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Reproductive tract development and puberty in two lines of Nellore heifers selected for postweaning weight

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ABSTRACT

The objective was to evaluate reproductive tract development (ovary and uterus) and onset of puberty in two lines of Nellore heifers (*Bos indicus*) selected for postweaning weight. A total of 123 heifers, including 46 from the control Nellore line (NeC) and 77 from the selection Nellore line (NeS) were used. Every 18 to 21 days from 12 to 24 months of age, average ovarian area (OVA), endometrial thickness (ETh), and diameter of the largest follicle in each ovary were evaluated (using transrectal ultrasonography), and body weight, hip height, and body condition score were measured. There were no differences between NeS and NeC heifers for ETh or OVA ($P < 0.05$). Genetic selection for higher postweaning weight had no negative influence on the onset of puberty, with 52% and 48% of NeC and NeS heifers, respectively, pubertal at 24 months of age ($P = 0.49$). Heifers that reached puberty at the end of the study were heavier (NeC, 296.9 vs. 276.7 kg; NeS, 343.5 vs. 327.9 kg; $P < 0.01$) and younger (NeC, 23.4 vs. 24.2 mo; NeS, 22.7 vs. 24.0 months; $P < 0.01$) than those that did not. Furthermore, heifers that were heavier at weaning reached puberty earlier. Pubertal heifers had a greater OVA (4.15 vs. 3.14 cm^2; $P < 0.01$) and ETh (12.15 vs. 9.93 mm; $P < 0.01$) than nonpubertal heifers. Taken together, OVA and ETh had positive effects ($P < 0.01$) on the onset of puberty and were suitable indicator traits of heifer sexual precocity in pasture management systems. However, selection for weight did not alter ovarian or endometrial development, or manifestation of puberty at 24 months of age. Among the growth traits studied, weaning weight and weight at puberty had significant positive effects on manifestation of first estrus.

1. Introduction

Age at puberty has a major effect on the productive, reproductive, and economic efficiency of female cattle. First calving at older ages is associated with economic losses in dairy and beef cattle. The late onset of puberty in pasture-fed Zebu breeds is the result of seasonal forage production, poor pasture management, lack of feed supplementation during the growth period, and relative lack of selection for precocious puberty [1]. In a review of studies on puberty in South American Zebu breeds, Nogueira [2] reported a high heritability of puberty in Zebu breeds. As a consequence, heifers selected for age at puberty and age at first calving should produce progeny that reach puberty early.

The onset of puberty is associated with various alterations in heifers, including changes in body and

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reproductive tract development. Increased endometrial thickness (ETh) is one parameter used to estimate whether cows and heifers are near ovulation in an AI program [3,4]. The selection of heifers and cows for evaluating ETh using ultrasound or transrectal palpation before insemination, can be associated with increased rates of fertility [3,4].

Several studies emphasized genetic gains in growth traits of Nellore populations. However, few investigations have evaluated the effect of selection for growth on the reproductive efficiency of Zebu breeds [5,6]. The objective of the present study was to evaluate reproductive tract development (ovary and endometrium) and its association with the onset of puberty in Nellore heifers. A second objective was to determine the effects of selection for growth on the traits studied.

2. Materials and methods

Humane animal care and handling procedures of the State of São Paulo (Brazil) law number 11.977 were followed for all experiments.

2.1. Study place and animals

The study was conducted at the Experimental Station of the Sertãozinho Institute of Zootechny (EEZS), which is affiliated with the Secretary of Agriculture of the State of São Paulo. The station is located in the northeastern region of the state (latitude: 21°10′ N and longitude: 48°5′ W). The climate of the region is tropical humid, with a mean annual temperature and rainfall of 24 °C and 1312 mm, respectively.

Animals from two Nellore lines selected for post-weaning weight were used. In 1980, the Nellore herd (*Bos indicus*) was divided into two selection lines: a control Nellore line (NeC; 60 cows) and a selection Nellore line (NeS; 120 cows). In the NeS line, male cows (7% to 10%) and female cows (55% to 65%) with higher selection differentials for weight adjusted to 378 and 550 days of age, respectively, were subjected to directional selection. In the NeC line, male and female cows with selection differentials close to zero were subjected to stabilizing selection [6,7]. As a classical selection experiment, cattle in the two selection lines were kept under the same environmental conditions and nutritional management until selection, and females were retained and assessed throughout their productive life (from birth to culling). The NeC line is used for monitoring environmental variation and thereby to estimate the genetic changes that occurred in NeS for various features. The heifers were maintained on pasture consisting of guinea grass (*Panicum maximum*) and palisade grass (*Brachiaria brizantha*).

