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Puberty and parturition on the antral follicle count of female Nelore beef cattle

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Abstract – The objective of this work was to investigate whether puberty and parturition alter the antral follicle count (AFC) in female Nelore (*Bos indicus*) beef cattle. Two experiments were carried out to compare AFC between the prepubertal and pubertal periods and between the prepubertal and postpartum periods. AFC and follicle concentrations did not differ between the prepubertal and pubertal periods. However, the ovarian area increased after puberty. In addition, AFC and the ovarian area were greater in the postpartum than in the prepubertal period. Therefore, AFC is not affected by the puberty of Nelore heifers, but increases significantly when the prepubertal heifers become cows.

Index terms: *Bos indicus*, artificial insemination, ovulation, pregnancy.

Puberdade e parto na contagem de folículos antrais de fêmeas Nelore de corte

Resumo – O objetivo deste trabalho foi avaliar se a puberdade e o parto alteram a contagem de folículos antrais (CFA) em fêmeas Nelore (*Bos indicus*) de corte. Foram realizados dois experimentos para comparar a CFA entre os períodos pré-púbere e púbere e entre os períodos pré-púbere e pós-parto. A CFA e a concentração folicular não diferiram entre os períodos pré-púbere e púbere. Entretanto, a área ovariana foi maior após a puberdade. Além disso, a CFA e a área do ovário foram maiores no período pós-parto quando comparado ao pré-púbere. Portanto, a CFA não é afetada pela puberdade de novilhas Nelore, mas aumenta significativamente quando as novilhas pré-púberes se tornam vacas.

Termos para indexação: *Bos indicus*, inseminação artificial, ovulação, prenhez.

The development of ovarian follicles in cattle occurs in a successive wave pattern beginning with the simultaneous recruitment of a group of follicles of 1.0–3.0 mm in diameter, followed by the selection of a single follicle and the regression of the others, the subordinate follicles (Ginther et al., 1989). The antral follicle count (AFC) in cattle ovaries is a commonly used phenotypic marker of fertility (Ireland et al., 2011; Mossa et al., 2012; Silva-Santos et al., 2014; Santos et al., 2016), allowing of the prediction of productive and reproductive longevity (Ireland et al., 2008; Jimenez-Krassel et al., 2017), as well as of growth and age at puberty (Santa Cruz et al., 2018). Therefore, depending on its classification, AFC is becoming an optimal target of reproductive biotechnologies (Silva-Santos et al., 2014). Ireland et al. (2008) added that antral follicles ≥ 3.0

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mm, observed under a single ultrasonography, can be an important marker for the early selection of female cattle.

The transition of female cattle from prepuberty to full reproductive development requires several body structure and hormonal changes to allow of ovulation, pregnancy, corpus luteum generation, and pregnancy maintenance until parturition. Although studies have already shown that AFC is highly repeatable in the same individual (Burns et al., 2005), there are no known studies on whether AFC remains constant during the different stages of the reproductive life of a same individual.

The objective of this work was to investigate whether puberty and parturition alter the AFC in female Nelore beef cattle.

All experimental procedures were approved by the ethics committee for animal use of Embrapa Rondônia, under protocol number 03/2017.

Two experiments were carried out to compare the AFC between the prepubertal and pubertal periods and between the prepubertal and postpartum periods.

The first experiment was conducted in the municipality of Presidente Médici, in the state of Rondônia, Brazil. Twenty prepubertal Nelore heifers (*B. indicus*, n=20), weighing 265.79±9.8 kg and with 20±1.3 months of age, were used. The heifers were kept on an *Urochloa brizantha* (A.Rich.) R.D.Webster pasture with free access to water and mineral supplementation. On a random day of the estrous cycle, the ovaries of the heifers were examined twice in an 11-day interval through transrectal ultrasonography using the A5V 7 MHz linear transducer (Sonoscape, Shenzhen, China). Since there was no corpus luteum in the ovaries, these females were classified as prepubertal. During the initial ultrasonographic evaluation of the ovaries, AFC, and ovarian area (cm^2) were recorded. Additional ultrasonography exams were performed monthly until all heifers developed a corpus luteum, which was defined as puberty. Once puberty was reached, the female was examined again to determine AFC and ovarian area. The antral follicles were counted as described in Burns et al. (2005) and Ireland et al. (2008). The total area of the ovary included parenchyma, follicles, and corpus luteum. In the prepubertal and the pubertal periods, the antral follicular concentration per ovarian area was determined by dividing the total AFC by the total area (cm^2) of both ovaries, using the following equation: follicular concentration = AFC / ovary area.

