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Antral follicle count classification selects fertile heifers without influencing the carcass traits

A classificação da contagem folicular antral seleciona novilhas mais férteis sem influenciar as características da carcaça

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Highlights

Cows and young heifers with low antral follicle count have high conception rates.
Antral follicle count (AFC) can be used as a selection tool.
Carcass traits (rib-eye area and fat thickness) and AFC are not correlated.

Abstract

This study aimed to evaluate the ovarian structure, estrus intensity, ultrasound carcass measurements, and pregnancy rate of Nelore breed heifers and cows in accordance with antral follicle counts (AFCs). We evaluated 503 heifers and 565 Nelore cows, with a mean age of 15.5±2.2 and 69.8±36.1 months, respectively, submitted to a fixed-time artificial insemination (FTAI) protocol. On day zero, all bovine females were examined using ultrasound to determine the AFC. The mean AFC of the heifers and cows were 20±8.6 and 22.5±8.4, respectively. The rib-eye area (REA) and fat thickness (FT) of the heifers (n = 119 for REA and n = 219 for FT) were measured using ultrasound imaging. The average conception rates at the first FTAI and at the end of the breeding season were 35.8% and 57.5%, respectively, for heifers and 45.1% and 78.9%, respectively, for cows. We demonstrated that the probability of pregnancy at the first FTAI and at the end of the breeding season for both young heifers and cows increased as the AFC decreased (P>0.001

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and P=0.0123, respectively). FT and REA showed no correlation with AFC in heifers. The intensity of estrus expression was negatively correlated with AFC (−0.46; P<0.0001). In conclusion, Nelore heifers and cows with low AFC had a high probability of pregnancy during the entire breeding season. Thus, AFC can be used as a tool to select heifers with increased fertility without affecting carcass traits (REA and FT).
Key words: Artificial insemination. Carcass. Follicular population. Ovaries. Pregnancy.

Resumo

Este estudo teve como objetivo avaliar as estruturas ovarianas, intensidade do cio, medidas de carcaça por ultrassonografia e taxa de gestação de novilhas e vacas da raça Nelore com diferentes contagens de folículos antrais (CFA). Avaliamos 503 novilhas e 565 vacas Nelore, com idade média de 15,5 ± 2,2 e 69,8 ± 36,1 meses, respectivamente, submetidas a um protocolo de inseminação artificial em tempo fixo (IATF). No dia zero, todas as fêmeas foram examinadas por ultrassom para determinar a CFA. A CFA média das novilhas foi de 20 ± 8,6 e a das vacas foi de 22,5 ± 8,4 folículos. A área de olho de lombo (AOL) e a espessura de gordura (EG) das novilhas (n = 119 para AOL e n = 219 para EG) foram medidas utilizando imagens ultrassonográficas. As taxas médias de concepção na primeira IATF e no final da estação de monta foram de 35,8% e 57,5% e 45,1% e 78,9% para as novilhas e vacas, respectivamente. Demonstramos que a probabilidade de gestação na primeira IATF e no final da estação de monta, tanto para novilhas jovens quanto para vacas, foi maior quando a CFA era menor (P>0,001 e P=0,0123, respectivamente). A espessura da gordura e a AOL não mostraram nenhuma correlação com a CFA. A intensidade da expressão do cio foi negativamente correlacionada com o CFA (-0,46; P < 0,0001). Em conclusão, novilhas jovens e vacas da raça Nelore com menor CFA apresentam maior probabilidade de prenhez durante toda a estação de monta. Nenhuma relação entre a CFA e as características de carcaça foram observadas em novilhas Nelore. A CFA pode ser usada como uma ferramenta de seleção de novilhas com maior fertilidade sem influenciar as características de carcaça (AOL e EG).
Palavras-chave: Inseminação artificial. Ovários. População folicular. Prenhez. Carcaça.

Introduction

Cattle production systems are increasingly seeking efficiency; therefore, efficient animals should be identified (Fontoura et al., 2016). The reproductive efficiency of cows and heifers, including those with high genetic value, may vary significantly; therefore, fertile animal selection might optimize herd productivity (Azevêdo et al., 2005). The efficiency could be increased by applying such criteria to young heifers, particularly when selecting replacement cows (Jimenez-Krassel et al., 2017).

Females that fail to become pregnant during the breeding season cause considerable economic losses for beef cattle farmers. Therefore, only pregnant females should be maintained in the herd after breeding, particularly those conceived at the beginning of the breeding season, to select the most fertile females (Lamb, 2013).

