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# Ovarian follicular dynamics and conception rate in *Bos indicus* cows with different antral follicle counts subjected to timed artificial insemination

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ABSTRACT

Two experiments in Nelore cows subjected to timed artificial insemination (TAI) were designed to compare the influence of the antral follicle count (AFC/AFCs) on (1) ovarian follicular dynamics and (2) conception rates. First, multiparous cows with high ($\geq$45 follicles; $n = 43$) or low ($\leq$15 follicles; $n = 32$) AFCs were selected to undergo a TAI protocol to monitor ovarian follicular dynamics. Second, the AFCs of 962 cows also subjected to TAI were determined and classified as groups of high (G-high; $\geq$45 follicles; $n = 194$), intermediate (G-intermediate; $\geq$20 $\leq$ 40 follicles, $n = 397$) or low (G-low; $\leq$15 follicles; $n = 243$) AFCs. In study I, the ovarian measurements (diameter, perimeter and area) were greater ($P \leq 0.05$) and there was a greater consistency in number of antral follicles during the period of synchronization for TAI in the high than low group. Effects of the AFC and ultrasonic evaluation interval on the follicular diameter were observed ($P \leq 0.05$); however, there was no interaction ($P > 0.05$). Dominant follicles had greater diameters ($P \leq 0.05$) in the G-low than in the G-high at $D_4$ (7.3 ± 2.2 *vs.* 6.2 ± 1.4 mm, respectively, $P = 0.06$), $D_8$ (11.2 ± 1.8 *vs.* 9.5 ± 1.8 mm, respectively), $D_9$ (12.3 ± 1.7 *vs.* 10.6 ± 1.7 mm, respectively), and $D_{10}$ (13.4 ± 1.3 *vs.* 12.2 ± 1.8 mm, respectively), as well as greater estimated diameters of ovulatory follicles (14.4 ± 1.5 *vs.* 13.4 ± 2.1 mm, respectively, $P = 0.08$). In study II, the cows with fewer AFCs had greater ($P \leq 0.05$) conception rates (61.7%) than the cows with the intermediate (52.9%) and greater (49.5%) AFCs. Nelore cows with fewer AFCs subjected to synchronization of time of ovulation had a larger follicular diameter and a greater conception rate than the groups with intermediate and a greater AFCs.

## 1. Introduction

The relationship between the antral follicle count (AFC) and fertility is of considerable importance in cattle, as the AFC and reproductive performance have been the focus of several studies in recent years (Burns et al., 2005; Ireland et al., 2011; Evans et al., 2012; Mossa et al., 2012; Silva-Santos et al., 2014a,b; Jimenez-Krassel et al., 2015; Santos et al., 2016; Morotti et al., 2017).

The great variability in the number of antral follicles is a remarkable characteristic of cattle. Even though there is great variability,

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the AFC observed in a given individual in consecutive AFC evaluations has considerable repeatability (Burns et al., 2005; Ireland et al., 2007). Thus, the consistency of the AFC in the same individual has become a strategic opportunity as a result of the possibility of classifying cattle with low, intermediate or high numbers of follicles by performing a single ultrasonic examination during the estrous cycle (Burns et al., 2005; Ireland et al., 2007, 2008; Silva-Santos et al., 2014a).

A low AFC in *Bos taurus* females is associated with various characteristics related to poor reproductive performance, such as small ovaries (Ireland et al., 2008), a lesser probability of pregnancy at the end of the breeding season (Mossa et al., 2012), reduced responsiveness to superovulatory treatment, less potential for embryo production and fewer viable embryos (Singh et al., 2004; Ireland et al., 2007), lesser 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 greater AFCs have the opposite physiological and endocrine characteristics as those with lesser AFCs, which suggests a linear correlation between greater AFCs and important aspects of reproductive fertility in *Bos taurus* cattle (Ireland et al., 2011; Evans et al., 2012).