A total of 123 Nellore heifers born between 2006 and 2007, including 46 from the NeC line and 77 from the NeS line, were used. For evaluation of differences between lines in growth traits, least square means (herd by year of birth) of age, body weight, hip height (HH) and body condition score (BCS) were estimated for all evaluations made during the experiment (18 evaluations per heifer), except for birth weight (BW) and weaning weight (WW). The last trait was adjusted to 210 days of age.

2.2. Evaluation of growth traits

Heifers were weighed and HH and BCS were measured at intervals of 18 to 21 days, from 12 to 24 months of age (beginning and end of the experiment, respectively). For all heifers, assessments of BCS, ranging from 1 (severely emaciated) to 9 (very obese), were done by two technicians [8]. Weight at puberty was determined as the first observation of a CL using transrectal ultrasonography.

2.3. Blood collection and radioimmunoassay

For confirmation of age at puberty, blood samples (10 mL) were collected from each heifer via puncture of the jugular vein into BD Vacutainer tubes containing sodium heparin (Franklin Lakes, NJ, USA) at intervals of 18 to 21 days between 12 and 24 months of age, as described [9]. Blood samples were immediately centrifuged (900 $\times$ *g* for 20 minutes) and plasma stored at −20 °C.

Blood samples of heifers with a CL visible on the ultrasound images were evaluated at two time points: before detection of the CL using ultrasound (confirmation that they were not cycling) and when the CL was detected using ultrasound (confirmation of cyclicity). Blood samples of heifers without a CL detected with ultrasongraphy were only evaluated in the second assessment (i.e., to confirm that they were not cycling).

Plasma progesterone concentrations were determined by radioimmunoassay (Coat-A-Count kit; Diagnostic Products Corporation, Los Angeles, CA, USA). The intra- and interassay coefficients of variation were 3.5% and 11.6%, respectively.

2.4. Ultrasonographic assessment

The following measures of reproductive tract development were evaluated every 18 to 21 days between 12 and 24 months of age using transrectal ultrasound (6.0-MHz transducer; Aquila System; Pie Medical Equipment B.V., Maastricht, Netherlands) adapted to the methods of Adams et al. [10] and Evans et al. [11]: average ovarian area (OVA), diameter of the largest follicle (LFD) and CL (if present), in each ovary. Images were stored for subsequent analysis with the Echo Image Viewer 1.0 program (Pie Medical Equipment B.V.).

Endometrial thickness was measured by placing the transducer on the middle third of the right uterine horn and exerting minimal pressure (to prevent artifacts). Endometrial thickness was the distance between the edge of the endometrial lumen to the visualized interface between the endometrium and myometrium, as described [4]. Ovarian area (cm^2) was the average of both ovaries (right and left). Heifers with a CL and concomitant plasma progesterone concentrations >1.5 ng/mL were classified as pubertal [3]. All evaluations (growth traits, ultrasonographic assessment, and blood collection) were performed concurrently.

2.5. Statistical analyses

Repeated measures for weight, HH, BCS, ETh, OVA, and LFD were analyzed by fitting a model that included the fixed effects of line (NeC and NeS), year of birth (2006 and 2007), evaluation (1–18), and the interaction of line by year of birth and lines by evaluation. Statistical analysis was performed using the PROC MIXED procedure of the SAS program version 9.3 (SAS Institute, Cary, NC, USA). The REPEATED command was used to model the residual covariance structure, employing unstructured, first-order ante-dependent and heterogeneous Toeplitz covariance matrices respectively for ETh, OVA, and LFD. Regressions of ETh, OVA, and LFD least square means on the evaluation and Pearson correlation between ETh and OVA was also determined.

Repeated measures for the presence or absence of the CL were analyzed using the PROC GENMOD with a probit link function. The model included the fixed effects of year of birth and the interaction of line by evaluation, without modeling residual variance of repeated measures. This analysis was also conducted using PROC LIFEREG with the variable age at puberty; results were similar to those obtained in the CL analysis with PROC GENMOD. Birth weight, WW, and age and weight at puberty estimated per line and per CL class (absence or presence of CL; 0 or 1, respectively), were obtained with the PROC MIXED procedure, without modeling residual variance of repeated measures. The model included the fixed effects of year and month of birth, age class of the dam (for BW and WW), and CL class.

