Research, Society and Development, v. 11, n. 6, e1211628576, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i6.28576

Anticipation of estrus and ovulation in Nelore heifers with low antral follicle count

Antecipação de estro e ovulação em novilhas Nelore com baixa contagem de folículos antrais

Anticipación del estro y ovulación en novillas Nelore con bajo conteo de foliculos antrales

Received: 03/08/2022 | Reviewed: 04/04/2022 | Accept: 04/13/2022 | Published: 04/18/2022

Jair Sábio de Oliveira Junior
ORCID: https://orcid.org/0000-0003-1042-2859
Centro Universitário UNIFACIMED, Brazil
E-mail: jairsabiojr@gmail.com
George Moreira da Silva
ORCID: https://orcid.org/0000-0002-3705-0170
Universidade Federal de Rondônia, Brazil
E-mail: georgmoreiradasilva@gmail.com
Elizângela Mirian Moreira
ORCID: https://orcid.org/0000-0001-7346-6571
Fundação de Amparo à Pesquisa do Estado de Rondônia, Brazil
E-mail: elizangelavet2005@gmail.com
Jéssica de Souza Andrade
ORCID: https://orcid.org/0000-0002-9595-6966
Rede BIONORTE, Brazil
E-mail: jessica_andrade_@hotmail.com
Vanessa Rachele Ribeiro Nunes
ORCID: https://orcid.org/0000-0002-2125-2472
Amazonpec, Brazil
E-mail: vanessarachele45@gmail.com
Fábio Morotti
ORCID: https://orcid.org/0000-0003-4476-6449
Universidade Estadual de Londrina, Brazil
E-mail: fabiomorotti@uel.br
Evelyn Rabelo Andrade
ORCID: https://orcid.org/0000-0003-0393-9884
Universidade Federal de Rondônia, Brazil
E-mail: evelyn.andrade@unir.br
Marcelo Marcondes Seneda
ORCID: https://orcid.org/0000-0002-5097-5119
Universidade Estadual de Londrina, Brazil
E-mail: marcelo.seneda@uel.br
Luiz Francisco Machado Pfeifer
ORCID: https://orcid.org/0000-0003-2069-951X
Empresa Brasileira de Pesquisa Agropecuária, Brazil
E-mail: luiz.pfeifer@embrapa.br

Abstract
This study aimed to establish the relationship between antral follicle count (AFC), the time of puberty, and ovarian response in Nelore heifers subjected to timed-AI (TAI) protocols. Prepubescent Nelore heifers (Bos indicus; n = 30) were examined by transrectal ultrasound and videos of the ovaries were recorded for later CFA (≥ 3 mm). After AFC, heifers were divided into 2 groups, low and high AFC and then further ultrasonic ovarian assessments were performed to determine the onset of puberty. Once puberty was established in all heifers, they were submitted to an FTAI protocol. There were no differences (P > 0.1) between groups on the time of puberty, ovulation rate, the proportion of heifers that expressed estrus, and on preovulatory follicle diameter. However, heifers with low AFC had a smaller ovarian area in the prepubertal period (4.36 ± 0.3 *vs*. 5.65 ± 0.2 cm2) and, in the FTAI, they had the moment of estrus (48 ± 0.0 *vs*. 58.5 ± 3.1 h) and earlier ovulation (60 ± 1.8 *vs*. 72.6 ± 4.7 h) compared to heifers with high AFC (P < 0.05). In addition, heifers in the low AFC group had a higher (P = 0.05) pregnancy per AI (76.9%, 10/13) than the high AFC group (29.4%, 5/17). In this study, heifers with low AFC had the moment of ovulation anticipated and greater pregnancy per AI compared to heifers with high AFC.
Keywords: Antral follicles; Beef; Cattle; Ovulation.

Resumo
O objetivo deste estudo foi estabelecer a relação entre contagem de folículos antrais (CFA), idade à puberdade e resposta ovariana em novilhas Nelore submetidas a protocolos de Inseminação Artificial em Tempo Fixo (IATF).

