Correlation between phenotype, genotype and antral follicle population in beef heifers

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Correlation between phenotype, genotype and antral follicle population in beef heifers

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ABSTRACT

The present study was performed in *indicus-taurus* heifers 1) to determine if the antral follicle count (AFC) exhibits repeatability from puberty to yearling age and 2) to evaluate whether the phenotypic and genotypic parameters used in genetic improvement programs are correlated with AFC. In study I, Braford heifers (3/8 Nelore x 5/8 Hereford, n = 137) were serially examined by ultrasonography (with 60-day intervals) from weaning (9 ± 1 mo of age) to yearling ages (20 ± 1 mo of age) to monitor the numbers of antral follicles. In study II, the AFC of animals from experiment I and contemporary (same farm, considered at same age and kept under same conditions heifers n = 270 18–24 months) was correlated with the records of a genetic selection program using four statistical models with different covariates: i) model 1 considered effects of contemporary group and covariates age, weight gain from birth to weaning and visual scores for carcass traits at weaning, ii) model 2 covered contemporary group, age, weight gain from weaning to yearling and visual scores for carcass traits at yearling. The effects, variables and covariates of models 1 and 2 were combined to form model 3. Model 4 included the model 3 with addition of paternal effect. In study I, AFC varied from 3 to 64 follicles among females but was highly repeatable (0.89–0.92) within individuals in the same group. In study II, the four models tested showed low correlations with AFC: 0.072, 0.056, 0.082 and 0.172 for models 1, 2, 3 and 4, respectively. However, the model with paternal effect provided 17% of correlation of AFC and genotypic and phenotypic characteristics. Models 1, 3 and 4 also showed that AFC in *indicus-taurus* heifers can be influenced by finishing precocity at weaning ($P < 0.05$) with a variation of three follicles. Based on these studies, AFC in heifers from weaning to yearling age is highly variable between individuals and repeatable within the same female. Additionally, there is no correlation between phenotypic or genotypic characteristics and the antral follicle population. However, AFC can be slightly affected by finishing precocity at weaning.

1. Introduction

The use of reproductive biotechnologies in association with genetic improvement programs has been a widespread strategy for improving livestock production in a short time. For this purpose, artificial insemination and embryo production are the tools with the highest potential for disseminating superior genetic qualities and improving reproductive performance in both beef and dairy cattle [1,2]. However, there is a greater emphasis on the *in vitro* production of embryos, which has been the most used technique for embryo production in the world during the last decade [3], with highly effective results reported in many large-scale studies in cattle [4–7]. Nevertheless, the number of antral follicles (follicles ≥ 3 mm of diameter visualized on ultrasound) or antral follicle count (AFC) is closely related to the number of embryos produced by the donor, in both *in vitro* and *in vivo* methods [4,6,8], and can affect the reproductive efficiency of cattle.

AFC is a determinant characteristic in the bovine female, and its influence on the efficiency of reproductive biotechnologies and reproductive performance in dairy cattle has been well documented in several studies performed especially in *Bos taurus* herds

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as revised by Ireland et al. [9] and Evans et al. [10]. This count has high variation among animals and high repeatability within individuals, which allows the classification of females as low-, intermediate- or high-AFC by a simple ultrasonography exam during follicular waves [11–13].

For some reproductive biotechnologies, especially embryo production, ultrasonography has become a strategic tool in donor selection due to the constancy of AFC throughout the estrous cycles. Additionally, there is evidence that variability in the number of antral follicles in *Bos taurus* cattle can affect some characteristics of female fertility and compromise the reproductive performance of the herd [9,10,14]. Studies have shown that high AFC is positively correlated with characteristics linked to female fertility, such as the total number of morphologically healthy follicles and oocytes in the ovary [13], responsiveness to gonadotrophin treatment during superovulation (SOV) and the number of transferable embryos [9,12], progesterone production during the estrous cycle [15], and a smaller interval between calving and improved reproductive performance [14]. In divergence, cows with low AFC (Holstein) have exhibited a negative impact on reproductive performance when several aspects related to fertility are compared with those of cows with a high follicle count [9,10,14].