The effects of puberty on ETh, OVA, and LFD were evaluated by estimating the means per CL in a model that included the effects of year of birth, evaluation, CL, and of evaluation by CL interaction. For this purpose, the PROC MIXED procedure with the REPEATED command was used (as described previously in this section). For heifers that reached puberty during the experiment, the CL class was equal to 1 in all evaluations.

3. Results

3.1. Weight, body conformation score, and hip height

Weight gain, body condition, and pasture growth occurred consistently, except for the beginning of the experiment and in July and August (because of reduced forage availability during the dry season). During this period, heifers did not lose weight or body condition, nor did they gain weight. The NeS heifers had better BCS in the first, second, sixth, ninth, 10th, 11th, 13th, and 14th evaluations ($P < 0.05$; Fig. 1). Therefore, a difference in HH and weight between the two lines from the beginning to the end of the study (Figs. 1 and 2) was evident.

There were differences between the nonpubertal and pubertal groups in age, WW, and weight at puberty (Table 1). In both lines, pubertal heifers were heavier at weaning and reached puberty at younger ages and heavier weights than nonpubertal heifers (Table 1). Regarding HH, pubertal NeC heifers were larger than nonpubertal animals. The BCS for pubertal heifers of the NeS line was greater than for nonpubertal heifers. There were no significant differences in BW between pubertal and nonpubertal heifers in either line (Table 1). Heifers of the NeC line reached puberty at a mean of 23.4 months and 296.9 kg, and those of the NeS line reached puberty at a mean of 22.7 months and 343.5 kg (Table 1).

3.2. Ultrasonographic assessment

The first heifers of the NeS line had reached puberty by 18.3 months, and heifers of the NeC line reached puberty later, by 20.4 months of age (Fig. 3). Although NeC heifers started to cycle later than NeS heifers, there was no difference ($P > 0.05$) between lines at the end of the experiment when the heifers averaged 24.4 months old (NeC, 52%; NeS, 48%) (Fig. 3).

Ovarian area did not differ between evaluations in the NeC or NeS lines (Fig. 4, left panel). However, in the combined evaluation of all heifers, the average OVA was greater in pubertal heifers after the eighth evaluation (17.4 months) than in nonpubertal heifers, except in the 10th ($P = 0.0683$) and 13th evaluations ($P = 0.0642$; Fig. 4, right panel). The average OVA was 4.15 ± 0.06 cm^2 for pubertal heifers and 3.15 ± 0.03 cm^2 for nonpubertal heifers ($P < 0.05$; Fig. 4, right panel).

Endometrial thickness was greater in the NeS than in the NeC line ($P = 0.048$) only in the third evaluation (14.4 months), with a linear increase in the two lines (Fig. 5, left panel). In the last evaluation, average ETh was 11.74 and 11.80 mm in the NeC and NeS lines, respectively ($P > 0.05$). Comparing lines according to puberty, there was

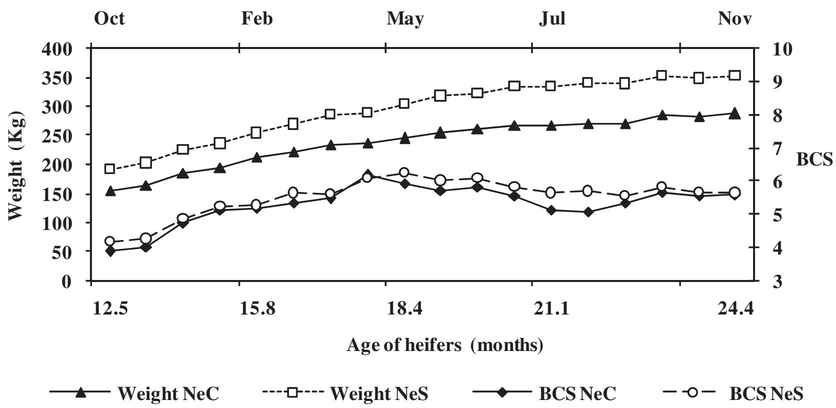

Fig. 1. Least square means of weight and BCS for the NeC and NeS lines. BCS, body condition score; NeC, control Nellore; NeS, selection Nellore.

