PRELIMINARY NOTE

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Low versus high antral follicle count on the fertility of timed AI Nelore heifers

Baixa versus alta contagem de folículos antrais na fertilidade de novilhas Nelore inseminadas em tempo-fixo

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ABSTRACT

This study aimed to determine the effect of antral follicle count (AFC), and pubertal status on the fertility of beef heifers. In this study, 230 Nelore heifers, 20±2 months of age, were subjected to an estradiol progesterone-based timed artificial insemination (TAI) program. On Day 0 of the TAI protocol, the heifers were examined by transrectal ultrasound to record videos of the ovaries. Later, in the darkroom of the laboratory of images, the videos were analyzed for AFC (≥ 3 mm) of each ovary. Females who failed the first TAI were resynchronized with the same hormonal protocol. The pregnancy status was evaluated by ultrasonography 30 days after each FTAI. The general mean of the AFC was 22.0 follicles. Thus, the heifers were divided into 2 groups according to AFC: Low AFC (< 22 follicles, n = 114), and High AFC (≥ 22 follicles, n = 116). No differences (P > 0.05) in the pregnancy per AI (P/AI) were observed between the Low and High AFC groups, and between pubertal and prepubertal categories. The P/AI was not different between heifers that displayed or did not estrus (P = 0.2). However, considering the estrus response of each AFC group, High AFC heifers that displayed estrus had greater P/AI (P = 0.01) than High AFC heifers that did not display estrus. In summary, AFC and pubertal status did not affect the fertility of Nelore heifers. In contrast, the P/AI of heifers that did not display estrus was lower than heifers observed in estrus only in the High AFC group.

Keywords: Bos indicus. Cattle. Ovulation. Pregnancy.

RESUMO

Esse estudo teve como objetivo determinar o efeito da contagem de folículos antrais (CFA) e da maturidade sexual na fertilidade de novilhas de corte. Neste estudo, 230 novilhas Nelore, com 20 ± 2 meses de idade, foram submetidas a um protocolo de inseminação em tempo-fixo (IATF) a base de estradiol e progesterona. No Dia 0 do protocolo de IATF as novilhas foram examinadas por ultrassonografia transretal e vídeos dos ovários foram gravados para posterior CFA (≥ 3 mm) realizada na sala escura do laboratório de imagens. Trinta dias após a ultrassonografia, as fêmeas que falharam na primeira IATF foram ressincronizadas com o mesmo protocolo hormonal. A prenhez foi avaliada por ultrassonografia 30 dias após cada IATF. A média geral da CFA foi de 22 folículos; assim, as novilhas foram divididas em 2 grupos de acordo com a CFA: CFA baixa (< 22 folículos, n=114) e CFA alta (≥ 22 folículos, n=116). A prenhez por IA (P/IA) foi semelhante (P > 0,05) entre os grupos CFA baixa e alta e entre novilhas púberes e pré-púberes. A P/IA não foi diferente entre as novilhas que apresentaram ou não cio (P = 0,2). No entanto, novilhas com CFA alta que apresentaram cio tiveram maior P/IA (P = 0,01) do que novilhas com CFA alta que não apresentaram cio. Em conclusão, a CFA e a maturidade sexual não afetaram a fertilidade de novilhas. Por outro lado, a P/IA das novilhas que apresentaram cio foi maior do que das novilhas não observadas em cio apenas no grupo CFA alta.

Palavras-chave: Bos indicus. Gado de corte. Ovulação. Prenhez.

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Age at puberty determines the age at sexual maturity, and as consequence, determines the age at first calving. In some tropical beef operations, especially those located in the Amazon biome, cattle are raised with a lack of forage quality and availability. Therefore, with such limited quality of feeding resources, B. indicus heifers did not attain puberty before 22 months of age and have their first conception at 36 months of age (Nogueira, 2004). Such aged heifers can compromise the female's reproductive longevity and the economic efficiency of beef operations systems (Melo-Filho & Queiroz, 2011). Therefore, alternatives to the earlier selection of more fertile heifers and the ones that attained puberty earlier may allow producers to increase the profitability of tropical beef operations.