This situation is poorly understood in *Bos indicus* cattle (both beef and dairy) because of the limited number of studies in this subspecies. Similar to the results of North American and European studies, the first studies in Brazil in *Bos indicus-taurus* and *Bos indicus* cattle indicated that *in vitro* embryo production was more efficient in donors with greater AFCs (Silva-Santos et al., 2014a; Santos et al., 2016). There were contrasting findings in studies in which the associations between AFCs and pregnancy were evaluated in *Bos indicus* (Nelore) cattle compared with findings from *Bos taurus* (Rodrigues et al., 2015; Santos et al., 2016). Although these studies have not completely clarified the influence of the AFC in zebu cattle, it is noteworthy that the relationship between the AFC and fertility (pregnancy timed artificial insemination [TAI]) in this subspecies does not appear to follow the same pattern described for taurine females.

The comprehension of the ovarian follicular dynamics in females with different AFCs may contribute to an enhanced understanding of ovarian and follicular characteristics in this subspecies. In the present study, the hypothesis was that cows with fewer as compared with those with greater AFCs would have a larger diameter of dominant follicles and a greater conception rate when subjected to a conventional protocol for TAI. Two experiments in Nelore cows were, therefore, designed to compare (1) the influence of greater or lesser AFCs on ovarian follicular dynamics during hormonal treatment for the synchronization of time of ovulation and (2) conception rates in females with low, intermediate and high AFCs after imposing a conventional TAI protocol.

## 2. Materials and methods

The present study was conducted according to the standards of the Ethics Committee for Animal Experimentation of the University of Londrina, based on Federal Law 11.794 of October 8, 2008, and was approved under number 5898.2014.76.

### *2.1. Experiment I – ovarian follicular dynamics in cows with high or low AFCs during hormonal treatment for the synchronization of time of ovulation*

#### *2.1.1. Location, animals and management*

This study was performed during the typical time of breeding beef cattle in South America at a latitude of 24°12′18″ and a longitude of 50°56′56″ on a single commercial beef farm in the South of Brazil. The climate in this region is humid and subtropical, with an average temperature greater than 25 °C during the summer and a rainy season that extends from November to February.

The animals were selected from 250 Nelore females (*Bos indicus*) after ultrasonic examinations to determine AFCs 7 days (day −7) prior to the beginning of the TAI protocol. Multiparous cows ($n = 75$, 48–96 mo. old) at 30–50 days postpartum (average 45 days), without a corpus luteum (CL) or large follicles (< 8 mm) (Baruselli et al., 2004), with a body condition score (BCS) between 2.5 and 4.0 (2.8 ± 0.2) on a scale of 1–5 (Lowman et al., 1976), maintained with continuous grazing of *Urochloa spp*. and provided with mineralized mix and water *ad libitum* were subsequently used in this study.

#### *2.1.2. AFC and experimental design*

To determine the AFC, the ovaries (right and left) of each animal were scanned ultrasonically with a 7.5 MHz transducer (Aquila PRO, Pie Medical, Maastricht, the Netherlands), and antral follicles (all follicles ≥ 3 mm) were counted as previously described (Burns et al., 2005; Ireland et al., 2008) to allocate the cows ($n = 75$) to two experimental groups according to the number of antral follicles. Cows that showed a consistently high (G-high, ≥45 follicles; n = 43) or low AFC (G-low, ≤15 follicles; n = 32) were defined based on the overall mean AFC (approximately 30 follicles) plus or minus one standard deviation (SD; approximately 15 follicles). Animals with intermediate AFCs (16–44 follicles) were not included in this study because there were no handling conditions to ultrasonically assess these animals simultaneously with the animals with high and low AFCs by the same operator.

Seven days after AFC evaluation, the animals were subjected to an ovulation synchronization protocol on a random day of the estrous cycle (day 0) to monitor ovarian follicular dynamics. The protocol consisted of the insertion of an ear implant that contained 3 mg of norgestomet (Crestar®, MSD Animal Health, Sao Paulo, Brazil) and intramuscular (i.m.) administration of 2 mg of estradiol benzoate (EB; Gonadiol®, MSD Animal Health, Sao Paulo, Brazil). On day 8, the implants were removed, and the animals received i.m. injections that contained 250 μg of cloprostenol ($PGF_{2\alpha}$, Ciosin®, MSD Animal Health, Sao Paulo, Brazil), 300 IU of equine chorionic gonadotropin (eCG; Novormon®, MSD Animal Health, Sao Paulo, Brazil) and 1.0 mg of estradiol cypionate (EC; ECP®, Zoetis, Sao Paulo, Brazil), as illustrated in Fig. 1.

