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Identification of parameters that affect the conception rate of precocious Nelore heifers before the start of the breeding season

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ABSTRACT - The objective of this study was to identify parameters, before the start of the breeding season, that affect the conception rate of precocious Nelore heifers through ultrasonographic ovarian evaluation and evaluation of weight and body condition score (BCS). A total of 407 heifers aged 404±69 days (≈14 months old), weighing 267.1±31.2 kg, and with mean BCS of 3.1±0.5, were used. Heifers were subjected to hormone treatment to induce cyclicity and, 30 days later, to a fixed-time artificial insemination (FTAI) program with three managements. Morphometric measurements of ovaries and ovarian structures were performed by transrectal ultrasonographic evaluation, as well as weighing and evaluation of BCS on D −30 and D0. Conception rate was evaluated 30 days after FTAI. The conception rate of heifers did not vary according to ovary diameter, weight, BCS, and age at first calving. However, heifers with the presence of follicle with a diameter > 6 mm at D −30 and D0 had a higher conception rate at the first FTAI. The presence of a dominant follicle greater than 6 mm at D −30 is an efficient complementary criterion to identify 14-month-old Nelore heifers with greater ability to conceive at the first FTAI before the breeding season. Antral follicle count performed on D −30 is not a good tool to predict fertility at the first FTAI, and the weight may not be a limiter to select heifers under good nutritional and health management.

Keywords: conception, heifer, precocity, reproduction

1. Introduction

Reproductive efficiency is crucial to obtain high productivity rates in beef cattle (Santos et al., 2021) and age at first calving is the most reliable parameter in assessing the fertility of heifers (Sá Filho et al., 2011). In general, zebu heifers have reached puberty between 22 and 36 months of age, which is directly reflected in the age at first calving, which ranges from 34 to 35 months in Brazilian herds (Dias et al., 2004; Azevêdo et al., 2006; Bolignon et al., 2007; Eler et al., 2010). In response to the demand for greater production efficiency, reducing age at first calving has been one of the most pursued zootechnical parameters, as it can generate greater profitability to the farmer, as the production cycle becomes shorter, increasing efficiency.

Puberty is given by the first ovulation, which is an indication that heifers have the potential to reproduce (Hafez and Hafez, 2004). However, identifying when the heifer enters puberty can be a challenge, as environmental, behavioral, and physiological factors are involved in a complex way. Factors such as nutrition, age, breed, and genetics influence puberty, mainly as regulators of ovarian hypothalamic-pituitary axis maturation (Perry, 2016). The complexity of the timing of puberty explains why the selection of replacement heifers based only on body weight may not be the most efficient (Santa Cruz et al., 2018).

The most widespread strategy in herds that make selection pressure for precocious females is based on challenging young heifers aged between 11 and 20 months (by artificial insemination [AI], fixed-time artificial insemination [FTAI], or natural breeding) to the breeding season, at the end of which pregnant heifers will be considered precocious, and the others classified as conventional (Vozzi, 2008; Gregianini et al., 2021; Bevilaqua et al., 2022). However, for economic and animal welfare reasons, it would be important to identify precocious heifers before they are subjected to the breeding season.

A practical way to identify this condition within the farm routine is through the evaluation of the ovaries through ultrasound, highlighting the antral follicular count (AFC), as reported by Gregianini et al. (2021) and Bevilaqua et al. (2022). The AFC has become the focus of many studies considering its influence on the reproductive performance of bovine females (Ireland et al., 2008; Ireland et al., 2011; Pontes et al., 2011; Morotti et al., 2015; Morotti et al., 2017; Gheller et al., 2023). A greater probability of conception of Nelore heifers and cows with lower AFC has already been demonstrated (Batista et al., 2014).

Thus, the objective of the present study was to identify parameters, before the start of the breeding season, that affect the conception rate of precocious Nelore heifers through ultrasonographic ovarian evaluation and evaluation of weight and body condition score (BCS).

2. Material and Methods

The study was carried out in accordance with the ethical principles of animal experimentation approved by the Ethics Committee on Animal Use under number 032/2018.