In that regard, the association between the antral follicles count (AFC) and reproductive performance has been studied in females subjected to timed artificial insemination AI (TAI) (Moraes et al., 2019; Morotti et al., 2018; Seneda et al., 2018), and in embryo production programs (Ireland et al., 2007; Silva-Santos et al., 2014). Earlier studies observed that AFC can be determined using a single ultrasound examination because it is repeatable over time in the same individual (Burns et al., 2005; Silva-Santos et al., 2014).

Low AFC in B. taurus cattle has been associated with smaller ovaries, lower embryo production (Ireland et al., 2007; Ireland et al., 2008), and lower pregnancy per AI (P/AI) (Evans et al., 2012; Mossa et al., 2012). Several studies suggested that B. taurus females with high AFC have better reproductive performance than females with low AFC (Ireland et al., 2008; Mossa et al., 2012). Although the positive association between AFC and fertility was observed in B. taurus females, the results observed in B. indicus females subjected to TAI protocols have shown conflicting results (Morotti et al., 2015). Bos indicus females with low AFC subjected to TAI protocols ovulated larger follicles and had greater P/AI (Moraes et al., 2019; Morotti et al., 2018).

Several studies have estimated the relationship between AFC and fertility in B. indicus (Moraes et al., 2019; Morotti et al., 2018; Lima et al., 2020). However, the relationship between AFC, pubertal status, and fertility in heifers has not been deeply investigated. Based on these considerations, this study aimed to compare the fertility between low and high AFC and between pubertal and prepubertal beef heifers subjected to TAI protocols. The hypothesis tested was that Low AFC have greater pregnancy per AI than High AFC heifers.

The experimental procedures of this study were approved by the Animal Use Ethics Committee of the Brazilian Agricultural Research Corporation (Embrapa Rondônia; Protocol 03/2017).

This experiment was performed on a commercial farm located in Ariquemes-RO, Brazil. Nelore heifers (n = 230, B. indicus) from 18 to 22 months of age and 280–320 kg of live weight were enrolled in this experiment. Heifers were maintained in Brachiaria brizantha pasture with free access to water and mineral supplements.

On a random day of the estrus cycle (Day 0), heifers were treated with an intramuscular (IM) injection of 2 mg of estradiol benzoate (EB, Gonadiol®, Zoetis, São Paulo, Brazil), and an intravaginal progesterone-releasing device (IVP, CIDR®, Zoetis, São Paulo, Brazil), previously used for 8 days. On Day 8, heifers were treated with IM 150 μg of D-Cloprostenol (Croniben®, Biogenesis Bagó, Buenos Aires, Argentina), 0.6 mg of estradiol cypionate (ECP®, Zoetis, São Paulo, Brazil), 200 IU of eCG (Novormon®, Zoetis, São Paulo, Brazil) and had the IVP device removed. On Day 8, the cows were painted with color chalk on the tail head. The TAI was performed on Day 10 (48 h after the IVP removal). At TAI the occurrence of estrus was observed based on the chalk removal (no estrus: 100% of chalk remaining; estrus: <25% of chalk remaining).

Timed AI was performed 48 h after IVP removal (D10), with semen from a single bull of proven fertility.

The pregnancy detection was performed by transrectal ultrasonography (Mindray M5; equipped with a 6 MHz linear transducer, Shenzhen, China) 30 days after the first TAI. The non-pregnant heifers were once again hormonally treated with the same TAI protocol previously described (2nd TAI).

On day 0, before the hormonal treatment of the first TAI protocol, ultrasound video clips of the ovaries were recorded and stored for the subsequent antral follicle counting (≥ 3 mm) and measurement of the ovarian area (cm2) according to Oliveira Junior et al. (2022). Later, in the darkroom of the laboratory of images, the cine-loop recordings (uncompressed TIFF sequences) could be viewed at any speed or direction including frame-by-frame viewing.

For the division of the experimental groups, the general mean of the AFC of the heifers was calculated. The general mean of AFC was 22.0. Thus, heifers were separated into two groups, such as 1) heifers with above-average AFC (high AFC, ≥ 22 follicles, n = 116), and 2) heifers with below-average AFC (low AFC, <22 follicles, n = 114).

The ovarian follicular concentration (OFC) was determined by dividing the total AFC (follicles) by the total area (cm2) of both ovaries, according to the following formula: OFC = AFC/area.

During the analysis of the videos, other ovarian structures were recorded, such as the presence of the corpus luteum (CL) or dominant follicle (DF, > 8 mm in diameter). Heifers that showed CL and DF were considered pubertal (n = 77) and those that exhibited neither of these structures were considered prepubertal (n = 153).