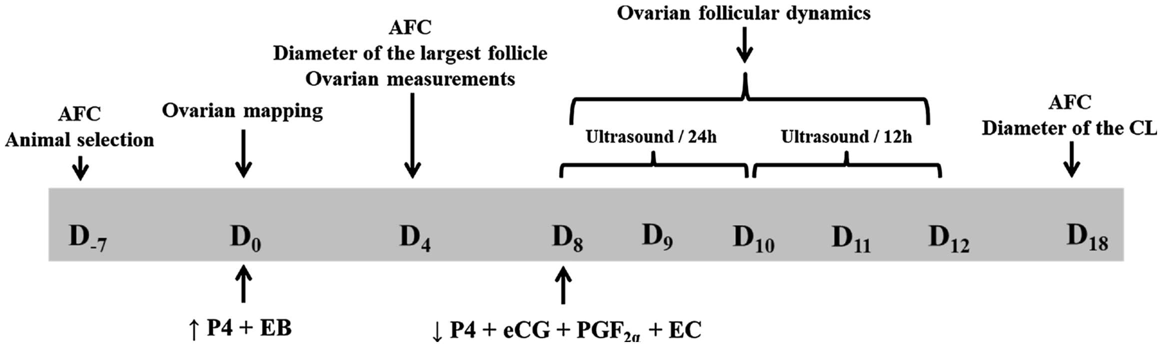


**Fig. 1.** Experimental design for assessing ovarian follicular dynamics and the hormonal treatment applied for the synchronization of time of ovulation in Nelore cows with consistently high (G-high, ≥45 follicles; n = 43) or low (G-low, ≤15 follicles; *n* = 32) AFCs. AFC – antral follicle count; P4 – progesterone/norgestomet; EB – estradiol benzoate; eCG – equine chorionic gonadotropin; $PGF_{2\alpha}$ – cloprostenol; EC – estradiol cypionate; and CL – corpus luteum.

#### *2.1.3. Ovarian follicular dynamics, blood sample collection and serum P4 concentrations*

Evaluations of follicular dynamics were performed by the same operator through blinded assessments on days 0, 4, and 8–10 (daily, to evaluate the follicular diameter), days 10–12 (every 12 h, to monitor ovulation) and day 18 (to evaluate the CL size and P4 plasma concentrations). All follicles ≥5 mm were identified and measured via ultrasonography, and the mean follicular diameter was calculated from two cross-sectional linear measurements of the follicular antrum captured on the ultrasound monitor, following which the diameter was drawn on an ovarian map (Ginther et al., 1989). The diameter, perimeter and area of both ovaries of the animals in the low and high groups were also determined through consecutive ultrasonography exams. After a previous ultrasonic scanning, the largest image of an ovary was captured on the monitor, and the measurements of each ovary image were determined.

During each evaluation of follicular dynamics, the following variables were determined: the AFCs (all follicles ≥3 mm) on days −7 (prior to the beginning of the TAI protocol), 4 and 18 (7 days after ovulation); the ovarian diameter, perimeter and area on day 4; the diameter of the largest follicle on days 4, 8, 9 and 10; the time of ovulation after ear implant removal; and the diameter of the CL on day 18. These data were obtained for both ovaries and were individually recorded for subsequent monitoring.

The dominant follicle (DF) was defined as a follicle that grew to at least > 8 mm in diameter and exceeded the diameter of all other follicles (Figueiredo et al., 1997). Ovulation was monitored every 12 h from 48 to 96 h after progesterone implant removal; it was initially detected based on the absence of the DF and was subsequently confirmed by the presence of a CL in the same ovary. On the seventh day after ovulation, the diameter of the CL was determined in the same ovary that previously contained the pre-ovulatory follicle. The diameter of the CL was determined based on the average of two linear measurements of the cross-sectional area of the CL (Ginther et al., 1989). Immediately after measurement of the CL, jugular blood samples were collected and subsequently centrifuged for 15 min at 3500 rpm; aliquots of serum were individually recovered in 3 mL polypropylene tubes, which were subsequently frozen at −20 °C until the time of analysis.