Routinely used reproductive parameters for selecting fertility include the age at first calving, calving interval, probability of pregnancy at 14 months, and ability to stay in the herd. Environmental,

nutritional, and management variables directly influence these parameters (Gressler et al., 2000; Pereira et al., 2000; Eler et al., 2002; Azevêdo et al., 2005). In addition, to obtain this information, it is necessary to wait for the females to grow and have one or more pregnancies, which is time-consuming and does not fulfill the objective of shortening the intergenerational interval.

In this regard, antral follicle count (AFC) has emerged as a simple alternative for selecting high-fertile Bos taurus females (Ireland et al., 2007; Mossa et al., 2012; Martinez et al., 2016) because their high individual repeatability enables AFC-based classification with a single ultrasound examination (Burns et al., 2005; Ireland et al., 2007; J. S. Oliveira et al., 2015). However, its relationship with fertility is opposite to that for Bos indicus (Mendonça et al., 2013; Santos et al., 2016; Morotti et al., 2018).

Although AFC is not considered for selection in breeding programs, it has been widely used as a selection criterion for oocytes and embryo donors. However, little is known about the consequences of this selection on fertility or production characteristics such as fat thickness (FT) and rib-eye area (REA), which have been the targets of selection in the Nelore breed for generations (Caetano et al., 2013).

FT of bovine females is positively related to fertility, unlike other productive traits antagonistic to reproduction (Staples et al., 1998; Ayres et al., 2014). Therefore, we aimed to clarify whether the selection for AFC for high fertility at fixed-time artificial insemination (FTAI) or improved embryo production would not affect carcass characteristics. This study aimed to evaluate carcass traits (REA and FT), estrous intensity and pregnancy rate with relation to AFC. In parallel, we intended to validate AFC as a tool for the selection of high fertile cows and heifers.

Methods

This study was conducted in accordance with the guidelines of the Animal Use Ethics Committee of the State University of Mato Grosso do Sul (approval number: 030/2017).

Study area and animals

This study was carried out during two breeding seasons (2017/2018 and 2018/2019, between October and February) in four farms in mid-western Brazil specializing in young bull and beef cattle production. This region has a tropical climate, with an annual average temperature of 27 °C and two well-marked seasons, with rainfall between October and April and drought from July to end-September.

The breeding seasons of 503 heifers and 565 Nelore cows (B. indicus) were monitored. The first FTAI protocol was started for heifers and cows at 15.5±2.2 and 69.8±36.1 months, respectively. The average weight and mean body condition score [BCS; on a scale of 1 to 5 according to Lowman et al. (1976)] were 292.7±28.1 kg and 3.3±0.4, respectively, for heifers and 432.9±63.1 kg and 3±0.4, respectively, for cows.

FTAI

In all farms, the FTAI protocol was performed using the progesterone intravaginal device, estradiol, prostaglandin, and equine chorionic gonadotropin; however, the protocol specifics and hormone manufacturers were chosen by veterinarians consulted by the farmers. The bovine females were inseminated with the bull semen indicated by the breeding programs 48-52 h after removing the P4 device. After artificial insemination (AI; D40), the pregnancy rate was evaluated by transrectal ultrasound, and the pregnancy diagnosis was repeated at the end of the breeding season, 30 days after bull removal. The conception rate (CR) and pregnancy rate at the end of the breeding season (PR) were calculated as

$$CR = \frac{number\ of\ pregnant\ cows}{number\ of\ inseminated\ cows} \times 100$$

$$PR = \frac{number\ of\ pregnant\ cows\ at\ the\ end\ of\ the\ breeding\ season}{number\ of\ cows\ submitted\ to\ the\ breeding\ season} \times 100$$

CR = number of pregnant cows / numbers of inseminated cows × 100, and the pregnancy rate at the end of the breeding season (PR) was calculated as,

PR = number of pregnant cows at the end of the breeding season / number of cows submitted to the breeding season × 100.

The heat scores (HEATSCs) of 80 cows were evaluated as described by Nogueira et al. (2019) by marking their sacral and tail head regions with ink (Raidl-Maxi; RAIDEX, Germany) soon after withdrawing the P_4 device. Immediately before AI, the animals were classified as HEATSC1 (no or low ink removal: no heat expression), HEATSC2 (partial ink removal: low heat expression), or HEATSC3 (near complete or complete removal of ink: high heat expression).

AFC

To determine the AFC, both ovaries of each female were evaluated on day zero (D0) of the FTAI protocol using an ultrasound device (SonoScape A5 VET) and a 7.5 MHz transrectal transducer for visualizing all antral follicles (≥3 mm) as described by Cardoso et al. (2018). To ensure that all follicles were counted once, the operator rotated the transducer slowly by 180°. The presence of CL was also evaluated. The total AFC of each animal was determined from the number of follicles visualized in each ovary.