The second experiment was carried out in the experimental farm of Embrapa Rondônia, in the municipality of Porto Velho, in the state of Rondônia, Brazil. Thirty prepubertal Nelore heifers (*B. indicus*, n=30), weighing 261.3±5.8 kg and with 6±0.7 months of age, were initially used. The heifers were kept on a *U. brizantha* pasture and had free access to water and mineral supplementation throughout the experimental period. The ovaries of the heifers were examined by transrectal ultrasonography using the M5 6 MHz linear transducer (Mindray, Shenzhen, China), in order to evaluate AFC, measure the ovarian area, and determine if all females were prepubertal as defined in the previous experiment. When the heifers reached a live weight of 300 kg, they were subjected to timed artificial insemination (TAI), using the CIDR intravaginal progesterone-releasing device (Zoetis, São Paulo, SP, Brazil) for eight days and 2.0 mg estradiol benzoate (Gonadiol, Zoetis, São Paulo, SP, Brazil) administered via intramuscular injection. Eight days later, the CIDR device was removed and 0.6 mg estradiol cypionate (ECP, Zoetis, São Paulo, SP, Brazil), 150 µg D-cloprostenol (Croniben, Biogenesis Bagó, Buenos Aires, Argentina), and 200 IU eCG (Novormon, Zoetis, São Paulo, SP, Brazil) were administered via intramuscular injection. Two days after the removal of the progesterone-releasing device, all heifers were inseminated using semen from a single bull with proven fertility. Thirty-five days after TAI, ultrasonography was used to detect pregnancies. The heifers that were not pregnant were inseminated again using the same TAI protocol and semen from the same bull. Only the pregnant and calved heifers were

included in the study, i.e., 25 of the 30 initially used, of which 18 were pregnant after the first TAI and 7, after the second.

Approximately 50 days after parturition (50±8.5 days), additional ultrasonographic examinations were performed to record AFC and measure ovarian area. Ovarian follicular concentration was calculated in the prepubertal and postpartum periods as described in the first experiment.

AFC, ovarian area, and follicular concentration data were normally distributed according to Shapiro-Wilk's test. Homoscedasticity and independence of residuals were checked using Levene's and Durbin-Watson's tests, respectively. Since regression was observed, linearity was checked using a plot of residual vs. fitted values. All the assumptions were met. The data were then analyzed using a paired data design in a linear mixed-effect model. Pairwise comparisons were performed using the corrected least-squares means. To evaluate the association between AFC and ovarian area, the linear regression analysis was performed, for which the statistical model was selected according to the significance of the coefficient of determination. A significance level of 0.05 was assumed.

In the first experiment, pubertal heifers had a larger ovarian area (p=0.0001) than the prepubertal ones (Figure 1 A), which was expected due to an increased ovarian activity and the presence of corpus luteum after the first ovulation. This result is in agreement with those obtained by Silva-Santos et al. (2014) when evaluating crossbreds (*Bos taurus* × *B. indicus*) at weaning from 9 to 24 months of age. The authors classified the heifers into groups with low, medium, and high AFCs, which remained stable throughout the evaluations, confirming that ovarian morphological changes during puberty do not result in an increased AFC. These findings suggest that the AFC in young females is similar before and after puberty.

In the second experiment, the ovarian area of the cows was 76% larger after parturition (p=0.0001) in comparison with prepuberty (Figure 1 C). Likewise, Monteiro et al. (2008), in slaughterhouses, found that the ovaries of cows were larger than those of heifers. Contrastingly, Fernandez et al. (2020) did not observe any differences in the size of the ovaries of females aged 12 to 36 months. Ireland et al. (2011) reported a positive relationship between AFC and ovarian size, as well as number of follicles and oocytes in the ovaries of young adult females. In another study, the heifers classified in the low AFC group had 60% smaller ovaries and a much lower total number of follicles and oocytes than those in the high AFC group (Ireland et al., 2008). In the present study, no reproductive status effect was observed on antral follicular concentrations in both experiments (p>0.05) (Figure 1 B and D).