The serum P4 concentrations were determined using a commercial solid-phase radioimmunoassay kit (RIA IM1188 kit; Beckman Coulter®, Immunotech, Czech Republic) in 100 µL samples. The sensitivity of the test was 0.001 ng/mL, and the intra-assay coefficients of variation were 3.0% for the greater value and 0.1% for the lesser value.

### *2.2. Experiment II – conception rate in cows with high, intermediate or low AFCs subjected to TAI*

#### *2.2.1. Location, animals and management*

This study was performed during the 2014/2015 breeding season in South America on three commercial beef farms located within a 100 km radius of the farm from study I. These farms were selected because of the similarities in the breed and in the nutritional, sanitary and reproductive management practices and physical installations.

Multiparous Nelore cows (*Bos indicus*, *n* = 962) with an average age of 72 ± 12 months, at 40 ± 15 days postpartum (suckling), with a BCS between 2.5 and 4.0 (2.9 ± 0.5) on a scale of 1–5 (Lowman et al., 1976), were subjected to time-of-ovulation synchronization for TAI. The animals were maintained with continuous grazing of *Urochloa spp*. and were provided a mineralized mix and water *ad libitum*.

#### *2.2.2. Hormonal protocol for TAI, AFCs and conception rates*

The animals received a TAI protocol similar to Study I. At Day 0, the ovaries of each animal (*n* = 962) were scanned via *trans*-rectal ultrasonography (Aquila PRO, Pie Medical, Maastricht, the Netherlands, with a 7.5 MHz linear transducer), and the number of antral follicles (≥3 mm) was counted as previously described for Study I.

At 48–52 h after implant removal, the cows were artificially inseminated by a single experienced inseminator using frozen-thawed semen from six sires with known fertility. The females were subsequently assessed via *trans*-rectal ultrasonography between 30 and 35 days after TAI to determine pregnancy status.

### 2.3. Evaluated variables and statistical analysis

In Study I, the AFCs of the cows in the experimental groups were evaluated on days 0, 4 and 18; the ovarian diameter (mm), perimeter (mm) and area ($mm^2$) were assessed on day 4; the diameter of the largest follicle was determined on day 4 (mm); the diameter of the DF was measured on days 8, 9 and 10 (mm); and the diameter of the CL was recorded on day 18 (mm). The average values for the follicular growth rate (mm/day), estimated diameter of the pre-ovulatory follicle (mm) and time of ovulation (h) were also determined in estrous synchronized females. Information for each individual animal was tabulated into a dataset for statistical analysis. The daily follicular growth rate was calculated for each cow and each group (Figueiredo et al., 1997).

The repeatability and correlation of the AFC were evaluated by the generalized linear mixed model adjusted to the Poisson distribution, according to Nakagawa and Schielzeth (2010). Two different models were adjusted to verify the repeatability of AFCs, being between groups and in the presence or absence of the effect of groups in the model. All adjustments were made to the R software through the lme4 and npmlreg packages, through the glm command. In all cases, the adjustments can be considered correct in the presence of high correlations between estimates and observed.

In Study II, the mean number of follicles (approximately 30 follicles) and the SD (approximately 15 follicles) for the whole population ($n = 962$) were established for the classification of females into three groups (G) according to the AFCs and SDs. For data analysis, females ($n = 834$) were classified as having a high (mean AFC of all 962 cows plus 1 SD; G-high AFC, ≥45 follicles; $n = 194$), low (mean AFC of all 962 cows minus 1 SD; G-low AFC, ≤15 follicles; $n = 243$) or intermediate (cows with an AFC ≥ 20 and ≤40, $n = 397$) AFC. The females with an AFC of 16, 17, 18 and 19 follicles (interface from low to intermediate group), as well as the cows with AFCs of 41, 42, 43 and 44 (interface from intermediate to high group) were excluded from the conception analyses because of the imprecision in the adequate determination of the AFC and the classification of groups as low or intermediate and intermediate or high AFC, respectively. Thus, a more trustworthy range of follicles was maintained among the groups.