REA and subcutaneous FT measurements

At the end of the breeding season, the REA and FT of young heifers (n = 119 for REA and n = 219 for FT) were measured between the 12th and 13th ribs (Yokoo et al., 2009) using ultrasound imaging with the ALOKA 500 V ultrasound device with a 17.2 cm linear probe (3.5 MHz) and a standoff coupler with an image capture system (Blackbox, Biotronics Inc.; Ames, IA, USA), which is a standard procedure for heifers enrolled in breeding programs. These images were later interpreted by the Data Quality Assurance Laboratory (Aval Technology Services S/C) using the Biosoft Toolbox software (Biotronics Inc.).

Statistical analysis

The average AFC, category, pregnancy (conception and final), semen, tail ink removal score (estrous intensity), REA, and FT were obtained by Proc MEANS using SAS (University Edition). Pearson's correlation was used to test all variables using the CORR procedure in SAS. The farm and semen variables were evaluated as covariates.

Analysis of variance (ANOVA) was used to compare the average AFC, conception, and final pregnancy rates between the categories (heifers and cows).

The relationship between AFC and pregnancy probability in a subset of heifers and cows was determined by logistic regression using the PROC LOGISTIC in SAS. The curves were plotted using the coefficients obtained from the interactive data analysis as

$$y = \frac{\exp(x+b)}{[1+exp(x+b)]}$$

For all analyses, P<0.05 was considered statistically significant.

Results and Discussion

The mean AFC for heifers and cows was 20±8.6 (8-64) and 22.5±8.4 (8-57) follicles, respectively (Figure 1) and different between categories (P<0.0001; Table 1). The average CRs and PRs were also higher than those in heifers, which was similar to the results obtained worldwide for beef cattle (Table 1).

The probability of pregnancy at the first FTAI and at the end of the breeding season decreased with increasing AFC (Figure 2), verifying that Nelore cows with low AFC exhibited excellent reproductive performance in FTAI programs, as previously reported (Morotti et al., 2018; Pegorari et al., 2018; F. L. Z. Moraes et al., 2019a). In all these studies, including the present study, approximately 700 heifers and 3,600 cows were evaluated in different regions of Brazil under different management conditions and the same reproductive performance pattern was observed, thus validating this characteristic as a reliable selection tool.

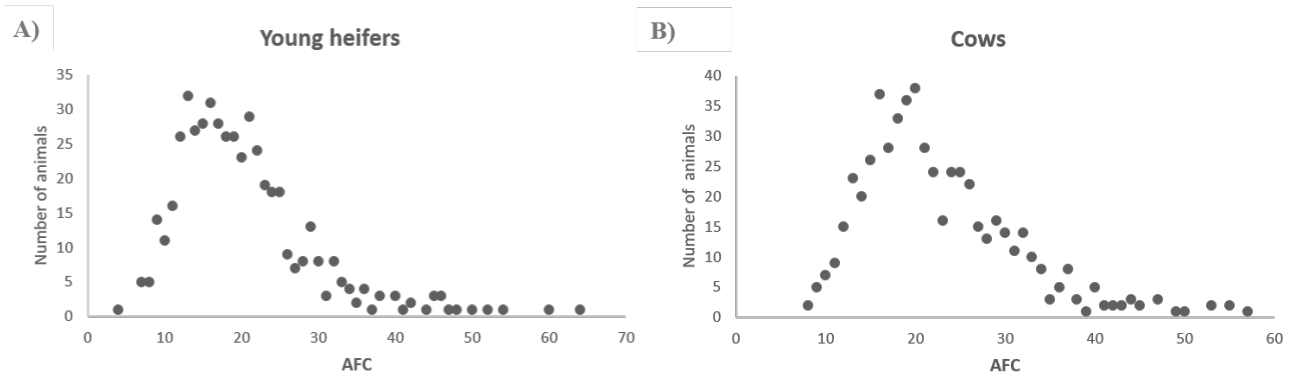

Figure 1. Distribution of total antral follicle count (AFC) in Nelore (A) young heifers and (B) cows in Midwest Brazil.