2.1. Location and animals

The study was carried out during the 2018/2019 (Farms 1, 2, and 3) and 2019/2020 (Farm 1) breeding seasons. The climate in the region is tropical, with an annual temperature average of 27 °C, with two distinct periods—rain between October and April and dry season from July to the end of September. A total of 407 heifers aged 404±69 days (≈14 months old) and weighing 267.1±31.2 kg was used. The mean BCS was 3.1±0.5, on a scale of 1 to 5 (Houghton et al., 1990). The heifers had not yet been exposed to the bull, hormone treatment, or AI.

2.2. Characterization of the farms and management system

Farm 1 (n = 230) is located in Terenos, Mato Grosso do Sul (MS) (20°43'48.38" S, 54°58'04.92" W). The heifers were maintained under a grazing system with *Brachiaria* sp. associated with multiple mixture (18% crude protein [CP]), provided at 0.8% of body weight/day. Previously to the breeding season, aiming at inducing puberty, the females received supplementation of 2.28 g/animal/day of melegestrol acetate (MGA® Premix) along with the multiple mixture, for 12 days. When supplementation with MGA® Premix ceased, 1 mg of estradiol cypionate (E.C.P.®, Zoetis, São Paulo, Brazil) was administered intramuscularly (IM).

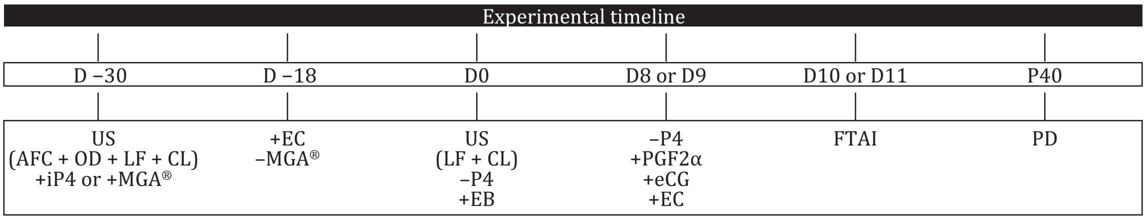
Eighteen days after the end of MGA supplementation, an FTAI protocol with three managements (D0, D9, and D11), using progestogen, estradiol, eCG, and prostaglandin, were carried out. Between 48 and 54 h after removal of the intravaginal progesterone device (P_4 device; DIB 0,5®, Zoetis, São Paulo, Brazil; D11), FTAI was performed.

Farm 2 (n = 24) is located in Anastácio (20°34'44.78" S, 55°57'47.38" W) and Farm 3 (n = 153) in Corguinho, MS (20°00'5131" S, 55°02'35.11" W). On both farms, the heifers were kept under a grazing system on *Brachiaria* sp. + protein mineral supplementation (25% CP), which was provided in the amount of 0.1% of body weight/day.

Before the start of the breeding season, to prepare the animals for reproduction, pre-synchronization management was carried out, with the administration of 150 mg of progesterone IM (Sincrogest®, Ourofino Saúde Animal, São Paulo, Brazil), and after 12 days, 1 mg of estradiol cypionate (E.C.P.®). After 18 days, the FTAI protocol with three managements (D0, D8, and D10) was carried out. Between 48 and 54 h after removal of the P_4 device (D10), FTAI was performed.

In all farms, the animals received water *ad libitum* and all vaccines required in the official health protocol.

The experimental design is described in Figure 1.



US - ultrasound; AFC - antral follicle count; OD - ovarian diameter; LF - follicle > 6 mm; CL - corpus luteum; iP4 - injectable progesterone; MGA - melegestrol acetate; EC - estradiol cypionate; FTAI - fixed-time artificial insemination; PD - pregnancy diagnosis.
Before the start of the breeding season, to prepare the animals for reproduction, pre-synchronization management was carried out (D −30/D −18). After 18 days, the FTAI protocol with three managements were carried out. Between 48 and 54 h after removal of the P4 device, FTAI was performed. Ultrasonographic evaluation was performed on D −30 (AFC + OD + LF + CL) and D0 (LF + CL). Thirty days after FTAI, the pregnancy diagnosis was performed.

Figure 1 - Experimental design.