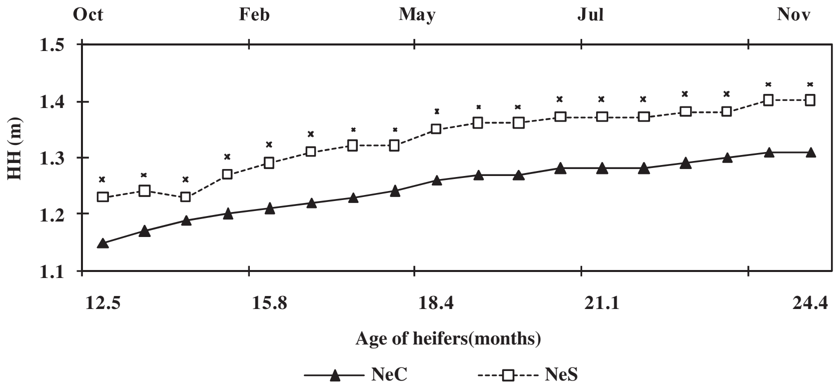

Fig. 2. Least square means of HH for the NeC and NeS lines (* $P < 0.05$). HH, hip height; NeC, control Nellore; NeS, selection Nellore.

a difference in ETh between pubertal and nonpubertal heifers in all evaluations after the fourth evaluation (14.9 months; $P < 0.05$; Fig. 5, right panel). Average ETh was 12.15 ± 0.08 mm for pubertal heifers and 9.93 ± 0.04 mm for nonpubertal heifers ($P < 0.05$; Fig. 5, right panel). The Pearson correlation between ETh and OVA was 0.58 ($P < 0.01$).

During ultrasonographic evaluation, the LFD was always measured in both ovaries (Fig. 6). There was a difference in LFD between the NeS and NeC lines in the following evaluations: fourth (15.1 months), sixth (16.4 months), seventh (17.1 months), eighth (17.7 months), 11th (19.7 months), 13th (21.1 months), 14th (21.7 months), and 17th (23.8 months) (Fig. 6, left panel). As can be seen in Figure 6 (right panel), pubertal heifers had a larger LFD in the third (14.1 months), fourth (14.9 months), fifth (15.6 months), and 12th (20.2 months) evaluations, and the LFD was greater in nonpubertal heifers in the 17th (23.8 months) and 18th evaluations (24.4 months). The LFD was 9.6 ± 0.14 mm for pubertal heifers and 9.19 ± 0.06 mm for nonpubertal heifers ($P < 0.05$; Fig. 6, right panel).

Regression analysis of reproductive traits means across evaluations (1–18) detected an increase of 0.08 ± 0.005 cm^2 in OVA ($R^2 = 0.94$), of 0.18 ± 0.004 mm in ETh ($R^2 = 0.99$), and of 0.09 ± 0.01 mm in LFD ($R^2 = 0.73$) per evaluation. Serum progesterone concentrations of pubertal heifers were 6.01 ± 2.73 ng/mL, compared with 0.54 ± 0.33 ng/mL for heifers that had not reached puberty.

4. Discussion

4.1. Reproductive traits and manifestation of puberty

One of the main issues addressed in this study was the influence of genetic gain in body weight on reproductive traits in *Bos indicus* heifers. Mercadante et al. [7] evaluated direct and correlated responses of selection for postweaning weight on reproductive performance (days to calving and calving success) in heifers and cows of the NeS and NeC lines. The authors concluded that selection for weight promoted high and consistent responses in postweaning weight and height without altering reproductive performance of female cows. Heifers born between 1993 and 1996 were analyzed in that study. Evaluating cattle from the same lines but born between 1997 and 2002, Razook and Mercadante [12] reported differences in the rate of calving success (higher in the NeC line compared with the NeS line). The authors suggested two possible explanations for this difference. The first explanation was that the difference in weight between selected and control females increased with increasing generations of selection for postweaning weight, as do nutritional requirements that also influence reproductive outcomes. The second explanation was the occurrence of a considerable decrease in the availability and quality of pasture forage, which impairs animal development and reduces pregnancy rates [12].

Table 1
Least square means (±SEM) of age, HH, BCS, BW, WW, and WP of heifers according to CL class (nonpubertal and pubertal) and line.