Studies also have shown that AFC can be affected by maternal environmental conditions such as nutritional and health status during pregnancy [9,10,16,17]. Additionally a single study has examined the relationship between AFC and genetic characteristics in dairy cattle (Holstein), which demonstrated that follicle count is also affected by lactation status and quality of milk; however, the level of milk production did not influence this count [18]. The effect of characteristics of genetic merit on AFC is an issue that needs to be better understood because the selection of females based on AFC has been a common practice before starting some reproductive biotechnologies; for example, due to the quantitative benefits, embryo production programs have prioritized donors with high follicle count. In this context, there is concern regarding whether AFC may be affected by characteristics linked to the genetic value of the female, especially in beef cattle, which has not been studied.

Considering the lack of information, we hypothesized that Braford heifers exhibit stable AFC from weaning to yearling age and as well as number of antral follicles can be influenced by characteristics of genetic merit. Therefore, the objectives of the present study in beef heifers were 1) to determine whether the number of antral follicles exhibits repeatability in its count between puberty and yearling age and 2) to evaluate whether the main phenotypic and genotypic parameters used in genetic improvement programs can be correlated with AFC in heifers.

2. Materials and methods

2.1. Location, animals and feed management

The experiments of the present study were performed in compliance with protocols approved by the Committee of Ethics in Animal Experimentation at the State University of Londrina, Parana, Brazil.

This study was performed in *Bos indicus-taurus* beef cattle that belonged to a large genetic improvement program (Conexão Delta G Group, Londrina, Parana, Brazil) during the 2012–2013 breeding season in South America at latitude 23° 17′ 34″ and longitude 51° 10′ 24″. The climate in this region is tropical, with an average temperature of 24 °C and a rainy season from November to January.

Braford cattle (3/8 Nelore x 5/8 Hereford) with adequate body-condition score (BCS; between 3.0 and 4.0 on a scale of 1–5 according to Lowman et al. [19] and normal health status were selected for the experiments. The animals were maintained by continuous grazing of *Urochloa brizantha* pasture and were given *ad libitum* access to mineralized salt and water.

2.1.1. Study I – antral follicle count and monitoring

To evaluate AFC, Braford heifers (n = 137) were serially examined by ultrasonography from weaning (Mean ± SD; 9 ± 1 mo of age) to yearling ages (20 ± 1 mo of age). At 24 mo of age, the mean BCS was 3.0 ± 0.5, and the average live body weight was 360 ± 10 kg.

On a random day of the estrous cycle (Day 0), both ovaries from each animal were assessed (from end-to-end) using a real-time B-mode ultrasound scanner (Scanner 200 Vet; Pie Medical, Maastricht, The Netherlands) equipped with a 7.5-MHz convex intravaginal array transducer, and antral follicles (all follicles ≥ 3 mm in diameter) were counted as described previously [11,13]. Additional similar ultrasound exams were performed at 60-day intervals (days 0, 60, 120, 180, 240 and 300) to monitor the numbers of antral follicles.

All of the ultrasound examinations were systematically performed by the same operator (without knowledge of the AFC of each animal - blind assessment), and all data were obtained in both ovaries and individually recorded for subsequent monitoring.

2.1.2. Study II - antral follicle count and variables of genetic merit

Heifers from experiment I (n = 137) and a new group of Braford heifers (n = 133) at the same age and identical conditions (contemporary) were included in the study to determine the correlation between AFC and genetic merit characteristics in beef cattle. The group II belonged to the same beef farm as experiment I and was established under the same conditions of health and nutritional management. Considering the previous results of study I, the AFCs of the contemporary group were determined by intravaginal ultrasonography in a single assessment on a random day of the estrous cycle, under the same criteria adopted in the previous study and by the same operator. Characteristics of genetic selection (zootechnical characteristics) were evaluated in all heifers (n = 270) by the same inspector and in accordance with criteria adopted by the genetic improvement program (GenSys®, Porto Alegre, Brazil) as described in Table 1. The effect of contemporary group (CG) as well the effect of the father of the heifer (effect of 23 bulls) were also considered depending on the models used (Fig. 1). The visual scores for conformation, precocity and musculature were individually assigned and ranged from 1 to 5 (Table 1) as described by Severo [20].