Novilhas Nelore pré-púberes (Bos indicus; n = 30) foram avaliadas por ultrassonografia transretal e vídeos dos ovários foram gravados para posterior CFA. Após a CFA, as novilhas foram divididas em 2 grupos, CFA baixa e alta e então avaliações ultrassonográficas dos ovários foram realizadas para determinar a puberdade. Uma vez estabelecida a puberdade em todas as novilhas, elas foram submetidas a protocolo de IATF. Não houve diferenças (P > 0,1) entre os grupos na idade à puberdade, na taxa de ovulação, na proporção de novilhas que expressaram estro e no diâmetro do folículo pré-ovulatório. No entanto, novilhas com baixa CFA apresentaram menor área ovariana no período pré-púbere (4,36 ± 0,3 vs. 5,65 ± 0,2 cm^2) e, na IATF, tiveram o momento do estro (48 ± 0,0 x 58,5 ± 3,1 h) e ovulação antecipados (60 ± 1,8 vs. 72,6 ± 4,7 h) em comparação com novilhas com alta CFA (P < 0,05). Além disso, as novilhas do grupo de baixa CFA apresentaram maior (P = 0,05) prenhez por IA (76,9%, 10/13) que o grupo de alta CFA (29,4%, 5/17). Nesse estudo, novilhas com baixa CFA tiveram o momento da ovulação antecipado e maior prenhez por IA em comparação com novilhas com alta CFA.
Palavras-chave: Folículos antrais; Sistemas de corte; Bovinos; Ovulação.

Resumen
El objetivo de este estudio fue establecer la relación entre el conteo de folículos antrales (AFC), el tiempo de pubertad y la respuesta ovárica en novillas Nelore sometidas a protocolos inseminación artificial a tiempo fijo (TAI). Novillas Nelore prepubescentes (Bos indicus; n = 30) fueron examinadas mediante ultrasonido transrectal y se grabaron videos de los ovarios para posterior CFA (≥ 3 mm). Después de AFC, las vaquillas se dividieron en 2 grupos, AFC bajo y entonces se realizaron evaluaciones de ovario para determinar el inicio de la pubertad. Una vez establecida la pubertad, las vaquillas fueron sometidas a un protocolo FTAI. No hubo diferencias (P > 0.1) entre los grupos en el momento de la pubertad, la tasa de ovulación, la proporción de vaquillas que expresaron celo y el diámetro del folículo preovulatorio. Sin embargo, las vaquillas con AFC bajo tuvieron menor área ovárica en el período prepuberal (4,36 ± 0,3 x 5,65 ± 0,2 cm2) y, en el IATF, tuvieron el momento del estro (48 ± 0,0 x 58,5 ± 3,1 h) y ovulación más temprana (60 ± 1,8 x 72,6 ± 4,7 h) en comparación con novillas con AFC alto (P < 0,05). Además, las vaquillas en el grupo de AFC bajo tuvieron una tasa de preñez más alta (P = 0.05) (76.9 %, 10/13) que el grupo de AFC alto (29.4 %, 5/17). Las vaquillas con AFC bajo tuvieron el momento de ovulación anticipado y mayor preñez por IA en comparación con las vaquillas con AFC alto.
Palabras clave: Folículos antrales; Operaciones bovinas; Bovinos; Ovulación.

1. Introduction

The age at which puberty is reached in heifers is considered one of the most relevant factors for the productive life of the future female (Funston et al., 2012). In some tropical beef operations, cattle performance under extensive range management is strongly limited by the availability and quality of feeding resources. Females commonly attain puberty after 22 months of age in these beef operations, with the average age at first conception of approximately 36 months, and the age at first calving of between 44 and 48 months (Nogueira, 2004). Such aged heifers can significantly compromise the female's reproductive longevity in the tropical beef production systems (Melo-Filho & Queiroz, 2011). Thus, the search for phenotypic markers related to puberty may allow producers to select younger, and consequently, more fertile animals that can generate greater profitability for production systems.