Table 1
Antral follicle count (AFC) and pregnancy rate of Nelore females after the first fixed-time artificial insemination (FTAI) protocol of the season (conception rate) and at the end of the breeding season (final pregnancy)

Animal category	Animals (*n*)	AFC (Mean ± SE)	Conception rate (% ± SE)	Final pregnancy (% ± SE)
Young heifers	503	20±8.6	35.8±0.5	57.5±0.5
Cows	565	22.5±8.4	45.1±0.5	78.9±0.4
P-value		<0.0001	0.0005	<0.0001

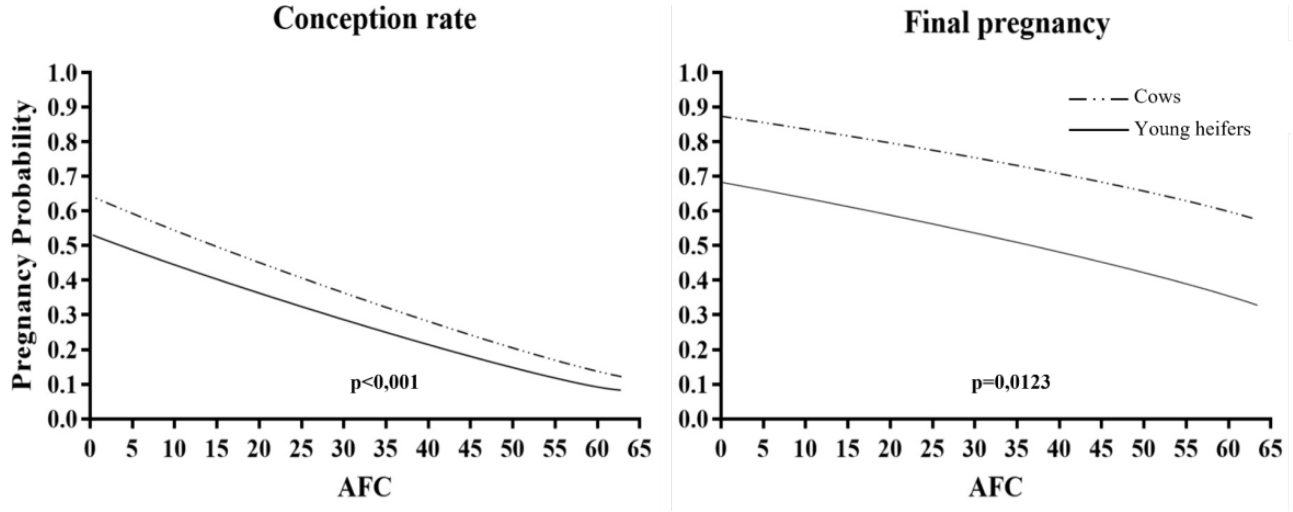

Figure 2. Logistic regression of pregnancy probability in relation to antral follicle count (AFC) for young heifers and multiparous Nelore cows (A) after the first fixed-time artificial insemination (FTAI) protocol of the season (conception rate) and (B) at the end of the breeding season (final pregnancy).

The CR at the first FTAI was high in cows with the CL at the beginning of the protocol (P<0.0001) and high body weight (P=0.0085). However, AFC was not significantly correlated to REA (P=0.1197) or FT (P=0.2235) in heifers (Table 2).

Table 2
Pearson correlations between reproductive and carcass parameters and antral follicle count (AFC) of Nelore young heifers and cows

	Young heifers			Cows		
	Animals (*n*)	*r*	*P-value*	Animals (*n*)	*r*	*P-value*
Estrous intensity *vs* AFC	-	-	-	80	−0.45881	<0.0001
Rib-eye area *vs* AFC	119	0.14343	0.1197	-	-	-
Fat thickness *vs* AFC	219	0.08258	0.2235	-	-	-

P<0.05 was considered significantly different.

Although most reproductive traits exhibit low heritability (Walsh et al., 2014), they are increasingly being used as selection criteria. AFC seems to be an interesting parameter for this purpose, since it exhibits moderate to high heritability in different breeds, such as Holstein cows and heifers (h^2 = 0.31 and 0.25, respectively; Walsh et al., 2014), Nelore (h^2 = 0.49; G. A. Oliveira et al., 2017) and Brangus (h^2 = 0.73; Snelling et al., 2012). This shows the potential of using AFC as a selection trait but also underscores the need for understanding of this trait and its relationship with fertility and productivity.

In both categories, >60% animals had a lower AFC than the average AFC for heifers and cows. The studied farms sought to combine genetic value with reproductive efficiency for over 10 years and rigorously eliminate non-pregnant animals at the end of the breeding season, regardless of their genetic value. Considering the hypothesis that animals with high AFC have low fertility over the years, it is possible that females with low AFC are involuntarily selected.