A statistical package (SAS 9.1, 2002) was used to perform all statistical analyses. Binomial variables, such as Pregnancy per AI (P/AI) at the first TAI, P/AI at the second TAI, Pregnancy rate (1st TAI + 2nd TAI), and proportion of heifers observed in estrus were analyzed by logistic regression. A chi-square test was used to determine differences in P/AI, at first TAI, according to the expression of estrus. Continuous variables were analyzed by two-way ANOVA to detect the effect of AFC (High vs. Low), category (pubertal vs. pre-pubertal), and their interactions Logistic regression was used to analyze the relationship between AFC and P/AI. In all analyses, significant differences were considered at P <0.05.

The ovarian characteristics according to the AFC (low and high) and category (prepubertal and pubertal) are described in Table 1. Heifers with low AFC showed smaller ovaries (P <0.001) and lower OFC (P <0.001). However, the P/AI was similar (P= 0.88) between heifers with low and high AFC.

Pubertal heifers showed lower AFC (P <0.01) and lower OFC (P <0.01). However, the P/AI was similar (P= 0.42) between prepubertal and pubertal heifers (Table 1).

The proportion of heifers observed in estrus was not different between AFC groups and categories (Table 1). No differences were detected for P/AI at 1st TAI, at 2nd TAI and pregnancy rate (1st + 2nd TAI) between AFC groups and between categories (Table 1).

Disregarding AFC groups and categories, the P/AI was not different (P=0.2) between heifers that displayed estrus (50.3%, 78/155) and heifers that did not display estrus at TAI (41.3%, 31/75). However, considering the estrus response for each AFC group, High AFC heifers that displayed estrus had greater P/AI (P = 0.01) than High AFC heifers that did not display estrus (Figure 1). In contrast, the estrus response did not affect P/AI in the Low AFC group (P = 0.45).

No relationship was observed between AFC and the probability of pregnancy in heifers subjected to TAI (Figure 2).

The hypothesis of this study was not supported. The fertility was not different between Low and High AFC and between prepubertal and pubertal heifers. In contrast, in the High AFC group, the P/AI of heifers that did not display estrus was lower than heifers observed in estrus only. As far as we are aware, this is the first study that demonstrates

Table 1 - Effect of AFC (Low vs. High), and category (pubertal vs. prepubertal) on ovarian responses and fertility in Nelore heifers subjected to TAI protocol

	AFC		Category		P-value		
	Low	High	Prepubertal	Pubertal	AFC	Category	AFC x Category
Number of heifers	114	116	153	77	-	-	-
AFC*	16.28±3.16	28.08±6.3	23.33±8.25	19.94±6.10	<0.001	<0.01	0.04
Ovarian area*, cm^2	6.83±0.13	7.73±0.13	7.29±0.12	7.27±0.16	<0.001	0.69	0.30
Ovarian follicular concentration*, follicles/cm^2	2.46±0.05	3.72±0.08	3.25±0.08	2.78±0.09	<0.001	<0.01	0.34
Proportion of heifers observed in estrus, % (n/n)	70.2 (80/114)	64.6 (75/116)	64.7 (99/153)	72.7 (56/77)	0.44	0.23	0.64
Pregnancy per IA (1^{st} AI), % (n/n)	46.5 (53/114)	48.3 (56/116)	49 (75/153)	44.1 (34/77)	0.28	0.66	0.23
Pregnancy per AI (2^{nd} AI), % (n/n)	47.5 (29/61)	55 (33/60)	50 (39/78)	53.5 (23/43)	0.53	0.65	0.9
Pregnancy rate (1^{st} TAI + 2^{nd} TAI), %, n/n)	71.9 (82/114)	76.7 (89/116)	74.5 (114/153)	74 (57/77)	0.28	0.54	0.44

*Data is presented as average±SE

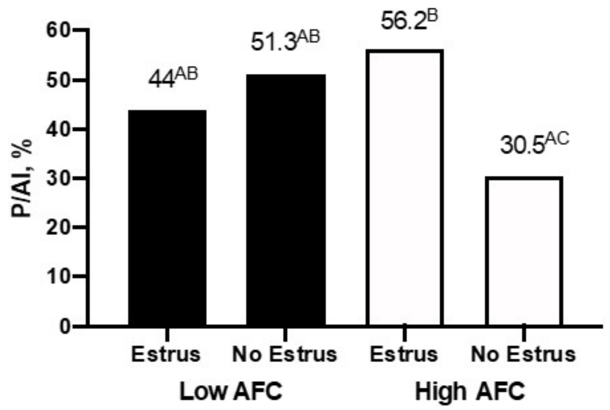

Figure 1 - Pregnancy per AI in Nelore heifers with Low and High AFC, according to the expression of estrus. Different letters indicate the effect between groups ($P < 0.05$).