2.3. Ultrasound of the ovaries and AFC

Morphometric measurements of ovaries and ovarian structures were performed by transrectal ultrasonographic evaluation. An ultrasound equipment coupled to a 7.5-MHz linear transducer (SonoScape® A5 VET) was used to evaluate both ovaries of each female, to identify the presence or absence of corpus luteum (CL) and the presence of the largest antral follicle (LF) > 6 mm, AFC, and measurement of the ovarian diameter. Images of these structures were stored for later measurement of diameters using the image J program (National Institutes of Health, NIH Bethesda). To determine the measurement of the diameter of the LF, CL, and ovary, the largest diameter observed in the images was used. This procedure was carried out twice on D −30 and on D0 on farms 1 and 2, and once on D −30 on Farm 3.

Antral follicle count was performed only once on D −30, and follicles with diameter ≥ 3 mm were counted in both ovaries to characterize AFC per animal. To count the follicles, through transrectal ultrasound, the operator promoted a slow rotation around the ovaries of about 180°, ensuring that all follicles were counted once (Cardoso et al., 2018a).

2.4. Statistical analysis

Data collected at the pre-synchronization and at the beginning of synchronization were evaluated. The fixed effects of the presence or absence of the largest follicle > 6 mm and CL were analyzed according to a model that also considered the linear effects of weight, age, BCS, and AFC as covariates. As just five animals with CL were observed on D −30, this effect was removed from the model when analyzing the data collected at this time.

To evaluate the fixed effects of weight, age, and AFC, three categories were considered, which were divided considering the mean and standard deviation (SD): a group containing the average values minus an SD, a group with average values plus an SD, and a group with the values between the cited groups. So, the categories were divided as follows: weight ≤ 234 (N = 132), > 234 and ≤ 297 (N = 206), > 297 (N = 45); age ≤ 331 (N = 76), > 331 and ≤ 466 (N = 221), > 466 (N = 86); AFC ≤ 9 (N = 28), > 9 and ≤ 36 (N = 314), > 36 (N = 41).

In all analysis, the models included the random effects of farm, year within farm, and batch within farm, and the Satterthwaite approximation for the denominator degrees of freedom was used. The PROC GLIMMIX from SAS (Statistical Analysis System, University version) was used in all statistical analyses. A significance level of 5% was adopted.

3. Results

In the first ultrasound assessment, it was verified that 1.25% (5/401) of the heifers had CL (Table 1). Furthermore, 66% (265/401) of them had a follicle greater than 6 mm. The ovarian diameter was 23.7±3.2 cm and AFC was 22.5±13.1. The heifers aged 404±69 days (≈14 months old), weighed 267.1±31.2 kg, and had BCS of 3.1±0.5. The incidence of CL at D0 was 24% (59/246) and of follicle greater than 6 mm was 72% (177/246).

The conception rate of Nelore heifers with LF was higher than those that did not have it, both at the time of pre-synchronization (P = 0.0312) and synchronization (P = 0.0158) for FTAI (Table 1).

The conception rate of heifers did not vary depending on the diameter of the ovary, AFC, age, weight, and BCS on D −30 (P>0.05). The AFC, age, and weight showed more variability and, therefore, we chose to evaluate them in categories (Figures 2, 3, and 4). However, even so, no significant difference of these parameters was observed on the conception rate at the first FTAI.

Table 1 - Conception rate of Nelore heifers with or without the presence of a follicle larger than > 6 mm (LF) on the first day of cycling induction (D −30) and first day of FTAI protocol (D0).

	Pregnant with LF (%)	Pregnant without LF (%)	P-value
D −30	50.19 (133/265)	36.76% (50/136)	0.0312
D0	50.28 (89/177)	37.68 (26/69)	0.0158

LF - largest follicle.