Item	NeC (N = 46)		NeS (N = 77)	
	Nonpubertal	Pubertal	Nonpubertal	Pubertal
N	22	24	40	37
Age (mo)	24.2 ± 0.2[a]	23.4 ± 0.2[b]	24.0 ± 0.2[a]	22.7 ± 0.2[b]
HH (m)	1.30 ± 0.008[a]	1.32 ± 0.009[b]	1.38 ± 0.007[a]	1.38 ± 0.008[a]
BCS	5.5 ± 0.09[a]	5.9 ± 0.10[a]	5.3 ± 0.10[a]	5.8 ± 0.13[b]
BW (kg)	24.2 ± 0.19[a]	24.4 ± 0.42[a]	30.5 ± 0.18[a]	30.9 ± 0.43[a]
WW (kg)	156.0 ± 0.75[a]	161.1 ± 1.80[b]	172.9 ± 1.03[a]	180.7 ± 2.43[b]
WP (kg)	276.7 ± 6.0[a,c]	296.9 ± 6.4[b]	327.9 ± 5.2[a,c]	343.5 ± 5.8[b]

Abbreviations: BCS, body condition score; BW, birth weight; HH, hip height; NeC, control Nellore line; NeS, selection Nellore line; WP, weight at puberty; WW, weaning weight.
[a,b] For each line, fitted means in the same row without a common superscript letter differed ($P < 0.05$).
[c] Weight of heifers that had not reached puberty was measured in the last evaluation (18th evaluation).

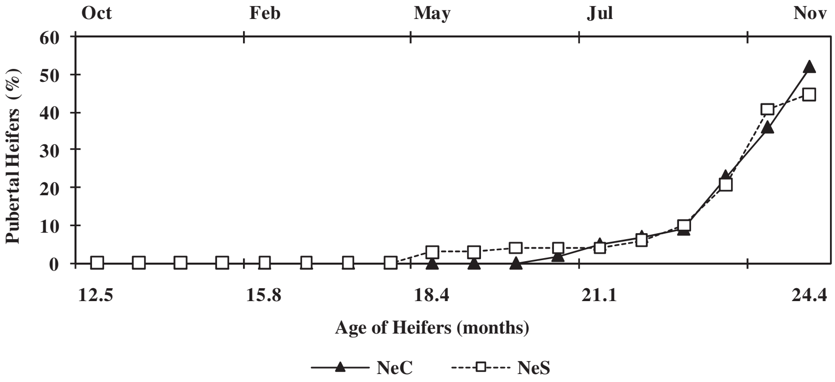

Fig. 3. Percentage (least square means) of heifers in the NeC and NeS lines that had reached puberty. NeC, control Nellore; NeS, selection Nellore.

Based on ultrasonographic evaluation of reproductive tracts (ovary and endometrium) no differences were detected between the NeC and NeS lines, especially at the onset of puberty before the heifers entered the breeding season at approximately 24 months of age. Endometrial thickness was greater in NeS heifers only in the third evaluation at 14.4 months of age ($P = 0.048$) and increased linearly across the 18th evaluations in the two lines. This parameter was similar in the NeC and NeS lines in the last evaluation (24.4 months of age; 11.74 and 11.80 mm, respectively; $P > 0.05$). Ovarian area was another important parameter analyzed, which did not differ between the NeS and NeC lines during the study period. In contrast, there were significant differences in LFD at some ages (15.1, 16.4, 17.1, 17.7, 19.7, 21.1, 21.7, and 23.8 months), being greater in the NeS line than in the NeC line. However, no difference was observed in the last evaluation at 24.4 months (NeS, 10.45 mm; NeC, 10.70 mm; $P > 0.05$).

The first heifer in which a CL was detected by ultrasound at 18.3 months (ninth evaluation) and subsequently confirmed by quantification of progesterone using a radioimmunoassay, belonged to the NeS line. In the NeC line, the onset of puberty occurred only at 20.4 months (12th evaluation). However, there was no significant difference between the two lines in the percentage of pubertal animals in the last (18th) evaluation at 24.4 months (NeC, 52.1% [24/46]; and NeS, 48.0% [37/77]). Excluding the effect of line on these traits, all heifers of the two lines (NeC and NeS) were analyzed to determine the relationship between the ultrasound-measured reproductive traits and onset of puberty. Heifers that reached puberty had an ETh (12.15 $\pm$ 0.08 mm) greater than that in nonpubertal heifers (9.93 $\pm$ 0.04 mm; $P < 0.01$). Therefore, the increase of ETh was an indicator of the manifestation of first estrus and consequent ovulation and elevated progesterone concentrations. Furthermore, the Pearson correlation between ETh and OVA (0.58) suggested a beneficial effect of ovarian function in the development of the uterine endometrium, probably because of increased estradiol concentrations just before ovulation.