After yearling ratings, heifers (n = 235) inserted in the breeding program were ranked based on the Decas (1–10), an index often used in breeding programs that is determined based on the value of Expected Progeny Differences (EPDs) [21]. The weighting of animals in the Decas allows the quick and objective classification of program members. In the present study, the animals were ranked into groups of 10% according to the expected normal distribution, i.e.,

Table 1
Weighting percentage applied to the expected progeny difference for ranking from Braford heifers (3/8 Nelore x 5/8 Hereford, n = 270) at weaning and yearling.

Productive characteristics	Weighting (%)
Weight gain from birth to weaning (GW, Kg)	25
Conformation at weaning (CW, score of 1–5)	4
Finishing precocity at weaning (PW, score of 1–5)	8
Musculature at weaning (MW, score of 1–5)	8
Weight gain from weaning to yearling (GY, Kg)	25
Conformation at yearling (CY, score of 1–5)	4
Finishing precocity at yearling (PY, score of 1–5)	8
Musculature at yearling (MY, score of 1–5)	8

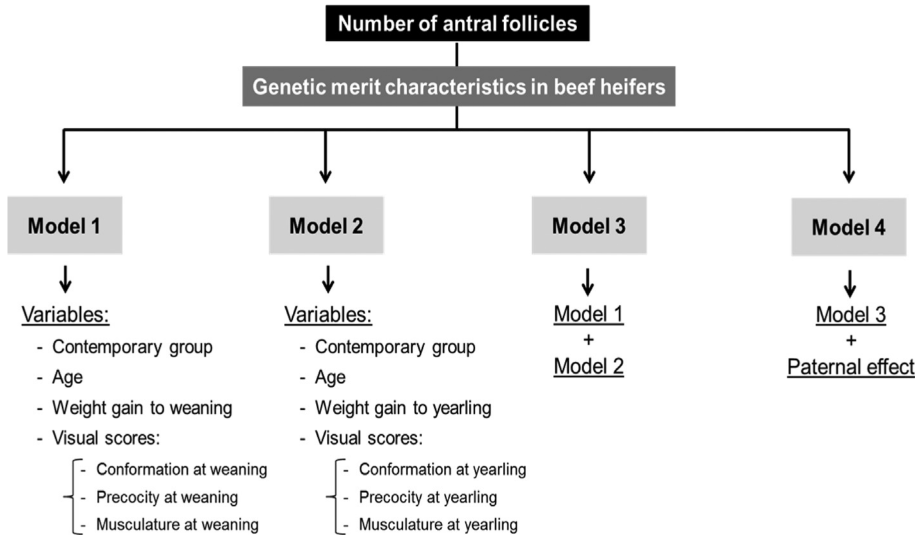

Fig. 1. Experimental design for correlation of the parameters of genetic merit and number of antral follicles in beef heifers. Model 1: Effects of contemporary group and covariates age, weight gain and visual scores at weaning (conformation, precocity and musculature). Model 2: Effects of contemporary group and covariates age, weight gain and visual scores in the yearling (conformation, precocity and musculature). Model 3: Effects, variables and covariates of models 1 and 2. Model 4: Parameters of the model 3 and paternal effect (father of the heifer).

Deca 1 corresponded to the group of animals in the top 10% relative to their contemporaries, Deca 2 corresponded to the group between the top 10% and the top 20%, and so on.