Quantitative variables were assessed for normal distributions of the data and homogeneity of variances and were subsequently analyzed via analysis of variance (one-way ANOVA). Variables with more than one source of variation were analyzed using an adjusted mixed effect model that included all possible effects and interactions. Differences in the conception rates between the groups (G-high, G-intermediate and G-low AFC) after TAI were analyzed using a model of logistic regression, considering the effects of the farm, BCS, sire and all possible interactions in the statistical model, in which no covariate demonstrated an effect on the conception rate.

All data were analyzed using the MINITAB 18® statistical software program, version 18.1. The significance level for rejecting H0 (the null hypothesis) was 5%; therefore, a level of significance ≤0.05 was considered to indicate an effect of the categorical variables and their interactions. A statistical tendency was indicated as probabilities > 0.05 and < 0.09. The data are presented as M ± SD or proportions for the descriptive statistical analysis.

## 3. Results

### 3.1. Experiment I

The AFC was consistent during hormonal treatment for the synchronization of ovulation in both groups, and the number of antral follicles was consistently greater ($P = 0.001$) in the high than low group on days −7, 4 and 18. The ovarian measurements, including the diameter, perimeter and area, were greater in the high group than in the low group ($P = 0.0001$, Table 1).

The repeatability in the two models was 0.9336 (93.36%) for Model I (no group effect) and 0.03 (3.45%) for Model II (with group effect). The correlation between the predicted values and true was 0.99 and 0.98 for Models I and II, respectively.

**Table 1**
Variables evaluated during ovarian follicular dynamics in Nelore cows with consistently high (G-high, ≥45 follicles) or low (G-low, ≤15 follicles) AFCs following a TAI protocol.

| Variables | G-high AFC | G-low AFC | *P*-value |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | |
| Body condition score (1–5) | 2.8 ± 0.2 | 2.9 ± 0.2 | 0.423 |
| AFC day $_{-7}$ (n) | 48.9 ± 5.8 | 12.6 ± 4.1 | 0.0001 |
| AFC day $_4$ (n) | 49.5 ± 5.7 | 12.5 ± 4.4 | 0.0001 |
| AFC day $_{18}$ (n) | 49.7 ± 6.9 | 12.9 ± 3.8 | 0.0001 |
| Ovarian diameter (mm) | 28.3 ± 3.9 | 20.5 ± 3.2 | 0.0001 |
| Ovarian perimeter (mm) | 100.9 ± 13.2 | 73.8 ± 13.9 | 0.0001 |
| Ovarian area ($mm^2$) | 67.5 ± 16.4 | 37.7 ± 11.7 | 0.0001 |
| Follicular growth D4 to D8 (mm) | 3.3 ± 0.5 | 4.0 ± 0.4 | 0.304 |
| Follicular growth D8 to D9 (mm) | 1.2 ± 0.2 | 1.1 ± 0.2 | 0.737 |
| Follicular growth (mm/day) | 1.4 ± 0.2 | 1.1 ± 0.1 | 0.160 |
| Time of ovulation (h) | 69.8 ± 7.1 | 70.2 ± 5.9 | 0.851 |
| CL diameter (mm) | 18.2 ± 3.1 | 19.4 ± 1.7 | 0.129 |
| P4 concentration (ng/mL) | 2.34 ± 0.6 | 2.84 ± 0.8 | 0.155 |