Reproductive tract scoring was developed at Colorado State University (Fort Collins, CO. USA), as a tool to evaluate pubertal status in heifers [13]. One of the parameters used was uterine horn diameter. In this case, a minimum diameter of 30 mm with good tone is required for the definition of puberty in *Bos taurus* heifers [13,14] and 25 mm for *Bos indicus* heifers [15]. This value for *Bos indicus* heifers differs from that observed in the present study in which heifers started to cycle at an ETh of 12.15 mm. This difference might be explained by the fact that in the above study [15] the animals were evaluated subjectively by transrectal palpation, and ultrasonography was used in the present investigation, which is a more accurate method. Recently, using transrectal palpation [16] and ultrasonography for

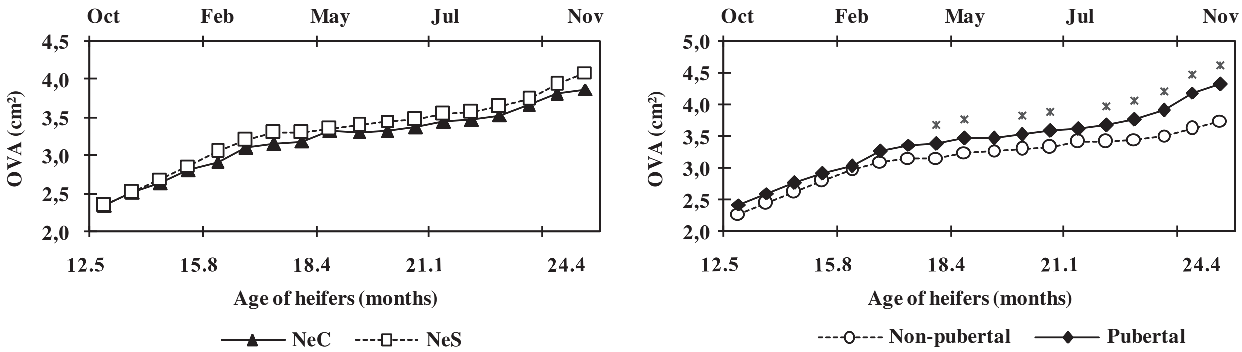

Fig. 4. Comparison of least square means of average OVA between the NeC and NeS lines (left panel) and between nonpubertal and pubertal heifers (right panel) at 12 and 24 months of age (* $P < 0.05$). NeC, control Nellore; NeS, selection Nellore; OVA, ovarian area.

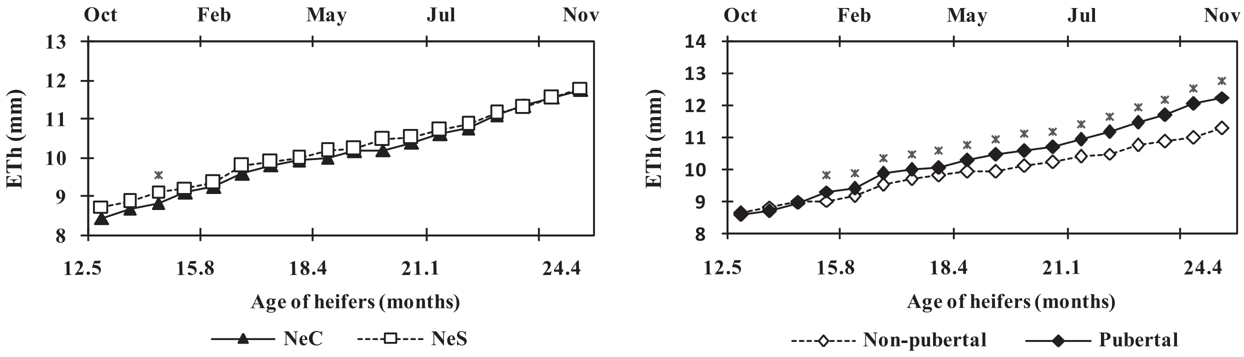

Fig. 5. Comparison of least square means of ETh between the NeC and NeS lines (left panel) and between nonpubertal and pubertal heifers (right panel) at 12 and 24 months of age (* P < 0.05). ETh, endometrial thickness; NeC, control Nellore; NeS, selection Nellore.

uterine horn analysis, diameters of 13.9 ± 0.3 and 13.5 ± 3.8 mm for *Bos indicus* heifers aged 24 to 27 months were reported, respectively [17], similar to that observed in the present study (12.15 ± 0.08 mm). In the case of pasture-fed animals, ETh has been indicated as an important parameter to select prepubertal heifers for AI programs and natural breeding of *Bos taurus* [13,14,17] and *Bos indicus* cattle [15,16]. This parameter could therefore be used for the selection of precocious heifers for early breeding programs.

