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Characterizing emergence and divergence in the first follicular wave in a tropically adapted *Bos taurus* breed

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ABSTRACT

Tropically adapted *Bos taurus* breeds are descended from the cattle which were brought from the Iberian Peninsula to Latin America by the colonizers and which adapted over time to local conditions. These breeds have become a genetic treasure that must be preserved. The objective of this study was to characterize ovarian follicular emergence and divergence in tropically adapted *B. taurus* cows and heifers. Cyclic heifers (n = 11) and nonlactating, multiparous cows (n = 11) were examined at 8-hour intervals using ultrasonography starting on the day following the final application of $PGF_{2\alpha}$, which was used to synchronize the estrous cycle, and ending 5 days after ovulation. Blood samples were collected immediately following the ultrasonographic examinations in order to evaluate FSH and estradiol-17β (E_2) concentrations. The interval between ovulation and follicular wave emergence (11.6 ± 3.3 hours vs. 20.3 ± 2.5 hours, $P < 0.05$) and ovulation and follicular wave divergence (52.4 ± 5.2 hours vs. 71.8 ± 4.1 hours, $P < 0.05$) was shorter in the cows than in the heifers, respectively. Plasma FSH concentrations increased ($P < 0.05$) and serum E_2 concentrations decreased earlier in cows than in heifers before ovulation. Following follicular wave emergence, no difference in follicular development was found between the cows and the heifers. Consequently, following follicular wave emergence, the data from both the cows and the heifers were combined and categorized by dominant follicle (DF) and second largest follicle (SF). The DF and SF were identified at the same time ($P > 0.05$). The mean number of small (≤4 mm, 7.2 ± 5.1) and medium (4 to ≤ 8 mm, 6.8 ± 3.5) follicles was greater than that of large follicles (≥8 mm, 0.6 ± 0.5) from ovulation until 5 days after ovulation. The DF diameter (8.1 ± 1 mm) did not differ ($P = 0.09$) from SF diameter (7.6 ± 0.9 mm) at the time of follicular divergence (around 45 hours after follicular emergence). The DF and SF growth rates were similar ($P > 0.05$) until follicular divergence, at which point the SF growth rate decreased, whereas the DF growth rate remained constant. Serum E_2 concentrations did not change ($P > 0.05$) during the divergence period, whereas FSH concentrations decreased between 48 and 32 hours before follicular wave divergence. In conclusion, cows and heifers differed only in the interval from ovulation to follicular wave emergence and divergence, which was confirmed by the different patterns of FSH and E_2 concentrations.

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1. Introduction

Tropically adapted *Bos taurus* breeds, such as the Curraleiro Pé-Duro, originate from Iberian cattle, brought to Latin America during the colonization more than 500 years ago, which have adapted to the different ecosystems and climatic conditions encountered in the various regions of Brazil [1]. Throughout years of natural selection, the animals have become extremely hardy and resistant to dry weather, high temperatures, low-quality pastures, diseases in general, endoparasites and ectoparasites [2,3].

At present, although still at risk of extinction, this genetic group has attracted the interest of some cattle raisers due to its rusticity and adaptability which gives it an advantage in systems with reduced inputs. This genetic group may contribute to the national cattle industry due to its high prolificacy and reproductive efficiency, small size, heat tolerance, and natural resistance to diseases and parasites, especially in less-favorable environments (cost × benefit relationships), or *via* the introduction of those traits into commercial breeds [4].

The use of reproductive biotechnologies such as fixed-time artificial insemination (FTAI), multiple ovulation and embryo transfer (MOET), and *in vitro* embryo production (IVP) would contribute greatly to the preservation and dissemination of this breed. Studies on follicular emergence [5,6] and divergence [7,8] in *B. taurus* and *Bos indicus* breeds have already been performed, allowing the practical application of these biotechnologies [9,10]. Similarities to some aspects of these studies were found. However, some differences between the *B. taurus* and *B. indicus* breeds [11,12] led to adjustments in hormonal therapy for the management of the reproductive cycle. Thus, it is important to study the physiology of reproduction of tropically adapted *B. taurus* breeds such as the Curraleiro Pé-Duro, for which there is no information regarding these physiological events, in order to increase the chances of success using FTAI, MOET, and IVP.