In the first experiment, the AFC of the heifers did not differ significantly (p=0.56) between the prepubertal and pubertal periods, which showed 19.3±1.8 and 20.35±2.3 units of AFC, respectively (Figure 2 A). However, in the second experiment, a higher AFC was observed after parturition (p<0.001) when compared with the prepubertal period, with 36.36±1.8 and 21.67±1.04 units of AFC (Figure 2 B), respectively.

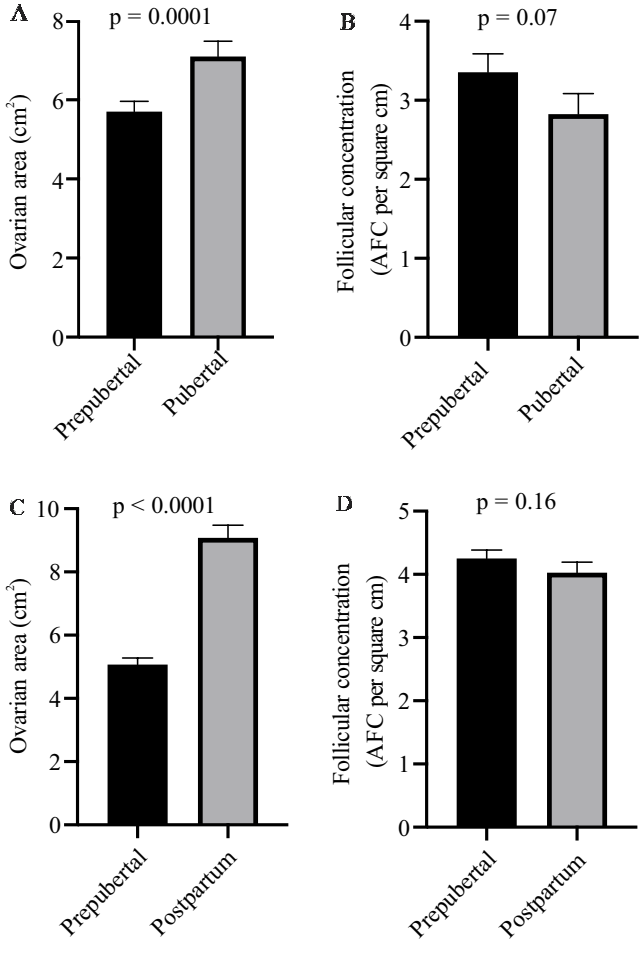

Figure 1. Ovarian area (A) and follicular concentration (B) in heifers in the prepubertal and pubertal periods in the first experiment and ovarian area (C) and follicular concentration (D) in cows in the prepubertal and postpartum periods in the second experiment. AFC, antral follicle count.

Burns et al. (2005) found that AFC varies widely among different females but has a high repeatability in the same individual. In the present study, the same female was compared at different ages (16 months vs. ~34 months), showing an increase of 67% in AFC from the prepubertal to the postpartum period. Unfortunately, the experimental design did not allow of concluding whether this difference between cows and heifers is related to parturition, or to maturity, or to both. In this regard, some studies have shown a positive relationship between AFC and progesterone concentrations in the estrus cycle (Jimenez-Krassel et al., 2009; Ireland et al., 2011). Therefore, it is reasonable to expect that the exposition of the reproductive tract, during pregnancy, to high levels of progesterone for a long period may have some influence on postpartum AFC. The main finding of the present study was that AFC changes dramatically from the prepubertal to the adult phase. Based on these results, prepubertal AFC may not be a good biological marker to predict accurately the AFC of an adult cow as parturition and aging may change its values.

In the first experiment, a positive linear correlation was observed between ovarian area and AFC in the same heifers at prepuberty ($p<0.001$; $R^2=0.49$) and after puberty ($p<0.01$, $R^2=0.5$) (Figure 3 A). Similarly, in the second experiment, there was a positive linear correlation between ovarian area and AFC for the same heifers in the prepubertal ($p<0.0001$, $R^2=0.54$) and postpartum ($p<0.01$, $R^2=0.45$) periods (Figure 3 B). The obtained results, therefore, indicate that puberty increases the size of the ovaries of Nelore heifers but not their AFC and follicular concentration.