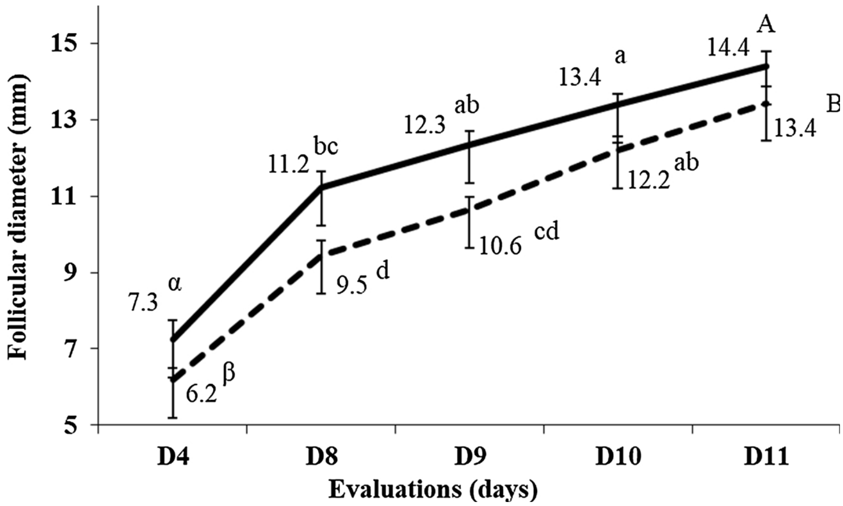


**Fig. 2.** Follicular diameter (mm) and estimated diameter of the ovulatory follicle (mm) during ovarian follicular dynamics in Nelore cows with consistently high (≥45 follicles; dashed line) or low (≤15 follicles; continuous line) AFCs synchronized with a TAI protocol. Effects of the AFC ($P = 0.0001$) and ultrasonic evaluation interval ($P = 0.0001$) were observed; however, there was no interaction ($P = 0.784$). Values denoted using different Greek letters (α-β; punctual evaluation on day 4), lowercase letters (a-d; evaluations with 24-h intervals) or capital letters (A-B; evaluations with 12-h intervals) were different. (D8, 9 and 10/P < 0.05, D4/P = 0.06 and D11/$P = 0.08$).

Considering the follicular diameter, effects of the AFC ($P = 0.0001$) and the ultrasonic evaluation interval ($P = 0.0001$) were observed; there was, however, no interaction ($P = 0.784$). The follicular diameter was greater ($P < 0.05$) in the cows with low AFCs than in the cows with high AFCs on day 4 ($P = 0.06$), day 8, day 9, and day 10. The time of ovulation after implant removal was similar between the cows with low and high AFCs ($P = 0.85$), as well as the CL diameter ($P = 0.12$) and the P4 concentration on day 18 ($P = 0.15$). The estimated diameter of the ovulatory follicle tended ($P = 0.08$) to be greater, however, in the low than high group (Fig. 2).

### 3.2. Experiment II

The mean number of antral follicles (mean ± SD) was 30.1 ± 15.6 (range 3–95 follicles), and the average conception rate after TAI was 54.7% (456/834). The cows with low AFCs had a greater ($P = 0.02$) conception rate than the cows with intermediate and high AFCs (Table 2).

## 4. Discussion

To our knowledge, this study was the first investigation to evaluate ovarian follicular dynamics in *Bos indicus* cows with high or low AFCs subjected to a hormonal protocol for time-of-ovulation synchronization. The results indicated that ovarian measurements (diameter, perimeter and area) were greater in cows with high AFCs than in cows with low AFCs. Interestingly, from the fourth day of the protocol for ovulation synchronization until the time at which the diameter of the ovulatory follicle was estimated, the follicular diameter was larger in cows with low AFCs than in cows with high AFCs. Furthermore, the present study provides the first evidence to support a positive influence of a low AFC on the conception of *Bos indicus* cattle subjected to TAI, thus the findings are consistent with the hypothesis of the present study.

This study provides applicable information regarding AFCs and the relationship with ovarian follicular dynamics in *Bos indicus* cattle, which suggests that the differences in follicular diameters between groups with high and low AFCs may affect the conception rate in cows of this subspecies subjected to TAI. This hypothesis was confirmed in Study II, in which females with low AFCs had a

**Table 2**
Means ± SDs for the antral follicle population and conception rates of Nelore cows with consistently high (G-high, ≥45 follicles), intermediate (G-intermediate, ≥20– ≤40 follicles) or low (G-low, ≤15 follicles) AFCs following a TAI protocol.

| AFC groups | Animals *n* | AFC Mean ± SD | Conception rate% (*n*) |
|---|---|---|---|
| G-low | 243 | 11.3 ± 2.8 [c] | 61.7 (150) [a] |
| G-intermediate | 397 | 29.4 ± 6.0 [b] | 52.9 (210) [b] |
| G-high | 194 | 52.8 ± 7.7 [a] | 49.5 (96) [b] |
| *P*-value | – | 0.001 | 0.027 |
| Total/Mean | 834 | 30.1 ± 15.6 | 54.7 (456) |

Values with different superscripted letters (a, b) were different ($P \leq 0.05$) between the AFCs and conception rates.

greater conception rate of more than 10% compared with the group with high AFCs. Furthermore, this study contributes to an enhanced understanding of the antral follicular population in *Bos indicus*, in which the results differed from those obtained in other studies conducted in *Bos taurus* cattle.