2.2. Statistical analysis

In the first study (on AFC and its repeatability), data were analyzed according to a normal distribution (parametric), and the females were assigned to three groups according to the number of antral follicles (follicles $\geq$ 3 mm diameters) and the standard deviation (SD), as follows: heifers with a consistently high (mean + ± SD; G-High, $\geq$ 41 follicles), intermediate ($\geq$12 and $\leq$ 40 follicles) or low AFC (mean − SD; G-Low, $\leq$ 11 follicles) in all ultrasound scans. Differences among assessments of AFC were compared using analysis of variance (ANOVA) with repeated measures on Minitab® statistical 16.1.1 software. The significance level was set at $P \leq 0.05$, and the values were expressed as the mean ± SD. Repeatability (proportion of the total variance that could be attributed to animal variance, range 0–1, 1 = perfect) was calculated according to Boni, Roelofsen [22].

To determine the association between characteristics of genetic merit (phenotypic and genotypic) and AFC in beef heifers, the data from the second study were analyzed using four different models, which considered different covariates, as shown in Fig. 1. Additionally, the correlation between genetic ranking (Deca) and AFC was evaluated by Pearson correlation coefficient. All data were analyzed by linear regression using the GLM procedure of SAS, considering $p \leq 0.05$ to be significant.

3. Results

Out of 137 heifers monitored in study I, 25 (18.3%) showed higher numbers of antral follicles $\geq$ 41 follicles (G-high) in all evaluations, 87 (63.5%) animals showed follicular count between 12 and 40 follicles (G-intermediate) and 25 (18.3%) heifers $\leq$ 10 follicles (G-Low) in all exams. The mean number of antral follicles varied about 21 folds among females (range 3–64 follicles) but was repeatable (G-High $r = 0.90$; G-intermediate $r = 0.89$ and G-Low $r = 0.92$) within individuals in the same group (Fig. 2).

The average weight at the beginning of the experiment (weaning - Day 0) and at the end of the study (yearling - Day 300) did not differ between heifer groups: Low- (188 ± 27 and 300 ± 35 kg), Intermediate (194 ± 33 and 303 ± 27 kg) and High-AFC (186 ± 22 and 301 ± 28 kg; $P > 0.05$).

Out of a total of 270 heifers included in study II, the average AFC was 26.82 ± 15.74 follicles, with females presenting variability from 2 to 90 antral follicles. Among the four models tested in this study, model 2, which includes the effects of CG and the covariates AG; GY; and visual scores for GY, PY and MY (yearling characteristics), showed no effect on the AFC ($p > 0.05$) and revealed a coefficient of determination (R^2) of 0.056. For the other models, the coefficients of determination were 0.072 (model 1 - effects, variables and covariates of weaning); 0.082 (model 3 - effects, variables and covariates of weaning and yearling); and 0.172 (model 4 - paternal effect, in addition to variables and covariates of weaning and yearling). Additionally, from all models that showed some correlation (models 1, 3 and 4), the visual scores for finishing precocity at weaning showed significant effects ($P < 0.05$; Table 2) on the numbers of antral follicles of the evaluated heifers.

The genetic ranking (Deca) did not correlate with the number of antral follicles ($P > 0.05$) in Braford heifers. In addition, the proportion of heifers that were ranked as Deca 1 (animals considered to be in the top 10% of the program) was similar between the groups of low, intermediate and high AFC (16.9, 15.3 and 15.6%, respectively; Fig. 3; $P > 0.05$).

4. Discussion

The present study showed that in beef heifers, the numbers of antral follicles at weaning and yearling ages are highly variable between individuals but undergo only slight variation, showing a high repeatability (0.90–0.92) within the same female. Additionally, to our knowledge, this is the first study to evaluate the correlation between number of antral follicles and genetic merit characteristics in beef heifers that were actively participating in a genetic improvement program.