In this context, the antral follicles count (AFC) is a reproductive characteristic that has been widely studied due to its influence on the performance of reproductive biotechniques (Silva-Santos et al., 2014; Morotti et al., 2018; Moraes et al., 2019; Seneda et al., 2019; Morotti et al., 2021). Using a simple ultrasound examination, it is possible to determine the number of antral follicles present in the ovaries of females (U-krit et al., 2022), and although this count is highly variable among animals, it is highly repeatable in the same individual (Burns et al., 2005; Silva-Santos et al., 2014; Oliveira Junior et al., 2015; Morotti et al., 2017).

Due to the high variability in the number of antral follicles (follicles ≥ 3 mm), some authors have suggested classifying females in a herd by low, intermediate, and high AFC (Burns et al., 2005; Ireland et al., 2007). Such classification presented strong reliability due to the strong repeatability of the follicular count (0.8 to 0.95) regardless of the season, the number of waves of follicular growth per cycle, age and lactational status (Burns et al., 2005), sexual maturity (Silva-Santos et al., 2014; Morotti et al., 2017; Seneda et al., 2019), and the day of the TAI protocol (Morotti et al., 2018).

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i6.28576

In *B. taurus* cattle, low AFC is associated with a series of characteristics linked to low fertility, such as smaller ovaries, less ovarian follicular reserve, less potential for embryo production (Ireland et al., 2007; Ireland et al., 2008), lower reproductive performance, and lower pregnancy per AI (P/AI) and pregnancy rate at the end of the reproductive season (Evans et al., 2012; Mossa et al., 2012). In contrast, females with high AFC showed opposite characteristics, suggesting that they were positively associated with several important aspects of fertility (Ireland et al., 2008; Ireland et al., 2011; Mossa et al., 2012). However, there are conflicting results when evaluating P/AI in *B. indicus* females subjected to TAI (Morotti et al., 2015; Morotti et al., 2017).

Therefore, AFC has been positively correlated with embryo production, since high AFC has resulted in greater embryo production in both, *B. indicus* and *B. taurus* cows (Santos et al., 2016; Garcia et al., 2020). However, the reproductive performance of *B. indicus* females subjected to TAI has revealed that females with low AFC have been associated with better synchronization of ovulation (Morotti et al., 2018), greater P/AI (Morotti et al., 2018; Moraes et al., 2019), and a better pattern of expression of important genes for oocyte competence and fertility (Lima et al., 2020). In addition, greater reproductive longevity and fertility in reproductive programs have been described in low-AFC Holstein cows (Jimenez-Krassel et al., 2017).

Although some studies have evaluated the relationship between AFC and the fertility of *B. indicus* (Morotti et al., 2018; Moraes et al., 2019; Lima et al., 2020), the correlation between AFC, puberty, and fertility in heifers is still poorly understood. Based on these considerations, this study aimed to establish the relationship between the number of antral follicles, the time of puberty, and the fertility of beef heifers.

2. Methodology

All experimental procedures were approved by the Animal Use Ethics Committee of the Embrapa (Protocol 03/2017).

This study was performed at the experimental research farm of Embrapa (08°48’12” S, 63°50’56” W). Nelore (*B. indicus*) prepubertal heifers, 16 ± 0.7 months old, were kept in a *Brachiaria brizantha* pasture with free access to water and mineral supplements.

At the beginning of the experiment, ultrasound videos of the ovaries were recorded and stored for the subsequent counting of the antral follicles (≥ 3 mm) and measurement of the ovarian area (cm^2). The average AFC was 21.6 ± 5.6 follicles; therefore, the females were divided into two groups: 1) AFC above the average (high AFC, ≥ 22 follicles, n = 17), and 2) AFC below the average (low AFC, <22 follicles, n = 13).

Ultrasound evaluations of the ovaries were performed every 15 days until puberty detection. Before each ultrasound assessment, the heifers were weighed.

After confirmation of puberty of all heifers, they were subjected to a TAI protocol. Heifers received an intravaginal progesterone-releasing device (CIDR®, Zoetis, São Paulo, Brazil), previously used for 8 days, and intramuscular (IM) 2 mg of estradiol benzoate (Gonadiol®, Zoetis, São Paulo, Brazil). Eight days later (D8), the CIDR device was removed and 0.6 mg of estradiol cypionate (ECP®, Zoetis, São Paulo, Brazil), 150 μg of D-Cloprostenol (Croniben®, Biogenesis Bagó, Buenos Aires, Argentina), and 200 IU of eCG (Novormon®, Zoetis, São Paulo, Brazil) were administered (IM). At CIDR removal, heifers received an estrus detector device (Estrotect™, Rockway Inc., Spring Valley, WI, USA) to monitor estrus expression. All heifers were inseminated 48 h after removal of the progesterone device (D10), with semen from a single bull of proven fertility.

