it is essential to establish strategies that improve reproductive efficiency. To enhance beef cattle productivity, numerous reproductive biotechnologies have been highlighted, such as the increasing use of hormonal protocols and fixed-time artificial insemination (FTAI) programs (Baruselli *et al.*, 2018). This strategy allows artificial insemination without the need for estrus detection, reducing cattle handling while

synchronizing breeding services, consolidating reproductive management, calving periods, and standardizing calf batches (Bó *et al.*, 2007).

Thus, the present study aimed to evaluate the reproductive efficiency of cattle females subjected to a fixed-time artificial insemination (FTAI) protocol.

2 Material and Methods

The present study was conducted through a retrospective analysis of reproductive data from three beef cattle farms located in southwestern Paraná, which used fixed-time artificial insemination (FTAI) technology during the breeding season. In all farms, Nelore or predominantly Zebu breed females were evaluated.

The females were categorized as follows: primiparous, females with one pregnancy; multiparous, females with two or more pregnancies; or heifers, females mated for the first time. Calved females were also classified based on their physiological stage according to the presence of a suckling calf: lactating cows, with a calf at foot; or non-lactating cows, without a calf at foot. None of the evaluated females remained with a calf at foot for more than 210 days. The females subjected to the FTAI protocol were managed similarly, being kept in pastures predominantly composed of grasses from the *Brachiaria* and *Panicum* genera. All animals received mineral supplementation based on NaCl, provided in troughs, and had access to water from streams, reservoirs, or drinking fountains.

The FTAI management was carried out in different farms between September and December 2021, totaling 530 protocols. The same hormonal protocol was used for all animals. On day 0 (D0), an implant containing 1.0 g of progesterone (SINCROGEST®) was placed along with an intramuscular application of 2.0 mg of estradiol benzoate (SINCRODIOL® 10%). For heifers, third-use implants were used.

On day 8 (D8), the progesterone implant was removed, and 0.5 mg of sodium cloprostenol (SINCROCIO® 2.5%), 1.0 mg of estradiol cypionate for primiparous and multiparous females or 0.5 mg of estradiol cypionate for heifers (SINCROCP® 10%), and 300 IU of equine chorionic gonadotropin (SINCROeCG®) were administered intramuscularly. During the same handling procedure, a marking was applied with a paint stick to the upper tail insertion region to assist in evaluating female mounting acceptance by other females.

On day 10 (D10), insemination was performed using semen from sires obtained from collection centers, all of the Angus breed. For females with intact dorsal paint markings, an intramuscular application of 2.5 mL of a GnRH analog (GONAXAL® 0.00042%) was administered along with artificial insemination.

At the beginning of the FTAI protocol, females were evaluated for body condition score (BCS) using the visual assessment methodology (Houghton *et al.*, 1990). They were classified on a scale from

1.0 to 5.0 points, in 0.25-point intervals, where 1.0 = very thin and 5.0 = very fat. Additionally, 105 animals exposed to the FTAI protocol were weighed using an electronic scale. The animals' age was obtained from farm records, as well as their category (heifers, primiparous, or multiparous). The presence of a calf at foot was also recorded.

The calving interval and protocol initiation were calculated by subtracting the number of days between protocol initiation and the calving date of the respective female. To assess the need for gonadotropin-releasing hormone (GnRH) administration, the presence of intact paint markings on the animal's back was evaluated. If the marking remained intact, the hormone was administered. The percentage of females requiring GnRH administration and the pregnancy rate of those receiving it were assessed. Pregnancy diagnosis was performed through transrectal ultrasound 32 days after artificial insemination.

The response variables for pregnancy probability were represented as 1 for pregnant females and 0 for non-pregnant females, analyzed through logistic regression using the LOGISTIC procedure in SAS (2009). Multicollinearity diagnosis among predictor variables was conducted by analyzing the Pearson correlation matrix and variance inflation factor (VIF), condition index, eigenvalues (λ), and variance proportions associated with each λ (Freund; Littell, 1991).