The results of this study are interesting because they provide more information about using AFC as tool for selecting beef heifers

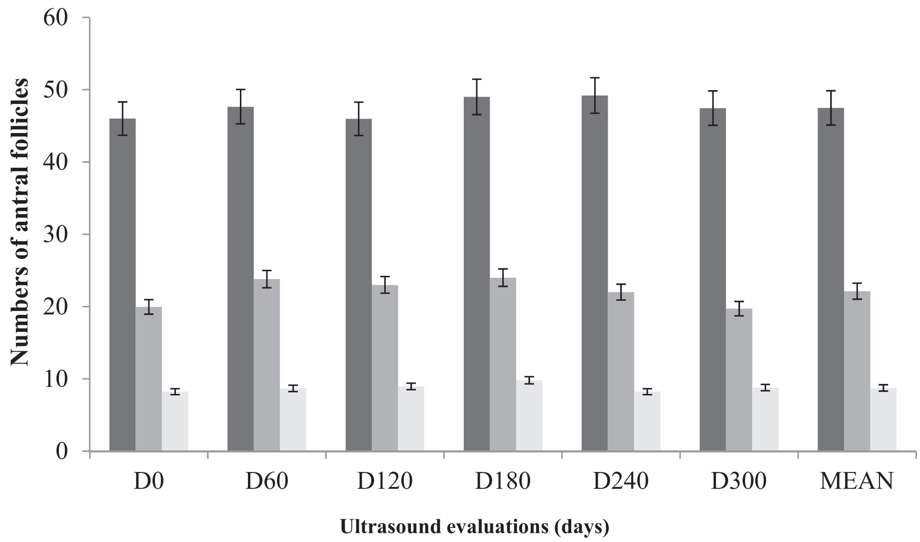

Fig. 2. Antral follicle count during ultrasound evaluations from weaning to yearling ages in beef heifers with high- (Dark Gray column, G-High ≥ 41 follicles, n = 25), intermediate (Gray column, G-Intermediate 12–40 follicles, n = 87) or low-AFC (Light Gray column, G-Low ≤ 11 follicles, n = 25).

in reproductive programs. This topic should be better discussed because full knowledge is still lacking regarding the influence of AFC on bovine fertility when comparing studies with *Bos taurus* [12–15,23] and *Bos indicus* [24,25] on the relationship between the number of antral follicles and genetic merit characteristics in beef cattle. In this context, understanding the relationship between AFC, fertility and genetic characteristics is important to develop the best strategies for livestock management.

Table 2
Coefficients of regression and P-values based on regression analysis for antral follicle count and characteristics of genetic merit in different models tested in *indicus-taurus* heifers.

Models	Variables and covariables	Coefficient of regression (±SE)	P-values
1	Contemporary group	4.26 (10.9)	0.137
	Age	0.20 (0.01)	0.230
	Weight gain to weaning	0.07 (0.61)	0.369
	Conformation at weaning	2.10 (1.66)	0.765
	Precocity at weaning	−2.81 (1.43)	0.018
	Musculature at weaning	−1.04 (1.65)	0.528
2	Contemporary group	1.26 (9.44)	0.091
	Age	0.02 (0.01)	0.108
	Weight gain to yearling	−0.04 (0.08)	0.531
	Conformation at yearling	−0.25 (1.42)	0.926
	Precocity at yearling	−1.36 (1.57)	0.603
	Musculature at yearling	1.68 (1.61)	0.297
3	Contemporary group	0.55 (9.41)	0.087
	Age	0.03 (0.01)	0.106
	Weight gain to weaning	0.06 (0.07)	0.396
	Conformation at weaning	2.92 (1.81)	0.449
	Precocity at weaning	−3.25 (1.55)	0.011
	Musculature at weaning	−1.02 (1.72)	0.556
	Weight gain to yearling	−0.01 (0.10)	0.714
	Conformation at yearling	−0.78 (1.57)	0.739
	Precocity at yearling	−0.65 (1.59)	0.886
	Musculature at yearling	1.26 (1.62)	0.438
4	Contemporary group	1.66 (9.42)	0.083
	Age	0.03 (0.01)	0.087
	Weight gain to weaning	0.09 (0.08)	0.131
	Conformation at weaning	2.71 (1.86)	0.414
	Precocity at weaning	−2.93 (1.58)	0.017
	Musculature at weaning	−0.65 (1.77)	0.684
	Weight gain to yearling	−0.01 (0.10)	0.614
	Conformation at yearling	−0.44 (1.63)	0.680
	Precocity at yearling	−1.75 (1.65)	0.379
	Musculature at yearling	1.13 (1.67)	0.499
	Bull	4.99 (8.13)	0.379