The final development of the pre-ovulatory follicle and ovulation were assessed by ultrasonography, from CIDR removal to ovulation at 12-h intervals. Ovulation was defined as the disappearance (from one scanning session to the next) of a

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i6.28576

previously identified follicle greater than or equal to 8 mm in diameter (Martinez et al., 2005). Ultrasound examinations for pregnancy diagnosis were performed 35 days after TAI.

The AFC was determined in both ovaries of each female, based on the visual count of the recorded videos. This counting methodology allowed for a more careful evaluation of the AFC, since the saved videos could be analyzed frame by frame at an appropriate speed, providing greater precision in the identification and counting of antral follicles.

All statistical analyses were performed using the SAS statistical package (SAS 9.1, 2002). Dichotomous variables, such as ovulation, estrus, and P/AI, were analyzed using the chi-square test. Continuous variables were analyzed by one way-ANOVA and the means were compared between groups using the Tukey test. In all analyses, significant differences were considered at P <0.05.

3. Results and Discussion

Ovarian and fertility responses of heifers according to AFC groups are shown in Table 1. Heifers with low AFC showed a smaller area of the ovary in the prepubertal period (P = 0.01).

At TAI heifers with low AFC had the moment of estrus (P <0.01) and ovulation (P = 0.01) anticipated in comparison to high AFC heifers. Similarly, heifers from the low AFC group had greater P/AI (P = 0.05) than those from the high AFC group (Table 1).

Table 1. Ovarian responses and fertility in Nelore heifers with Low (< 22 follicles) and high (≥ 22 follicles) AFC.

	Low AFC n = 13	High AFC n = 17	P-value
Average AFC	16.6 ± 4.3	25.5 ± 2.8	-
Ovarian area,cm^2	4.36 ± 0.3	5.65 ± 0.2	0.01
Moment of estrus detection. h*	48 ± 0.0	58.5 ± 3.1	0.004
Moment of ovulation, h*	60 ± 1.8	72.6 ± 4.7	0.02
Ovulation rate, % (n/n)	92.3 (12/13)	64.7 (11/17)	0.8
Estrus rate, % (n/n)	100 (13/13)	94.1 (16/17)	0.4
Diameter of POF, mm	12.7 ± 0.4	12.2 ± 0.5	0.4
Pregnancy per AI, % (n/n)	76.9 (10/13)	29.4 (5/17)	0.05

* After intravaginal insert removal. Source: Authors (2022).

Age, weight, and average daily gain of heifers at puberty did not differ (P > 0.05) between the low and high AFC groups (Table 2).

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i6.28576

Table 2. Age, live weight, and daily weight gain at puberty in Nelore heifers (n = 30) with Low (< 22 follicles) and high (≥ 22 follicles) AFC.

	Low AFC	High AFC	Valor de P
Age at puberty,m*	27.5 ± 1.2	28.2 ± 1.5	0.5
Weight at puberty, kg	360.3 ± 13.6	372.0 ± 9.6	0.5
Daily weight gain, g	407.6 ± 6.4	422.8 ± 6.4	0.10

*m, months. Source: Authors (2022).

This study presents the reproductive performance of beef heifers (*B. indicus*, Nelore) subjected to TAI according to the number of antral follicles. The results demonstrated, for the first time, that Nelore heifers with low AFC anticipated the moment of estrus and ovulation, as well as a greater P/AI compared to heifers with high AFC, confirming the studies carried out with *B. indicus* cows (Morotti et al., 2018; Lima et al., 2020).