From this analysis, a set of covariates was selected for model construction, based on the significance of each covariate obtained in the likelihood ratio test. Multiple regression models with linear and quadratic effects, as well as their interactions, were tested using the stepwise method. The probability threshold for both inclusion and retention in the model was set at 0.25 and 0.30, respectively (Hosmer; Lemeshow; Sturdivant, 2013). The best model selection was based on the Hosmer, Lemeshow, and Sturdivant (2013) goodness-of-fit test, with $p > 0.30$.

After model adjustment (estimating the β_i parameters), the significance of the resulting variables was tested to determine whether the independent variables were significantly related to the likelihood of calving. The Wald test and Score test were used to evaluate the quality of the adjusted model and the individual significance of the set of parameters used in the model.

The data were subjected to analysis of variance using the following mathematical model:

$$Y_{ijk} = \mu + A_i + \beta_j + T_k + \epsilon_{ijk}$$

where:

- Y_{ijk} represents the dependent variables;
- μ is the general mean of observations;
- A_i is the farm effect (used as a covariate);
- B_i is the effect of female category;
- T_k is the effect of the cow's physiological stage;

- ϵ_{ij} is the random residual error.

Means were classified using the F-test, and parameters with a significant effect for category were compared using Student's *t*-test, with $\alpha = 0.05$.

$$P_i = \frac{\exp(y_i)}{1 + \exp(y_i)} = \left[1 + \exp(-y_i)\right]^{-1}$$

In Model I, P_i represents the pregnancy diagnosis for the *i*-th cow:

$$y_i = \mu + \beta_1 X_{1i} + \beta_2 X_{2i} + \epsilon_i$$

where:

- μ is a constant;
- X_{1i} is the age of the *i*-th cow;
- X_{2i} is the body condition score of the *i*-th cow;
- ϵ_i is the random error associated with the *i*-th cow.

To interpret the coefficients, the estimated odds ratio was used:

OR = $\exp(\beta_k)$ which represents the ratio between two possible outcomes, i.e., between success (π_j) or failure ($1 - \pi_j$) of pregnancy. The odds ratios were based on the average denominator of the dataset for each model. The unit changes in the regressor variables were 12 months for age and 0.25 points for BCS.

3 Results and Discussion

The weight of the animals among the different categories of females evaluated showed a difference between them (Table 1). Multiparous cows had a weight 24.0% higher than heifers, with primiparous cows showing intermediate behavior. The weight variations observed in this study are related to the development stage and the animals' age, in which adult cows exhibited greater body development and, consequently, higher weight. Freneau *et al.* (2008) observed that 1,012 cows had an average weight of 434 kg, while 1,664 heifers had an average weight of 384 kg, values close to those obtained in this study.

Table 1 - Productive and reproductive performance of Nelore female cattle of different categories subjected to a FTAI protocol

Variables	Category			p-value
	Heifer	Primiparous	Multiparous	
Weight (kg)	294.9B	340.1AB	365.9A	<0.0001
Body condition score (points)	3.17	2.85	2.94	0.9413
Pregnancy rate (%)	45.0	41.3	50.3	0.1419

Means followed by different letters in the row differ by the t-test with $p < 0.05$.

Source: research data.

Weight is a good indicator of animals' productive performance but it does not reflect energy reserves, which would be a better indicator of meeting the female cattle's needs. The body condition score (BCS) of females was 2.98 points, which is considered medium on the 1.0 to 5.0-point scale. This similarity in BCS was reflected in the pregnancy rate, which averaged 45.5% for the female categories. The results of this study are similar to those reported by Hartmann and Machado (2022), who indicated an average pregnancy rate of 54.2% for previously calved cows, with a positive effect of BCS on the pregnancy rate. The average pregnancy rate for multiparous cows was 50.3%, with an average BCS of 2.94 points (Table 1).