The high variability of AFC among beef heifers in the present study (range 3–64 follicles) and high repeatability (0.90–0.92) within the same animal were similar to results obtained in studies with *Bos taurus* that found variation from 8 to 56 follicles per wave among females and repeatability of 0.85–0.95 within the same individual, regardless of race, age, breeding season, lactation or pregnancy conditions [11,12]. Therefore, although this characteristic is already known in *taurus* animals, we highlighted the fact that in *indicus-taurus* cattle, a single ultrasound examination during follicular waves can identify females with low, intermediate or high AFC even at weaning (9 ± 1 mo of age), due to the high degree of repeatability within the same female.

Currently, large-scale programs for the production of embryos in cattle perform ultrasound examination of the donor prior to the OPU or SOV procedure to select females with the highest number of antral follicles. This ultrasonography evaluation has become a widely used routine because the number of antral follicles has a close relationship with the number of embryos produced per donor, via both *in vitro* and *in vivo* methods [4,6]. This relationship exists because the high variabilities in oocyte recovery rate and superovulatory response are important factors that affect the success of bovine embryo production [6,8,12,26].

In this context, several studies involving *Bos taurus* [12,26,27], *Bos indicus* [4] and cattle with *indicus-taurus* blood [6,8] have shown that a greater number of follicles has been associated with quantitative benefits to the success of OPU/IVP and SOV)/embryo collection. In a recent study, our research group also found these benefits to the evaluated reproductive performance of *indicus-taurus* females with high (≥40 follicles) and low AFC (≤10 follicles), comparing embryo production following *in vitro* (OPU/IVP) and *in vivo* (SOV/embryo collection) procedures. The study showed higher production of embryos in females with high than low AFC in both OPU/IVP (6.10 ± 4.51 *versus* 0.55 ± 0.83) and SOV/embryo collection (6.95 ± 5.34 *versus* 1.9 ± 2.13) [8].

For *Bos indicus* (Nelore) cattle we also showed that high numbers of antral follicles are positively associated with *in vitro* embryo production but not with conception rate in females subjected to timed artificial insemination (TAI). The number of viable embryos was 18.4 from donors with high AFC, 6.1 from females