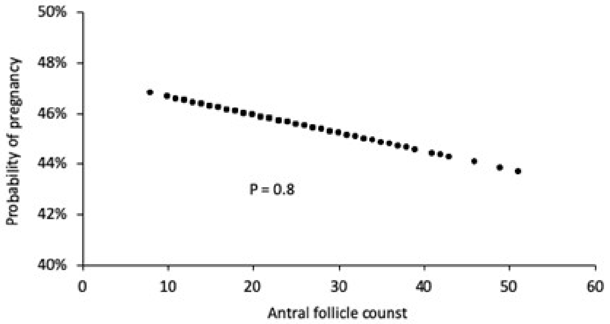

Figure 2 - Relationship between AFC and the probability of pregnancy in beef heifers submitted to the TAI protocol (P = 0.8; probability of pregnancy = e(-0.106*0.003AFC)/1+e(-0.106*0.003AFC)).

that the occurrence of estrus affects only High AFC heifers subjected to TAI programs.

As observed in the present study, Souza et al. (2017) also did not observe a difference in the P/AI in prepubertal and pubertal heifers subjected to estradiol-progesterone-based TAI programs. In contrast, Cunha et al. (2020) observed that heifers with low AFC tended to conceive later than heifers with high AFC. Altogether, these results demonstrate that the relationship between reproductive performance and AFC is still not clear, and only a few studies have been performed with heifers. The Low AFC heifers presented smaller ovaries and lower OFC. Ireland et al. (2008) also observed that females with low AFC have smaller ovaries.

Although the present study did not detect a difference in the P/AI between Low and High AFC heifers, another study performed with Nelore cows observed a negative association between AFC and P/AI (Morotti et al., 2018). In addition, Nelore females with high AFC had lower serum progesterone concentrations after ovulation and lower fertility in TAI programs (Lima et al., 2020). The difference in the cut-off to determine low and high AFC females could be another source of variation in the present study. In previous studies that observed greater P/AI in females with Low AFC, the cut-off used to determine low AFC females was ≤ 10 AFC (Moraes et al., 2019), and < 12 AFC (Morotti et al., 2015). In contrast, in the present study Low AFC heifers had < 22 AFC.

One main concern is that, different from other studies, the present study separated the AFC into only two groups (low and High AFC). In that regard, it is important to mention that the ovarian measurements performed in the present study were determined in laboratory conditions, directly from the videos recorded and stored in the ultrasound machine. Therefore, the operator (LFMP) did not experience the field difficulties inherent with the ultrasonography when the follicles were counted, including operator errors and misinterpretation. In that regard, Broekmans et al. (2010) identified limitations of the AFC in the field, especially those associated with the overcounting of follicles and the ultrasound artifacts, such as the sonolucent effect of a single or two adjacent follicles. Moreover, when the AFC is performed in conventional corrals and concurrently with hormonal injections in TAI programs, the operator may experience a lack of time and darkness enough to evaluate more accurately the ovaries of every single female enrolled in the reproductive program. Therefore, different from other studies in which females were split into 3 tertiles of AFC (low, intermediate, and high AFC), the more accurately AFC performed in the present study encouraged us to split the heifers into only two distinct groups. There is great divergence among the studies on the numerical limits for each AFC group.

In conclusion, fertility was not different between Low and High AFC and between prepubertal and pubertal heifers. In contrast, in the High AFC group, the P/AI of heifers that did not display estrus was lower than heifers observed in estrus. The estrus effect in the fertility of High AFC heifers requires further investigation to verify whether estrus occurrence is more important for High AFC than Low AFC heifers.

Conflict of Interest

The authors have no conflicts of interest in this study.

Ethics Statement

All procedures performed in this experiment were approved by the Committee for Ethics in Animal Experimentation from the Embrapa.

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