In general, primiparous cows showed inferior reproductive performance compared to multiparous cows or heifers, associated with the fact that these females demand energy for lactation and their body development. Grillo *et al.* (2015), in a study with 481 breeding females, observed that primiparous cows had the lowest pregnancy rate at the end of the breeding season due to ongoing growth, the onset of lactation, and dietary restrictions, resulting in lower BCS and hormonal depletion, especially of LH, which is essential for ovulation.

Separating cows by physiological stage - lactating (with calf at foot) and non-lactating (without calf at foot) - showed a difference in BCS (Table 2), where lactating females had an average of 2.81 points, and non-lactating females had 3.16 points. This difference in BCS suggests the mobilization of body reserves in lactating animals to maintain lactation and support their offspring nursing.

Table 2 - Productive and reproductive performance of Nelore female cattle in different physiological stages subjected to a FTAI protocol

Variables	Physiological Stage		p-value
	Non-lactating	Lactating	
Weight (kg)	358.6	368.2	0.4982
Body condition score (points)	3.16	2.81	<0.0001
Pregnancy rate (%)	46.6	48.9	0.4487

Source: research data.

A pregnancy rate of 46.6% was observed for females without calves at foot, while lactating cows had a pregnancy rate of 48.9%. Although the pregnancy rate did not show a significant difference, BCS

was higher for non-lactating females, though both physiological stages had scores close to the medium classification. Hartmann and Machado (2022) demonstrated that BCS directly affects the pregnancy rate, with rates of 62.2% for BCS above 3.1 points, 52.1% for BCS between 2.5 and 3.0 points, and 33.3% for BCS below 2.5 points. The similarity in pregnancy rates between the cows' physiological stages can be partly explained by management practices, where more attention is given to lactating females, designating them the best pastures over non-lactating females. In the past two years, the study region faced long droughts, affecting forage quality and availability and even access to water. Under these conditions, lactating cows, despite having a lower BCS, were prioritized in feeding, suffering less impact from negative energy balance. Meanwhile, non-lactating cows, although recovering their reserves better, had limited recovery due to lower pasture availability.

Negative energy balance occurs when feed intake is insufficient to meet demands, leading to the mobilization of body reserves and a reduction in BCS. In postpartum animals, limited dry matter intake, combined with increased milk production and low forage availability, delays ovulation due to reduced LH hormone, essential for returning to cyclicity and a new pregnancy (Sartori; Guardiero, 2010). There was no difference in GnRH use, with usage frequencies of 22.1% for heifers, 19.1% for primiparous cows, and 18.4% for multiparous cows (Table 3). GnRH, produced by the hypothalamus, stimulates the release of FSH and LH by the pituitary gland. LH, essential for ovulation, reaches its peak in response to increased estrogen produced by the growing follicle. The application of GnRH analogs at insemination promotes the LH surge, enhancing stimulation in the pre-ovulatory follicle (Peixoto *et al.*, 2021). Estrogen also induces estrus, with receptive animals showing higher hormonal levels. Protocols monitor estrus acceptance to identify animals with lower estrus expression and supplement GnRH, optimizing results.

Table 3 - Frequency of GnRH use and reproductive performance with its application in Nelore female cattle of different categories subjected to a FTAI protocol

Variables	Category			p-value
	Heifer	Primiparous	Multiparous	
GnRH use (%)	22.1	19.1	18.4	0.4103
Pregnancy rate with GnRH use (%)	34.8	32.3	43.3	0.2769

Source: research data.

The pregnancy rate of females that received GnRH was 34.8% for heifers, 32.3% for primiparous cows, and 43.3% for multiparous cows, with no differences among the evaluated categories. This result is likely associated with the similarity in body condition score (BCS), as this parameter directly influences the cattle's reproductive performance. According to Sonohata *et al.* (2009), BCS is a determinant of reproductive rates and can vary depending on nutritional and health management practices, as well as the herds' genetic composition. Torres *et al.* (2015) emphasized that visual

assessment of BCS provides support for assisting in the formation of management groups, defining nutritional supplementation strategies, and consequently improving the herds' reproductive indices.