A recent study has reported that pubertal dairy heifers (11-15 months) with high AFC (≥25 follicles with ≥3 mm diameter) have suboptimal fertility and a shorter productive herd life than heifers with fewer follicles (Jimenez-Krassel et al., 2017). This emphasizes the potential of AFC as a selection criterion for the entire productive life of a cow, which has a considerable economic value. In this study, we demonstrated that AFC could identify bovine females more likely to become pregnant at the beginning of the breeding season, independent of the category. Calves born at the beginning of the birth season are heavy (Alencar et al., 1999), and increasing the number of such animals can increase economic returns. The highest probability of pregnancy at the end of the breeding season was also observed in bovine females with lower AFC independent of category, demonstrating that this tool selects fertile females, even after the breeding with the bull.

REA and FT are parameters used by genetic improvement programs for animal selection in herds because of the improvement of the carcass in the subsequent generations (Abreu et al., 2019). G. F. Moraes et al. (2019b) demonstrated that selecting bulls with adequate backfat thickness negatively correlates with the scrotal circumference measured at 450 days of age; therefore, the authors suggested caution and preselection for reproductive traits to avoid reproductive

impairments in the herd. However, selection for the probability of precocious calving at 24 months of age is not genetically and phenotypically correlated with subcutaneous and rump FT and considered a reliable trait for herd selection (Bonamy et al., 2018). Therefore, we attempted to understand the relationship between AFC and carcass traits.

The carcass traits (REA and FT) of heifers in this study were not related with AFC. An REA study conducted by Morotti et al. (2017) found no significant correlations between AFC and the key characteristics of Brangus females used in the improvement program for beef cattle, similar to the observations of this study on Nelore heifers. Therefore, we can confirm that the AFC is a reliable tool for selection.

In this study, estrus intensity was classified according to the tail ink removal score (Nogueira et al., 2019). We revealed that cows with low AFC had high estrus intensity. The estrus intensity observed by this method is directly proportional to the dominant follicle size, since large follicles might generate high plasma concentrations of estradiol, the hormone responsible for estrus intensity behavior. Morotti et al. (2018) have reported that preovulatory follicle diameter and low AFC are positively related, which might explain our observations. They have also suggested that low competition for hormonal action in low AFC animals increased gonadotropic hormone efficiency. A similar hypothesis was suggested by Burns et al. (2005), who found an inversely proportional relationship between follicle stimulating hormone concentration and the number of follicles (≥3 mm) viewed during the ultrasound examination.

A very consistent analysis carried out in Brazil (Grupo Especializado em Reprodução Aplicada ao Rebanho [GERAR], 2020) showed 52.3% pregnancy rate for beef cattle in the breeding season of 2019 after analyzing more than 1.3 million AIs. They observed that approximately 20% and 17% multiparous zebu cows had >60% and <49% CR, respectively. However, approximately 9% and 49% zebu heifers had >60% and <49% CR, respectively (GERAR, 2020). The pregnancy rate is the result of different choices regarding nutrition, health, genetics, welfare, and reproduction. Each improvement in these areas can significantly impact the results (Grimard et al., 2006; Robinson et al., 2006; Lucy, 2019); therefore, remarkable differences in pregnancy rates can be observed after FTAI. We believe that AFC can help improve reproductive efficiency if used appropriately.

In this study, AFC differed between heifers and cows, which has already been reported for animals of the same breed (J. S. Oliveira et al., 2015). However, this result differs from that reported by Silva-Santos et al. (2014), indicating no difference between the AFC of prepubertal (6-month-old) and 24-month-old Braford individuals. Although these studies exhibited different results, it is difficult to compare them because the breeds and experimental conditions were different. Despite these differences, the relationship between AFC and CR are similar.

In our study, 20% cows with up to 15 follicles had 54.6% pregnancy rate at the first FTAI (Figure 2), which was 20% greater than that in cows with >30 AFC (34.8%). A similar pattern was observed for young heifers with up to 15 follicles, who achieved

a 39.2% pregnancy rate at the first FTAI during the breeding season to which they were challenged (Figure 2). The pregnancy rate was 17.1% greater than that of heifers with >30 AFC. Therefore, we suggest using AFC as an additional tool for selecting heifers for herd replacement or selecting heifers or cows that will be inseminated with the semen from valuable bulls.

Conclusion

We conclude that Nelore cows and heifers (11-15 months) with low AFC have a high probability of pregnancy during the entire breeding season. Thus, AFC can be used as a tool to select heifers with increased fertility for FTAI without affecting carcass traits (REA and FT).

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