In the present study, AFC did not affect the age at puberty, weight at puberty, or weight gain. These results corroborate the study by Cunha et al. (2020), who also found no differences in age and weight at puberty in heifers with high and low AFC. In contrast to the results observed in this study, heifers with high AFC tended to conceive earlier than heifers with low AFC (Cunha et al., 2020). In addition, Bradford heifers with greater AFC reached puberty earlier (Santa Cruz et al., 2018). These findings indicate that the relationship between puberty, AFC, and fertility differs between studies and requires further investigation before determining whether or not AFC is associated with the reproductive performance of beef heifers.

Although AFC is a widely studied topic in cattle, many findings are still conflicting, and only a limited amount of this research has been conducted on heifers. In addition, it is worth mentioning that, unlike other studies that also evaluated the relationship between AFC and fertility in cattle, in the present study, the AFC (≥ 3 mm) and the measurement of the ovarian area (cm2) were determined precisely in our laboratory from ultrasound images of the ovaries recorded during the experiment. Difficulties inherent in ultrasonography in AFC performed in the field have been reported, including operator errors and misinterpretation. Two of the main limitations are related to determining whether the ovarian sonolucent effect corresponds to a single follicle or two adjacent follicles, and in the re-counting of follicles that have already been identified (Broekmans et al., 2010). In addition, AFC is usually performed during reproductive management (e.g., at the time of application of hormones or TAI). Thus, it is performed quickly and without the ability to go back-and-forth in the cineloops of the video clips to return an accurate AFC.

Retrieving more precise AFCs encouraged us to separate animals into only two experimental groups, unlike other studies with AFC in which females were separated into low, intermediate, and high AFC groups (Santos et al., 2016; Morotti et al., 2018). In general, there is still no consensus on the numerical limits for each group, and there is a great divergence between authors. Some recent studies that evaluated AFC in *B. indicus* females have used the mean and standard deviation of the number of antral follicles to classify the groups. Therefore, the AFC average plus the standard deviation is defined as the high AFC group, the AFC average minus the standard deviation is defined as low AFC, and the intervals between these are considered the limits for the intermediate group (Santos et al., 2016; Morotti et al., 2018). Santos et al. (2016) defined low AFC females with ≤ 10 follicles and high AFC with ³ 25 follicles, and Morotti et al. (2018) defined low AFC cows with ≤15 follicles and high AFC animals with ³ 45 follicles. This variability in the AFC groups is a natural result of the differences in herds and reflects significant fluctuations in the AFC and interpretation of the results.

In this experiment, although heifers with low AFC had an increase in ovulation synchronization and P/AI, there was no difference in the diameter of the POF in comparison with heifers with high AFC. In contrast, other studies that evaluated the relationship between AFC, fertility, and follicular dynamics of Nelore cows, observed that cows with low AFC (≤ 15 follicles) demonstrated a larger follicular diameter in the TAI (13.4 vs. 12.2 mm; Morotti et al., 2018; Bonato et al., 2022). Despite this difference between studies, both reported increased fertility in females with low AFC compared to those with high AFC. In addition, the expression of genes related to cell division and maintenance, follicular growth, steroidogenesis and response to cell stress were positively regulated in oocytes and cumulus cells from Nelore females with low AFC, which also showed higher P4 concentration and greater fertility to TAI (Lima et al., 2020). In general, these studies corroborate the results of this experiment.

In contrast to the results observed in this experiment, earlier studies performed with *B. taurus* females demonstrated that low AFC in this subspecies is associated with several characteristics related to low reproductive performance, such as reduced ovary size (Ireland et al., 2008), low pregnancy rate at the end of the reproductive season (Mossa et al., 2012), reduced responsiveness to superovulatory treatment, lower potential for embryo production, a lower number of viable embryos (Singh et al., 2004; Ireland et al., 2007), lower circulating concentrations of progesterone and anti-Müllerian hormone (Ireland et al., 2011; Evans et al., 2012; Jimenez-Krassel et al., 2015), and reduced endometrial thickness (Jimenez-Krassel et al., 2009). In contrast, females with high AFC have opposite physiological and endocrine characteristics, suggesting a linear correlation between high AFCs and relevant aspects of reproductive fertility in *B. taurus* cattle (Ireland et al., 2011; Evans et al., 2012). Although there is no consensus on fertility in the AFC of animals subjected to the TAI protocol, large-scale embryo production programs have started to use ultrasound evaluation in donors to select females with a greater number of antral follicles, as females with high AFC have a higher number of embryos produced by *B. taurus* (Ireland et al., 2008), crossbreeds (Silva-Santos et al., 2014), and *B. indicus* (Santos et al., 2016) donors.