There is a direct relationship between estrus expression and pregnancy rate since animals that exhibit estrus have a larger pre-ovulatory follicle, which will form a corpus luteum of greater diameter, subsequently having a higher capacity for progesterone production, the hormone responsible for pregnancy maintenance. The pre-ovulatory follicle produces estrogen, the hormone responsible for estrus expression. Therefore, when estrus occurs, the pre-ovulatory follicle tends to be larger, leading to greater progesterone production and resulting in higher pregnancy rates (Ribeiro Filho *et al.*, 2013).

Bonato *et al.* (2021) did not observe differences in pregnancy rates among bovine females of different categories (nulliparous, primiparous, and multiparous) subjected to a FTAI protocol with GnRH use when they did not exhibit estrus. Gottschall *et al.* (2012) observed a pregnancy rate of 48.4% in females that did not express estrus and were administered a GnRH analog, similar to the present study, in which 43.3% of multiparous cows were diagnosed as pregnant when artificial insemination was performed after the administration of a GnRH analog. In the same study, females that did not exhibit estrus and did not receive GnRH achieved a pregnancy rate of 33.3%, demonstrating the importance of this hormonal tool in improving pregnancy rates in FTAI programs.

There was no difference between the physiological stages of lactating and non-lactating females, suggesting the necessity of GnRH use (Table 4). Even though BCS was higher for females without a calf at foot (Table 2), this improvement in energy reserves was not sufficient to increase the pregnancy rate, which was 32.3% for non-lactating cows and 38.6% for lactating cows.

Table 4 - Frequency of GnRH use and reproductive performance with its application in Nelore female cattle in different physiological stages subjected to a FTAI protocol

Variables	Physiological Stage		p-value
	Non-lactating	Lactating	
GnRH use (%)	15.3	20.7	0.8510
Pregnancy rate with GnRH use (%)	32.3	38.6	0.6911

Source: research data.

The use of GnRH occurred in 15.3% of cows without a calf at foot and in 20.7% of lactating cows. It is important to highlight that, although there was no difference in the use of GnRH between cows in different physiological stages, the strategy of using FTAI is highly useful in reproductive management with cattle, as it eliminates the need for field observation of estrus acceptance. Additionally, the application of GnRH at the time of artificial insemination proved to be effective in improving the pregnancy rate.

For logistic regression analysis, data from 437 FTAI protocols were used, in which the females had an average age of 69.9 (\pm 30.6) months, a body condition score of 2.92 (\pm 0.51) points, and a

pregnancy rate of 47.37 (\pm 4.99)%. For the logistic regression equation, only the variables that showed significance were considered (Table 5).

Table 5 - Multicollinearity diagnosis among the coefficients included in the pregnancy diagnosis equation

Number	Variance Inflation Factor	Eigenvalue (λ)	Condition Index	Proportion of Variance Decomposition Associated with Eigenvalues	
				Age	BCS
1	0	3.70628	1.00000	0.01086	0.00188
2	1.00646	0.18700	4.45195	0.39906	0.00039387
3	1.00530	0.09429	6.26966	0.56133	0.08911

BCS = Body Condition Score.

Source: Research data.

In "Model I," the effect of cow age and body condition score was included in the logistic regression equation to explain the probability of pregnancy in cows. Based on the observed values for the variance inflation factor, eigenvalue (λ), condition index, and proportion of variance associated with the eigenvalues, no adjustments were made to reduce multicollinearity. According to the Hosmer, Lemeshow, and Sturdivant (2013) statistic, there is no evidence of model misfit for the HLT ($p=0.5666$) (Table 6).