The objectives of this study were to characterize the first follicular wave and the concentrations of FSH and estradiol-17β (E_2) during follicular wave emergence and follicular wave divergence in Curraleiro Pé-Duro (tropically adapted *B. taurus*) heifers and cows that were born and raised in a dry tropical environment, as their ancestors had been for hundreds of years.

2. Materials and methods

All procedures described herein were approved by the Animal Health and Welfare Committee at the Embrapa Genetic Resources and Biotechnology Center (protocol number 02/2013).

2.1. Location, feed management, and animals

This study was conducted between March and April at a ranch located in Brasília, DF, Brazil (15°52′-15°56′S e 48°00′-48°02′O), with altitudes ranging from 1050 m to 1250 m above sea level. The predominant climate is the Koppen Aw, indicating dry winters (relative humidity can be as low as 10%) and rainy summers.

Eleven multiparous Curraleiro Pé-Duro (tropically adapted *B. taurus*) cows ranging from 3 to 8 year old (mean of 5 ± 1 year old), and 11 Curraleiro Pé-Duro heifers between 2 and 3 year old (mean of 2.5 ± 0.5 year old) with a mean body condition score (BCS) of 3 (ranging from 2.5–3.5) on a scale from 1 to 5 (where 1 = emaciated and 5 = obese; [13]) were used in this study. During the experiment, the animals were kept in a pasture of *Brachiaria decumbens* with mineralized salt and water provided ad libitum.

2.2. Estrus synchronization and ultrasonographic examinations

Before the beginning of the study, the cows were evaluated *via* transrectal palpation, ultrasonographic examinations (Mindray 2200 Vet, Shenzhen, China, equipped with a 7.5-MHz transrectal transducer), and vaginoscopic examinations to determine the absence of diseases and abnormalities in their reproductive tracts.

Estrous cycles of nonlactating, tropically adapted *B. taurus* females (n = 22) were synchronized with two im doses of D-Cloprostenol (0.150 mg $PGF_{2\alpha}$; Prolise, Tecnopec LTDA, São Paulo, Brazil) administered 11 days apart. After the second $PGF_{2\alpha}$ application, the animals were observed for 0.5 hour three times a day for estrous behavior with the aid of a vasectomized bull. Onset of estrus was considered to have occurred when a cow stood to be mounted by another cow or by a vasectomized bull.

Ultrasonographic examinations were performed thrice daily (8 AM, 4 PM and 12 PM) starting on the day following the last $PGF_{2\alpha}$ application until 5 days after ovulation, and subsequently every 24 hours for two consecutive days to clearly identify the dominant follicle (DF). During each examination, ovarian maps were drawn to record the diameter, the number, and relative position of small (≤4 mm), medium (4 to ≤8 mm), and large (>8 mm) follicles and corpus luteum (CL). Follicular diameter was determined by the average of two perpendicularly measured diameters of each follicle. Ovulation was defined as the disappearance of a previously identified DF (≥10 mm) between one ultrasound examination and the next (8 hours apart), and was confirmed by the subsequent formation of a CL.

Follicular wave emergence was defined as occurring on the last examination in which the retrospectively identified DF was 4 mm [14]. The DF was identified as being the largest follicle that grew to a diameter of greater than 10 mm and that was at least 2 mm larger than the second largest follicle (SF). The beginning of follicular wave divergence was defined as the beginning of the greatest difference in growth rates (follicular diameter changes between successive ultrasound examinations) between DF and SF at or before the examination in which the SF reached its maximum diameter [14].