Ovarian development was monitored by ultrasonography in NeC and NeS heifers. The ovaries increased in size until 24 months of age, with no significant difference between herds. However, the OVA was higher in pubertal heifers in comparison with nonpubertal heifers (pubertal: 4.15 ± 0.01 and nonpubertal: 3.15 ± 0.01 cm^2, respectively; $P < 0.01$). These results were consistent with Andersen et al. [13], Ferreira et al. [15], and Holm et al. [14], who reported a higher percentage of estrus manifestation and higher pregnancy rate among prepubertal heifers with larger ovaries. According to Honaramooz et al. [18], the most marked growth of the ovaries during reproductive tract development occurred between 2 and 14 weeks of age and again between 34 and 60 weeks; thereafter, the size of the ovary remains unchanged until the heifer ovulates. These results were in contrast to the present study in which a continuous increase of ovarian size occurred until heifers reached puberty.

Preovulatory follicular diameter has been used over the past years as an important indicator of cow and heifer fertility [19,20], and a parameter for reproductive tract scoring [13,14]. In the present study, LFD was always measured in both ovaries. Heifers of the NeS line had a greater LFD than NeC heifers in eight of the 18 evaluations; however, there was no significant difference between lines in the last assessment (24.4 months of age). Again, when heifers were classified according to pubertal status, pubertal heifers had a larger follicle diameter (9.56 ± 0.15 mm) than nonpubertal heifers (9.19 ± 0.06 mm; $P < 0.01$).

Preovulatory follicles of *Bos taurus* and *Bos indicus* are morphologically different. To determine the diameter when follicles acquired ovulatory capacity, Gimenes et al. [21] treated *Bos indicus* heifers with follicles of various sizes (7–8.4, 8.5–10, and >10 mm) with LH 25 mg. In that study, three of nine heifers ovulated when the follicles had a diameter of 7.0 to 8.4 mm and the response to LH increased when the follicle diameter reached 8.5 to 10 mm (80%) or exceeded 10 mm (90%). Based on these results, it was speculated ovulatory capacity was acquired at smaller diameters in *Bos indicus* than in *Bos taurus*. Although the heifers studied here possessed follicles that had acquired ovulatory capacity according to Gimenes et al. [21], follicle diameter was greater in pubertal heifers (9.56 ± 0.15 mm) than in those that had not reached puberty at 24 months of age (9.19 ± 0.06 mm).

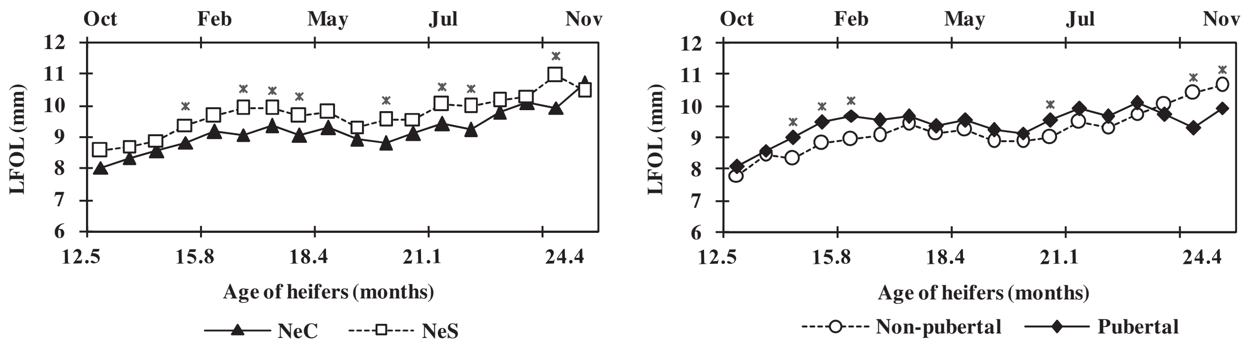

Fig. 6. Comparison of least square means of LFOL between the NeC and NeS lines (left panel) and between nonpubertal and pubertal heifers (right panel) at 12 and 24 months of age (* P < 0.05). LFOL, largest follicle diameter; NeC, control Nellore; NeS, selection Nellore.