Table 6 - Regressor variables and confidence limits on pregnancy rate

	Estimate	Standard Error	95% CI		p-value	HLT
Intercept	-1.7924	0.19450	-3.2546	-0.3303	0.0163	0.5666
Idade	0.00947	0.00102	0.00115	0.0178	0.0257	
ECC	0.3923	0.05956	-0.0868	0.8714	0.1085	

BCS = Body Condition Score.

Source: Research data.

According to the odds ratio statistic over a 12-month period, in addition to the average age of the herd analyzed in this study, the likelihood of cow pregnancy increased by 24.1%. However, when there was a 12-month reduction relative to the herd's average age, the likelihood of pregnancy decreased by 10.7% (Table 7). Shorten, Morris, and Cullen (2015) observed an increase in the reproductive indices of bovine females as their age rose from two to six years, followed by a decline when their age progressed from seven to 11 years. According to Hafez and Hafez (2004), puberty was mainly influenced by age, breed, and nutrition-related factors such as growth rate and weight. Thus, for the use of reproductive techniques in young bovine females to be effective and yield good reproductive outcomes, their application should be accompanied by dietary improvements, including high-quality forage, supplementation when necessary, and genotypes better adapted to different production environments.

Table 7 - Estimate of the Odds of Each Regressor Variable on Pregnancy Rate

Variables	Estimated Score	Increase	Estimated Score	Reduction
Age	+ 12.0 meses	1.241	- 12.0 meses	0.893
BCS	+ 0.25 pontos	1.245	- 0.25 pontos	0.907

BCS = Body Condition Score.

Source: research data.

In our study, variations in body weight did not explain the variations in the cows' pregnancy rate. This finding is significant as it highlights that other widely studied variables in bovine reproduction, such as cow's age and body condition score (BCS), have a high-impact representation in reproductive performance. These three variables (age, BCS, and body weight) are highly correlated, and when one of them does not limit reproduction, the others become more representative.

Studies that aimed to eliminate the heteroscedasticity effect among independent variables indicate that modeling pregnancy variations remains a major challenge. Some studies have explained variations in cows' pregnancy rates based on age and body weight (Pacheco *et al.*, 2022; Vaz *et al.*, 2023); the interaction between body weight and age (Pacheco *et al.*, 2020); age, weight, and Julian calving date (Pacheco *et al.*, 2020); as well as weight gain intensity (Reis *et al.*, 2023; Vaz *et al.*, 2023) and milk production (Reis *et al.*, 2023).

The positive effect of BCS was reflected in a way that each 0.25-point increase above the herd's average body condition score resulted in a 24.5% increase in the pregnancy likelihood, whereas a 0.25-point decrease in BCS led to a 9.3% reduction in pregnancy likelihood. Torres *et al.* (2015) found a 52.03% pregnancy rate in a group of 5,082 animals with an average body condition score of 3.0 points, subjected to fixed-time artificial insemination (FTAI) protocols. Furthermore, these authors reported that increasing the body condition score by 0.5 points could lead to a 39% increase in pregnancy rates, demonstrating the importance of this indicator for the success of reproductive programs.

According to Sá Filho *et al.* (2011), the bovine females' fertility subjected to FTAI depends on several factors, including nutrition, health, thermal comfort, breed, production system, and follicular diameter. Therefore, to achieve the desired reproductive indices, it is essential to consider factors preceding the selection and implementation of different hormonal protocols, ensuring that the females exposed to reproduction are provided with optimal conditions to express their productive potential.

4 Conclusion

The use of FTAI does not alter the reproductive performance of bovine females across different categories, although adult cows have a higher body weight.

Cows without calves present a better body condition; however, they do not show improved reproductive indices compared to lactating cows.

The category of females and the occurrence of lactation do not affect the frequency of GnRH use or the reproductive indices in females subjected to FTAI, highlighting the effectiveness of the protocol regardless of individual characteristics.

Improvement in body condition score and increasing age enhance the bovine females' reproductive performance.

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