4. Conclusion

Although the relationship between AFC and fertility in TAI is not entirely clear, our results have shown that the time of estrus and ovulation of heifers with low AFC are anticipated in comparison to those with high AFC. These results may explain why the likelihood of pregnancy of B. indicus heifers with low AFC subjected to TAI programs is greater than heifers with high AFC. However, more studies are necessary to investigate if the results observed in the present study will confirm this hypothesis. Furthermore, future research is needed to elucidate the relationship between AFC and fertility in timed AI Bos indicus beef heifers.

Acknowledgments

The authors are thank to Fundação de Amparo à Pesquisa do Estado de Rondônia (FAPERO; Universal Project 042/2018) for the financial support.

Conflict of interest

The authors have declared no conflict of interest.

References

Bonato, D. V., Ferreira, E. B., Gomes, D. N. Bonato, F. G. C., Droher, R. G., Morotti, F., & Seneda, M. M. (2022). Follicular dynamics, luteal characteristics, and progesterone concentrations in synchronized lactating Holstein cows with high and low antral follicle counts. *Theriogenology*, 179, 223-229. https://doi.org/10.1016/j.theriogenology.2021.12.006 0093-691X

Broekmans, F. J., Ziegler, D., Howles, C. M., Gougeon, A., Trew, G., & Olivennes, F. (2010). The antral follicle count: practical recommendations for better standardization. *Fertil Steril*, *94*(3), 1044-51. http://dx.doi.org/10.1016/j.fertnstert.2009.04.040.

Burns, D. S., Jimenez-Krassel. F., Ireland, J. L. H., Knight, P. G, & Ireland, J. J. (2005). Numbers of antral follicles during follicular waves in cattle: evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum follicle-stimulating hormone concentrations. *Biol Reprod*, *73*(1), 54-62. http://dx.doi.org/ 10.1095/biolreprod.104.036277.

Cunha, F., Cushman, R. A., Santa Cruz, R., De Nava. G., & Viñoles, C. (2020). Antral follicular count has limited impact in the selection of more fertile beef heifers. *Livest Sci*, 241:104230. https://doi.org/10.1016/j.livsci.2020.104230

Evans, A. C. O., Mossa, F., Walsh, S. W., Scheetz, D., Jimenez-Krassel, F., Ireland, J. L. H., Smith, G. W., & Ireland, J. J (2012). Effects of maternal environment during gestation on ovarian folliculogenesis and consequences for fertility in bovine offspring. *Reprod Domest Anim*, *47*(4), 31-37. https://doi.org/ 10.1111/j.1439-0531.2012.02052.x.

Funston, R. N., Martin, J. L., Larson, D. M, & Roberts, A. J. (2012). Physiology and endocrinology symposium: nutritional aspects of developing replacement heifers. *J Anim Sci*, *90*(4):1166-71. https://doi: 10.2527/jas.2011-4569

Garcia, S. M., Morotti, F., Cavalieri, F. L. B., Lunardelli, P. A., Santos, A. O., Membrive, C. M. B., Castilho, C., Puelker, R. Z., Silva, J. O. F., Zangirolamo, A. F., & Seneda, M. M. (2020). Synchronization of stage of follicle development before OPU improves embryo production in cows with large antral follicle counts. *Anim Reprod Sci*, 221:106601. https://doi.org/10.1016/j.anireprosci.2020.106601

Ireland, J. J., Ward, F., Jimenez-Krassel, F., Ireland, J. L. H., Smith, G. W, Lonergan, P., & Evans ACO. (2007). Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation in cattle. *Hum Reprod*, *22*(6), 1687–1695. https://doi.org/10.1093/humrep/dem071

Ireland, J. L. H., Scheetz, D., Jimenez-Krassel, F., Themmen, A. P. N., Ward, F., Lonergan, P., Smith, G. W., Perez, G. I., Evans, A. C. O., & Ireland, J. J. (2008). Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. *Biol Reprod*, *79*(6):1219-25. https://doi.org/10.1095/biolreprod.108.071670.