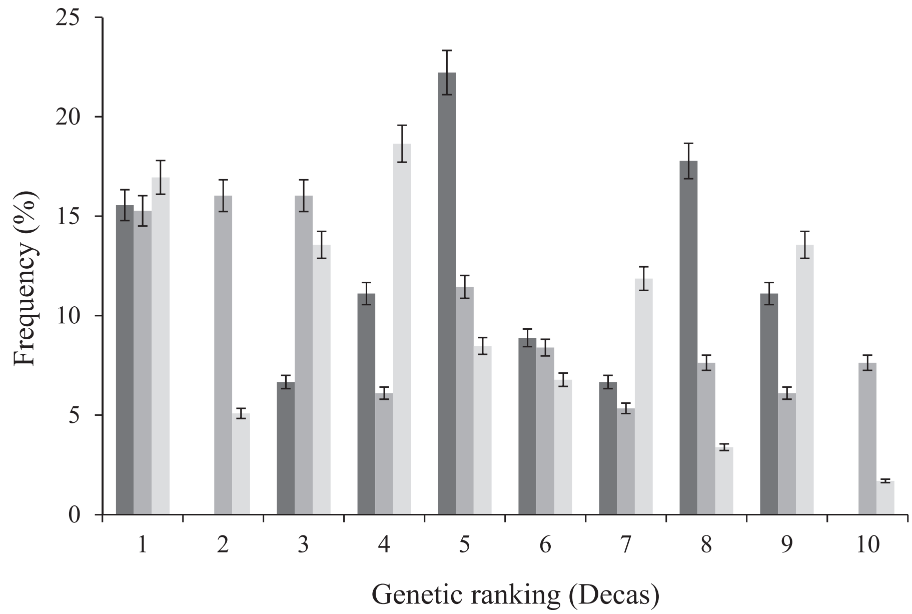

Fig. 3. Frequency distribution of genetic ranking (Deca) in beef heifers according to antral follicle count as high (G-High, ≥ 41 follicles, n = 45), intermediate (G-Intermediate, 12–40 follicles, n = 131) or low (G-Low, ≤ 11 follicles, n = 59). Deca 1: Corresponded to the group of animals in the top 10% relative to their contemporaries. Deca 2: Corresponded to the group between the top 10% and the top 20% relative to their contemporaries. Deca 10: Animals considered to be in the bottom 10% relative to their contemporaries.

with intermediate AFC and 0.6 from those with low AFC ($P < 0.05$). However, there was no difference in the conception rates after TAI between cows with high, intermediate or low AFC (51.9 vs. 48.6 vs. 58.6% [28].

In fact, it is well established that donors with higher numbers of follicles are associated with higher numbers of IVF embryos than females with few follicles. This value is a numerical indicator that is strongly considered for improving the efficiency of the IVF industry. However, little was known about genetic merit and the number of antral follicles, particularly in beef cattle. Here, we present consistent data to clarify this issue. We did not find any correlation between the number of antral follicles and relevant parameters of evaluation in beef cattle, like quality of carcass and weight gain. It is also important to emphasize that the animals of the three follicular categories (low, intermediate and high AFC) were similarly distributed among the best 10% of females (Deca 1) that were selected based on genetic merit for beef cattle. Thus, we consider that AFC may be an additional criterion for donor selection because no negative correlations were identified between number of follicles and characteristics of genetic gain. Therefore, considering the importance and impact of embryo production for the genetic gain and reproductive performance of livestock, the present study, which adopts a genetic improvement perspective, reinforces the expected concept that AFC is a useful tool for embryo production in cattle due to the quantitative benefits of females with high follicle counts.

In general, all models tested in this study showed a low correlation with the number of antral follicles (0.072, 0.056, 0.082 and 0.172 for models 1, 2, 3 and 4, respectively). However, it is noteworthy that the inclusion of paternal effect (model 4) was the correlation with AFC that had the highest score (17%) among the examined genotypic and phenotypic characteristics, suggesting some paternal influences on the number of antral follicles in heifers. Furthermore, considering all models that were statistically significant, the covariate visual scores for finishing precocity at weaning showed a negative correlation with the number of antral follicles, but only for a number of three follicles. This means that for each point that increases the visual scores for finishing precocity at weaning, there is a reduction of about three antral follicles (coefficient of regression −2.81, −3.25 and −2.93 for models 1, 3 and 4, respectively). However, as there was no effect of other variables in the tested models, it is plausible that this small number of follicles - three - is not indicative of a dramatic reduction in the AFC of the offspring. Additionally, assessing the heritability and impact of environmental effects during pregnancy on AFC in dairy cattle [18], a negative ($P < 0.05$) linear relationship between milk fat concentration and AFC was also found, with AFC decreasing by 0.4 per 1% -unit increase in fat concentration (genetic correlation of - 0.53).

In the global context of embryo production in cattle, AFC has been widely used as a donor selection criterion for both SOV and IVP. A traditional research group supervised by Evans and collaborators has revealed that several studies have shown that female fertility is positively correlated with a high number of antral follicles mostly in *Bos taurus* cattle [9,10,14,15,17,18,28–30]. Despite the differences among the herds (beef cattle with *indicus* cross breeding), this study did not show a correlation between AFC and genetic merit characteristics used in breeding programs for beef cattle. Therefore, the inconstancy of results between different studies highlights the need to always prioritize genetic merit in donor selection, not just the number of antral follicles [25,28].

In conclusion, the number of antral follicles in beef heifers from weaning to yearling age is highly variable between individuals and repeatable within the same female. Additionally, there was no correlation between the number of antral follicles and the most important genetic merit characteristics used in improvement programs for beef cattle.

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