Many studies on AFCs and fertility in cattle have associated low AFCs with several negative factors related to fertility, particularly in *Bos taurus* dairy cattle (Ireland et al., 2008; Ireland et al., 2011; Evans et al., 2012; Mossa et al., 2012; Jimenez-Krassel et al., 2015; Martinez et al., 2016). Small ovaries (Ireland et al., 2008), a lesser probability of pregnancy at the end of the breeding period (Mossa et al., 2012), reduced responsiveness to superovulation treatment, fewer viable embryos produced *in vivo* or *in vitro* (Singh et al., 2004; Ireland et al., 2007, 2011), lesser circulating concentrations of progesterone and AMH (Jimenez-Krassel et al., 2009, 2015) and a reduced endometrial thickness during estrous cycles (Jimenez-Krassel et al., 2009) have been associated with females with low AFCs.

In the present study, cows with high AFCs had a greater ovarian diameter, perimeter and area than cows with low AFCs. Although Ireland et al. (2008) investigated different ovarian measurements, these authors reported differences between the ovarian height and length in cows with high as compared with low AFCs (15.5 ± 0.8 *vs.* 12.3 ± 1.1 mm, respectively, $P < 0.05$ and 28.3 ± 1.3 *vs.* 23.3 ± 1.4 mm, respectively, $P < 0.05$). Similarly, based on the assessment of lactating dairy cows with high, intermediate or low AFCs, Martinez et al. (2016) identified a greater total ovarian area in animals with a high AFC (53.56 ± 15.6 $mm^2$) than in animals with intermediate and low AFCs (47.50 ± 13.5 and 43.04 ± 11.3 $mm^2$, respectively, $P = 0.001$). The data from the present study also suggest, therefore, that a larger ovary size (diameter, perimeter and area) in *Bos indicus* cattle is associated with a greater number of antral follicles.

The AFC repeatability changed in the presence of the group effect, but in both studies (I and II) the correlations between the predicted values and the true values remained above 0.98, indicating a high accuracy of the models. With Model I, the effect of the group was not considered, and the data were adjusted only for the animal effect and, therefore, the greatest repeatability value (93.36%) was obtained. This pattern is confirmed by observing the means and standard deviations that were consistent at each ultrasonic examination (12.6 ± 4.1, 12.5 ± 4.4 and 12.9 ± 3.8 for the group with low and 48.9 ± 5.8, 49.5 ± 5.7 and 49.7 ± 6.9 for the group with high scores on days −7, 4 and 18, respectively).

Cows with low AFCs had greater follicular diameters beginning on day 4 of the hormonal treatment, with a larger dominant follicle diameter until the presumed time of TAI. Moreover, the diameter of the pre-ovulatory follicle was greater in the group with low AFCs than in the high AFC group. In contrast, in Holstein cows with low (≤15 follicles), intermediate (16–20 follicles), high (21–25 follicles) or very high (≥25 follicles) numbers of follicles during follicular wave development, Burns et al. (2005) reported there were no differences ($P > 0.1$) between the groups regarding the length of time the dominant follicle persisted, interval between ovulations, day of emergence, day of deviation, largest diameter of the dominant and subordinate follicles during dominance, and diameter of the dominant and subordinate follicles at deviation. It is noteworthy that these aspects appear to be intriguing differences between the reproductive physiology in *taurus* and *indicus* cows.

Interestingly, a recent article also described a greater reproductive performance for *Bos taurus* with a low AFC. Considering Holstein heifers, Jimenez-Krassel et al. (2017) reported suboptimal fertility (a lesser conception rate and a greater period of days non-pregnant) and a shorter productive life (a lesser number of lactations with a greater herd culling rate) for females with high AFCs than heifers with low AFCs. The inconsistent data regarding AFCs reinforces the need for further investigations and a careful analysis of the antral follicle populations.