Ireland, J. J., Smith, G. W., Scheetz, D., Jimenez-Krassel, F., Folger, J. K., Ireland, J. L. H., Mossa, F., Lonergan, P., & Evans, A. C. O. (2011). Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Müllerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. *Reprod Fertil Dev, 23*(1):1-14. https://doi.org/10.1071/RD10226.

Jimenez-Krassel, F., Folger, J. K., Ireland, J. L. H., Smith, G. W., Hou, X., Davis, J. S., Lonergan, P., Evans, A. C. O., & Ireland, J. J. (2009). Evidence That High Variation in Ovarian Reserves of Healthy Young Adults Has a Negative Impact on the Corpus Luteum and Endometrium During Estrous Cycles in Cattle. *Biol Reprod, 80*(6):1272-81. https://doi.org/ 10.1095/biolreprod.108.075093

Jimenez-Krassel, F., Scheetz, D. M., Neuder, L. M., Ireland, J. L. H., Pursley, J. R., Smith, G. W., & Ireland, J. J. (2015). Concentration of anti-Mullerian hormone in dairy heifers is positively associated with productive herd life. *J Dairy Sci*, *98*(05), 3036–45. https ://doi.org/10.3168/jds.2014-8130

Jimenez-Krassel, F., Scheetz, D. M., Neuder, L. M., Pursley, J. R., & Ireland, J. J. (2017). A single ultrasound determination of ≥ 25 follicles ≥ 3 mm in diameter in dairy heifers is predictive of a reduced productive herd life. *J Dairy Sci, 100*(6), 5019–27. https ://doi.org/10.3168/jds.2016-12277

Lima, M. A., Morotti, F., Bayeux, B. M., De Rezende, R. G., Botigelli, R. C., De Bem, T. H. C., Fontes, P. K., Nogueira, M. F. G., Meirelles, F. V., Baruselli, O. S., Da Silveira, J. C., Perecin, F., & Seneda, M. M. (2020). Ovarian follicular dynamics, progesterone concentrations, pregnancy rates and transcriptional patterns in Bos indicus females with a high or low antral follicle count. *Sci Rep-UK, 10*(1), 1–13. https://doi.org/10.1038/s41598-020-76601-5

Martinez, M. F., Kastelic, J. P., Bó, G.A., Caccia, M., & Mapletoft, R. J. (2005). Effects of oestradiol and some of its esters on gonadotrophin release and ovarian follicular dynamics in CIDR-treated beef cattle. *Animal Reproduction Science*, 86(1-2), 37-52. https://doi.org/10.1016/j.anireprosci.2004.06.005.

Melo Filho, G. A., & Queiroz, H. P. (2011). *Gado de corte: o produtor pergunta, a Embrapa responde.* 2 ed. Brasília, DF:Embrapa Informação Tecnológica. 261 p.

Moraes, F. L. Z., Morotti, F., Costa, C. B., Lunardelli, P. A., & Seneda, M. M. (2019). Relationships between antral follicle count, body condition, and pregnancy rates afer timed-AI in Bos indicu cattle. *Theriogenology, 136*(15), 10–14. https://doi.org/10.1016/j.theriogenology.2019.06.024

Morotti, F., Barreiros, T. R. R., Machado, F. Z., Gonzalez, S. M., Marinho, L. S. R., & Seneda, M. M. (2015). Is the number of antral follicles an interesting selection criterium for fertility in cattle? *Anim Reprod*, *12*(3), 479-86.