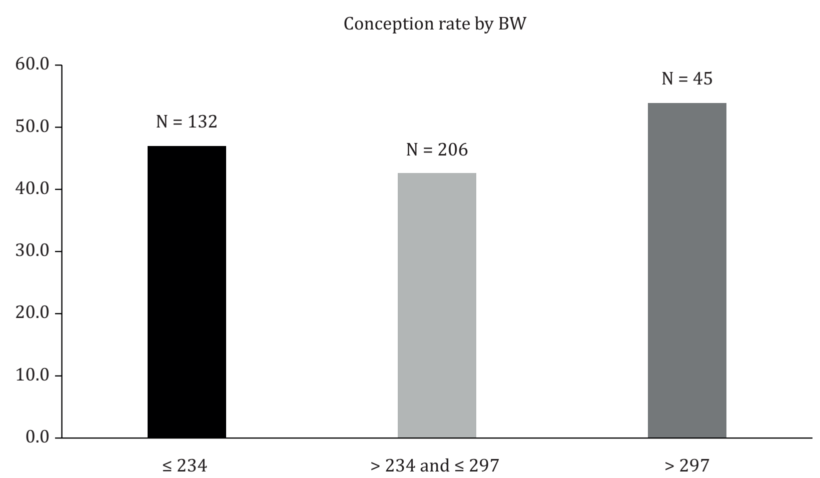


Figure 2 - Conception rate of Nelore heifers with different body weight (BW) ranges (P>0.05) 30 days before the start of the FTAI program (D −30).

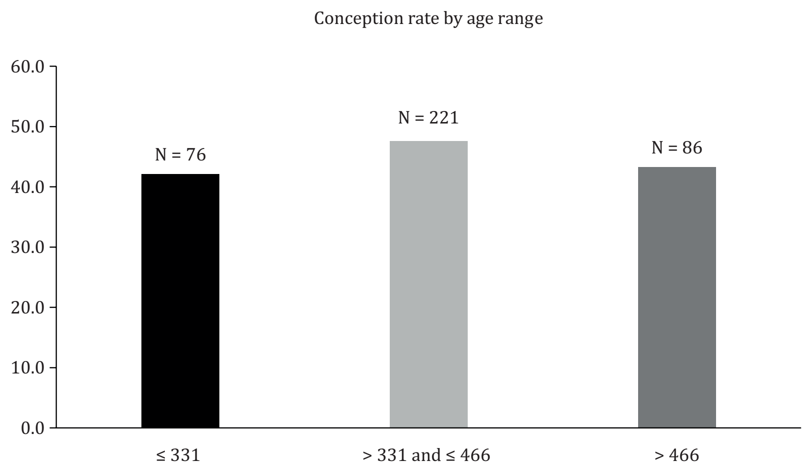
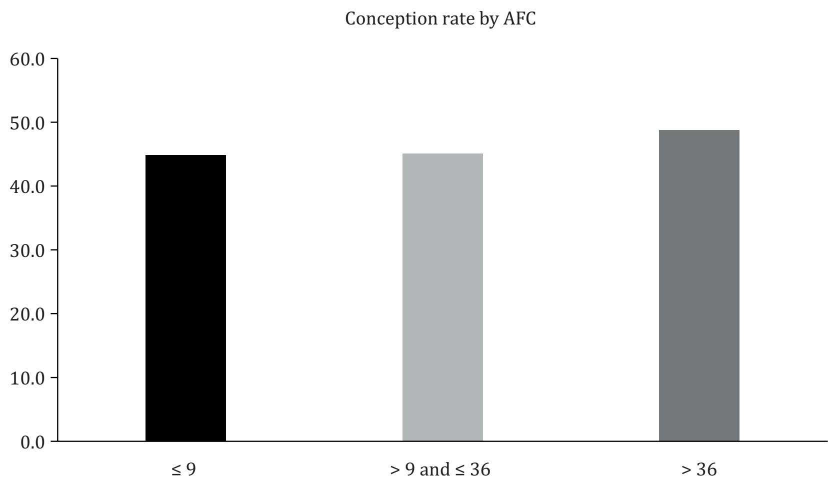


Figure 3 - Conception rate of Nelore heifers with different age ranges (P>0.05) 30 days before the start of the FTAI program (D −30).



Low AFC ≤ 9 (N = 28); intermediate AFC > 9 and ≤ 36 (N = 314); high AFC > 36 (N = 41).

Figure 4 - Conception rate of Nelore heifers with different antral follicle count (AFC) ranges (P>0.05) 30 days before the start of the FTAI program (D −30).

4. Discussion

In the present study, we evaluated three farms that presented its particularities in relation to nutritional, pre-synchronization protocols, and FTAI program. These differences are important for the applicability of the results in a greater variety of management scenarios in the field reality, and therefore were considered in our statistical model.