In the present study, the diameter of the dominant follicle 48 h after progesterone implant removal was greater in cows with low AFCs than high AFCs (13.4 ± 1.3 *vs.* 12.2 ± 1.8 mm, respectively, $P < 0.05$). This finding is consistent with studies that have associated the largest follicular diameter at TAI with greater pregnancy rates (Sá Filho et al., 2010; Pfeifer et al., 2012; Sales et al., 2012; Morotti et al., 2013; Pfeifer et al., 2015). A dominant follicle that attains a larger diameter after follicular deviation has a greater ovulatory potential (Gimenes et al., 2008), thus resulting in a larger CL and, consequently, greater progesterone concentrations (Pfeifer et al., 2009). In *Bos indicus* females, the larger ovulatory follicles detected at TAI is associated with a greater probability of pregnancy (Meneghetti et al., 2009; Sá Filho et al., 2010; Pfeifer et al., 2012; Pfeifer et al., 2015).

A study performed in *Bos indicus* cattle evaluated whether changing the interval from progesterone removal to TAI according to the pre-ovulatory follicle diameter would improve pregnancy per AI in cows (Pfeifer et al., 2015). In lactating Nelore cows ($n = 412$), improved pregnancy rates occurred after TAI was performed in blocks according to the dominant follicle diameter. These studies clearly indicate that a larger diameter of the dominant follicle at TAI is associated with greater pregnancy rates.

There are several hypotheses to explain the greater follicular diameter observed in cows with low AFCs. The first possibility is that the large number of antral follicles provided a more competitive environment through hormonal action, in which case the more desirable outcome in cows with low AFCs may have been a result of an enhanced gonadotropin action among the smaller number of follicles. For example, there is evidence that FSH concentrations are inversely, rather than directly, correlated with the number of follicles ≥3 mm in diameter during waves of ovarian follicular development in cattle (Burns et al., 2005).

Considering data of the ovarian dynamics in the present study, it is interesting to emphasize that the second study conducted confirmed the initial hypothesis, which indicates that *Bos indicus* cows with low AFCs have greater conception rates than cows with intermediate and high AFCs. It is noteworthy that this study was based on a significant number of animals, thus reflecting the reality of TAI programs on commercial farms. Although Burns et al. (2005) reported there was no effect of AFCs on follicular dynamics during emergence, selection or dominance, many other studies (particularly studies performed in dairy cattle, *Bos taurus*) have indicated a lesser reproductive performance of cows with low AFCs (Ireland et al., 2007, 2008; Jimenez-Krassel et al., 2009; Ireland et al., 2011; Evans et al., 2012; Mossa et al., 2012; Jimenez-Krassel et al., 2015), which is clearly suggestive of different follicular dynamics between *Bos taurus* and *Bos indicus* cattle with low and high AFCs.

Although the present study provided different results than other studies conducted in *Bos taurus*, it should be emphasized that there are numerous physiological differences between subspecies, and it is plausible for the reproductive patterns observed in one subspecies to be different from that in another subspecies. The great variability of AFCs between studies, different herds and categories of females evaluated, different management practices adopted, and absence of standardization in group classifications, in particular, make it more challenging to obtain a complete understanding of the effect of AFCs on the fertility of female cattle. The present research suggested, however, a positive influence of a low AFC in cows subjected to TAI, at least in *Bos indicus* beef cattle, as a greater follicular diameter and conception rate were identified in animals with low AFCs.

In conclusion, Nelore cows with high AFCs (≥45 follicles) had greater ovarian measurements (diameter, perimeter and area) than cows with low AFCs (≤15 follicles). The females with high and low AFC had a greater consistency of the number of antral follicles through the period of synchronization for TAI. The follicular diameter was, however, greater in the group with low AFCs during hormonal treatment for the synchronization of time of ovulation. Moreover, cows with low AFCs had a greater conception rate than the groups with intermediate and high AFCs, which suggests that the number of antral follicles may influence the reproductive performance of *Bos indicus* beef cattle in TAI programs.

## Conflict of interest

None.

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