While hormonal, metabolic, and nutritional aspects have been identified as important factors

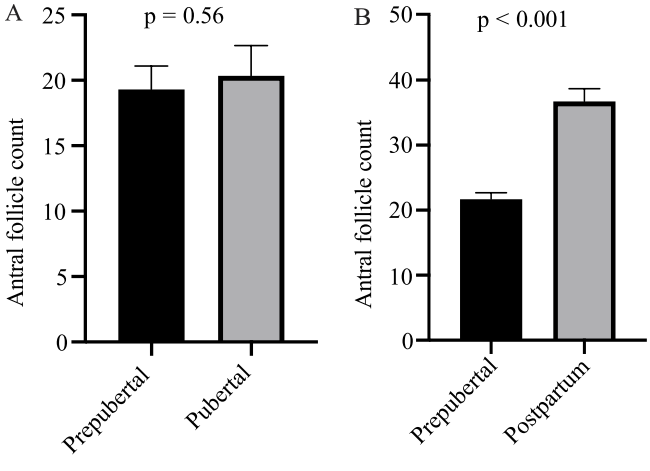

Figure 2. Number of antral follicles in prepubertal heifers that became pubertal (A) and in prepubertal heifers that became cows (B).

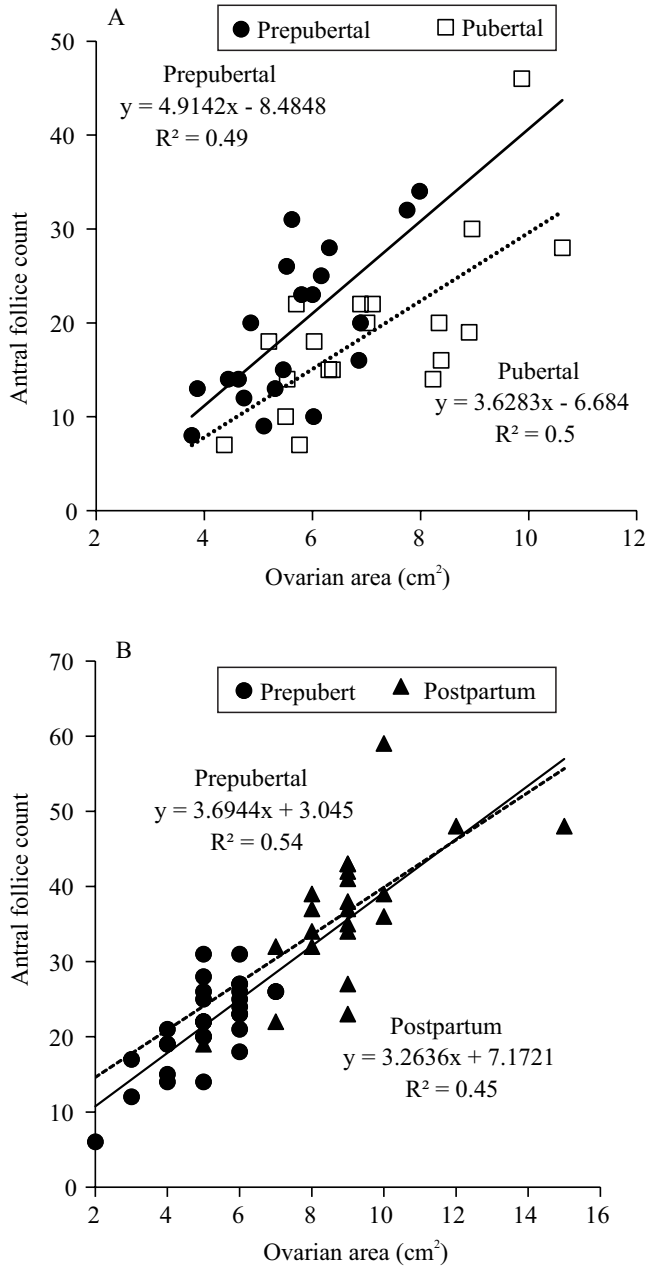

Figure 3. Relationship between ovarian area and number of antral follicles in prepubertal heifers that became pubertal (A) and in prepubertal heifers that became cows (B).

affecting the ovarian follicle population in cattle (Moraes et al., 2019), the relationship between heifer AFC and postpartum timing is poorly understood. Therefore, more comprehensive studies should be conducted to investigate the variation in AFC from the prepubertal to postpartum periods. Although the present work is limited due to the low number of females evaluated and the lack of an extra ultrasound after puberty in the second experiment, the obtained results may be used as a reference for further studies.

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