Morotti, F., Santos, G. M. G., Júnior, C. K., Silva-Santos, K. C., Roso, V. M., & Seneda, M. M. (2017). Correlation between phenotype, genotype and antral follicle population in beef heifers. *Theriogenology*, 91, 21–26. https://doi.org/10.1016/j.theriogenology.2016.12.025

Morotti, F., Moretti, R., Dos Santos, G. M. G., Silva-Santos, K. C., Ramos, C. P. H., & Seneda, M. M. (2018). Ovarian follicular dynamics and conception rate in Bos indicus cows with different antral follicle counts subjected to timed artificial insemination. *Anim Reprod Sci*, 188, 170–177. https://doi.org/10.1016/j.anireprosci.2017.12.001

Morotti, F., Camargo, H. S., Silva, L. O. L., Lorenzetti, E., Zangirolamo, A. F., & Seneda**,** M. M. (2021). Contagem de folículos antrais: relação com a eficiência das biotécnicas e fertilidade. *Revista Brasileira de Reprodução Animal*, 45(4), 202-209. https://doi.org/10.21451/1809-3000.RBRA2021.026

Mossa, F., Walsh, S. W., Butler, S. T., Berry, D. P., Carter, F., Lonergan, P., Smith, G. W., Ireland, J. J., & Evans, A. C. O. (2012). Low numbers of ovarian follicles ≥3mm in diameter are associated with low fertility in dairy cows. *J Dairy Sci, 95*(5), 2355–2361. https://doi.org/10.3168/jds.2011-4325

Nogueira, G. P. (2004). Puberty in South American Bos indicus (Zebu) cattle. *Anim Reprod Sci,* 82–83, 361–72. https://doi.org/10.1016/j.anireprosci.2004.04.007

Oliveira Junior, J. S., Cardoso, C. J. T., Silva, W. A. L., Kischel, H., Souza, M. B., Andrade, E. R., Nogueira, E., Silva-Santos, K. C., Seneda, M. M., & Melo-Sterza FA (2015). Antral follicles population in heifers and cows of Nelore and Girolando breeds. *Semina: Ciências Agrárias (Online),* 36, 3741-49. http://dx.doi.org/10.5433/1679-0359.2015v36n6p3741

Santa Cruz, R., Cushman, R. A., & Viñoles, C. (2018). Antral follicular count is a tool that may allow the selection of more precocious Bradford heifers at weaning. *Theriogenology,* 119, 35–42. https://doi.org/10.1016/j.theriogenology.2018.06.010

Santos, G. M. G., Silva-Santos, K. C., Barreiros, T. R. R., Morotti, F., Sanches, B. V., Moraes, F. L. Z., Blasch, W., & Seneda, M. M. (2016). High numbers of antral follicles are positively associated with in vitro embryo production but not the conception rate for FTAI in Nelore cattle. *Anim Reprod Sci*, 165, 17-21. https://doi.org/10.1016/j.anireprosci.2015.11.024.

Seneda, M. M., Morotti, F., Zangirolamo, A. F., Da Silva, N. C., Sanches, T. K., Blaschi, W., & Barreiros, T. R. R. (2019). Antral follicle population in prepubertal and pubertal heifers. *Reprod Fert Develop,* 31(1), 10–16. https://doi.org/10.1071/RD18344

Silva-Santos, K. C., Santos, G. M. G., Júnior, C. K., Morotti, F., Siloto, L. S., Marcantonio, T. N., Urbano, M. R., Oliveira, R. L., Lima, D. C. M., & Seneda, M. M. (2014). Antral follicle populations and embryo production - in vitro and in vivo - of Bos indicus-taurus donors from weaning to yearling ages. *Reprod* Domest Anim, 49(2), 228-232. https://doi.org/10.1111/rda.12255

Singh, J., Dominguez, M., Jaiswal, R., & Adams, G. P. (2004). A simple ultrasound test to predict the superstimulatory response in cattle. Theriogenology, 62(1-2), 227–243. https://doi.org/10.1016/j.theriogenology.2003.09.020.

U-krit, W., Wadsungnoen, S., Yama, P., Jitjumnong, J., Sangkate, M., Promsao, N., Montha, N., Sudwan, P., Mektrirat, R., Panatuk, J., (2022). Understanding the Ovarian Interrelationship with Low Antral Follicle Counts (AFC) in the In Vivo Bos indicus Cow Model: Unilateral and Bilateral Main AFC as Possible Biomarkers of Ovarian Response to Hormonal Synchronisation. Biology, 11, 523. https://doi.org/ 10.3390/biology11040523