4.2. Growth traits and manifestation of puberty

Although NeS heifers, on average, reached puberty 2.1 months earlier and were 55 kg heavier than NeC heifers, there was no significant difference between lines in manifestation of puberty at 24 months of age. Therefore, the present results differed from anecdotal evidence in tropical regions that smaller heifers (NeC) reached puberty earlier than larger heifers (NeS), because of lower nutritional requirements. According to Wolfe et al. [22], experiments using lines selected for weaning and postweaning weight had small differences between selection lines in age and weight at puberty.

Weight gain, body condition, and pasture growth remained unchanged in heifers of the two lines (NeC and NeS), except for the beginning of the experiment and in July and August as a consequence of the dry season. There were differences between NeS and NeC heifers in terms of weight, HH, BW, and WW in all evaluations. However, in each line, no differences were detected in BW between nonpubertal and pubertal heifers, whereas WW was greater in pubertal heifers in both lines ($P < 0.05$).

In addition, heifers reached puberty at heavier weight and at an earlier age. According to Roberts et al. [23], age at puberty is more affected by the growth rate before weaning and during the early stages after weaning than by the growth immediately before mating.

The mean adjusted weight of heifers that reached puberty was 296.9 ± 6.4 and 343.5 ± 5.8 kg at 23.4 ± 0.2 and 22.7 ± 0.2 months in the NeC and NeS lines, respectively. These results agreed with other studies reporting an age at puberty for *Bos indicus* of 22 to 36 months and age at first calving of 34 to 48 months [24–27]. The age of heifers at puberty is directly related to weight and body condition. According to Ferrell [28], heifers that consumed more energy and had a higher daily weight gain rate reached puberty at younger ages. Similarly, in the present study, pubertal heifers were heavier and younger than nonpubertal animals. In *Bos taurus* beef heifers, the onset of puberty is defined when the animals reaches approximately 60% of its mature weight. This percentage is approximately 65% for *Bos indicus* heifers [28,29]. The mean weight of adult cows (5–6 years of age) of the NeS line, born between 2005 and 2009, was 506 kg. Therefore, NeS heifers reached puberty at 68% (343.5/506.2 kg) of their mature weight, a percentage similar to that related for several other research studies [28–30]. In contrast, NeC heifers needed to reach a higher percentage of mature weight (76.4%; 296.9/388.4 kg) to initiate puberty.

Environmental factors, nutritional status, BCS, and pre- and postweaning weight gain are important factors to reduce age at puberty [30]. Postweaning nutrition plays a key role in the determination of age at puberty [31]. In this respect, the main reasons for the late onset of puberty in Zebu herds are the seasonal forage production, poor pasture management, and lack of dietary supplementation during growth. Although Zebu breeds reach puberty later than Taurine cattle, it is possible to reduce the age at puberty as demonstrated in studies in which first breeding occurred at 14 months of age [32]. Similar results have been reported by De Lucia et al. [33], who reported a pregnancy rate of 32% for Nellore heifers at 15 months of age. Recently, Pinheiro et al. [34] studied a line of Nellore heifers selected for weight similar to the NeS line. The animals were kept in a feed lot and were given nutritional supplementation for 140 days after weaning. Ultrasound detected a CL in 21.3% of the heifers at 14 months of age and in 71% at 24 months. These results appeared to differ from the present study in which none of the heifers had reached puberty at 15 months of age and only 50% had reached puberty at 24 months. In a literature review, 50% of half-blood *Bos indicus* heifers were able to breed at 15 months of age when fed a diet designed to obtain high daily weight gain [35]. However, a high proportion of prepubertal heifers were observed in *Bos indicus* herds even among animals older than 2 years, probably because of a low body weight and lack of fat reserves [35]. These results agreed with the present study in which only 52% and 48% of NeC and NeS heifers, respectively, had reached puberty at 24 months.

4.3. Conclusions

Among the reproductive traits studied in heifers, OVA and ETh were suitable indicator traits of sexual precocity in pasture management systems. Selection for body weight did not alter ovarian or endometrial development, nor manifestation of puberty at 24 months of age. Weaning weight and weight at puberty had a significant positive effect on manifestation of first estrus.

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