Our hypothesis was that ovarian parameters, such as the presence of a CL or follicle larger than 6 mm and especially AFC, would be more efficient than weight in predicting the greater probability of conception in precocious Nelore heifers before the start of the breeding season.

The identification of cyclic heifers by visualizing the CL would undoubtedly be the best parameter to indicate precocity and consequently the greater probability of conception. However, it is well known that puberty in zebu heifers occurs later than in taurine heifers, and even with genetic improvement for this trait, few animals become cyclic before the start of the FTAI protocol without prior hormonal treatment (Day and Nogueira, 2013; Lima et al., 2020). So, to identify young heifers (≈14 months old) as pubertal looking for a CL in a single ultrasound evaluation before the start of breeding season is not feasible, as we observed in this study, in which only 1% of the heifers had a CL.

The only feature that had a positive relationship with early conception was the presence of the LF on D −30 and on D0. It is known (Evans et al., 1992) that the presence of an LF is related to ovulation and, consequently, to cycling. However, although the presence of the LF indicates most likely that heifers are pubertal or at a peripubertal period, it is a cyclic structure and cannot be used as an isolated criterion for identifying precocious heifers. But it can be recognized as an important complementary parameter to select the heifers that should enter the breeding season and receive the most valuable semen.

We believed that AFC could predict sexual precocity and conception probability in prepubertal heifers. The hypothesis had been motivated considering previous studies that demonstrated a high repeatability of AFC throughout life (Ireland et al., 2008; Silva-Santos et al., 2014; Morotti, 2017), and because of the relationship of AFC with the likelihood of pregnancy in zebu heifers and cows (Batista et al., 2014; Cardoso et al., 2018b). One of the possibilities that can justify the controversy of the results of the present study with those of Cardoso et al. (2018b) is the fact that AFC assessment was performed in the present study when most of Nelore heifers were probably prepubertal.

In a traditional production system, the minimum weight of a Nelore heifer for reproduction entry is 270 kg (Hess, 2002). Unlike expected, in the present study, there was no relationship of age with early puberty. It is important to note that with genetic improvement focused on sexual precocity, many precocious heifers have become pregnant with age and weight lower than conventional, and this was the case of the farms evaluated in this study. The veterinarians of the farms discarded all heifers with child uterus and/or BCS less than or equal to 2, disregarding the weight and age of contemporaneous heifers.

Dickinson et al. (2019) showed that BCS is one of the main parameters related to the probability of pregnancy in heifers. A study by Moraes et al. (2019), showed that cows with BCS between 2 and 2.9 and lower AFC had higher conception rate. In the present study, no variation of the conception rate was observed as a function of the BCS, probably because of the small variation among BCS of the heifers (3.1±0.5). We emphasize that a good BCS is essential, because, in addition to conceiving, the heifers need to be able to maintain pregnancy, give birth, and conceive in the next breeding season in a moment of high energy demand for its development.

5. Conclusions

The presence of a follicle greater than 6 mm at the D −30 is an efficient complementary criterion to identify 14-month-old Nelore heifers with greater ability to conceive at the first FTAI before the breeding season. The antral follicle count performed on D −30 is not a good tool to predict fertility at the first FTAI of these heifers and the weight may not be a limiter to select young heifers under good nutritional and health management.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Silva, G. C.; Costa-e-Silva, E. V. and Melo-Sterza, F. A. **Data curation:** Melo-Sterza, F. A. **Formal analysis:** Silva, W. A. L.; Fernandes, H. J. and Costa-e-Silva, E. V. **Funding acquisition:** Costa-e-Silva, E. V. and Melo-Sterza, F. A. **Investigation:** Silva, G. C.; Silveira, M. V.; Silva, A. F. and Gheller, J. M. **Methodology:** Silva, G. C. and Silveira, M. V. **Project administration:** Silva, G. C. and

Melo-Sterza, F. A. **Resources:** Costa-e-Silva, E. V. and Melo-Sterza, F. A. **Supervision:** Melo-Sterza, F. A. **Writing – original draft:** Silva, G. C.; Silva, A. F.; Gheller, J. M. and Silva, W. A. L. **Writing – review & editing:** Gheller, J. M.; Fernandes, H. J.; Costa-e-Silva, E. V. and Melo-Sterza, F. A.

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