2.3. Blood collection and sample assays

Immediately after the ultrasonographic examinations (8 hours apart), blood samples for the analysis of serum E_2 and plasma FSH levels were collected *via* jugular vein

using 9 mL tubes (Labor Import, Osasco, São Paulo, Brazil) without EDTA and 5 mL tubes (Labor Import) containing EDTA, respectively, from 2 days before ovulation until 4 days after ovulation. Immediately after collection, the blood samples were kept at 4 °C in an isothermal box with ice for 10 to 15 minutes and then centrifuged at ×1500*g* for 20 minutes. Subsequently, serum and plasma were removed and stored at −20 °C until they could be tested for E_2 and FSH concentrations, respectively. Serum E_2 concentrations were determined using a solid-phase RIA kit with antibody-coated tubes (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA, USA). Test sensitivity was 2.2 pg/mL, and the intra-assay and interassay coefficients of variation were 8.9 and 12.2%, respectively, for high control (102.8 pg/mL) and low control (5.7 pg/mL). Plasma FSH concentrations were determined using an in-house RIA kit which has been adapted and validated for bovine FSH using USDA-bFSH for iodination and Bolt and Rollins [15] reference standards, and NIDDK oFSH antiserum. Test sensitivity was 0.025 ng/mL and interassay and intra-assay coefficients of variation were 10.55% and 13.22% for high control (2.5 ng/mL) and 12.1% and 14.8% for low control (1.5 ng/mL), respectively.

2.4. Statistical analysis

The statistical analyses were carried out using R Core Team 2013 free statistical software (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) and the level of significance was considered to be $P < 0.05$.

Fitting of the follicular development curve was performed using a linear regression model (least squares method), in which the dependent variable is the size of the dominant follicle and subordinate follicle, respectively, and the covariate is the time of evaluation. To evaluate the goodness of fit, the coefficient of determination (R^2) was used as a criterion.

The data on follicular size and growth rate were analyzed after normalization to the time of follicular emergence and divergence. The main effects of the sequential tests were examined for differences between averages using paired t-tests. Through the generalized linear model (GLM) using Gaussian distribution, the effects of follicular category, time, and follicle category by time interaction on follicular size and growth rate were ascertained. The suitability of a normal distribution (Gaussian) was analyzed using graphs of simulated waste envelopes. If there was no accommodation of the classic normal distribution, the binding function was changed to log, inverse, or square root. Follicular growth rate (mm/8h or mm/16h) in each wave was calculated by the ratio of the variation observed in the follicular diameter and the number of observation intervals. Data was included from the first day on which the follicle was observed until the day the maximum diameter was reached. FSH and E_2 concentrations were normalized to the time of ovulation and follicle divergence, and the effect of category and time on these hormones was detected through GLMs with normal distribution and binding function. Given the existence of a significant time or category effect, orthogonal contrasts were applied to obtain comparisons between the means. The data were presented as mean ± standard deviation (SD).

3. Results

Through regression analysis (data not shown) and comparison of means, most of the characteristics studied showed no differences between the cows and the heifers. Only the intervals between ovulation and follicular wave emergence and ovulation and follicular wave divergence differed, with both being shorter in the cows. Consequently, the data (DF and SF growth) from both cows and heifers were combined and normalized to follicular wave emergence or follicular wave divergence. Table 1 summarizes the follicular characteristics in the cows (n = 11) and heifers (n = 11) at follicular wave emergence and follicular wave divergence.

The mean (±SD) number of small (≤4 mm, 7.2 ± 5.1) and medium (4 to ≤8 mm, 6.8 ± 3.5) follicles was greater than the amount of large follicles (≥8 mm, 0.6 ± 0.5) from ovulation until 5 days after ovulation in tropically adapted *B. taurus* females (n = 22, Fig. 1). There were effects corresponding to follicular category, day, and follicular category by day interaction ($P < 0.0001$) throughout the 5 days analyzed, in which the number of small follicles decreased and the number of medium and large follicles increased.

Table 1
Characteristics (mean ± SD) of estrous cycles in tropically adapted *Bos taurus* cows (n = 11) and heifers (n = 11).

Follicular characteristics	Cows (n = 11)	Heifers (n = 11)
Interval from onset of estrus to ovulation (hours)[c]	21.8 ± 3	21.4 ± 1.4
Beginning of follicular wave emergence		
Interval from ovulation to follicular wave emergence (hours)[c]	11.6 ± 3.3[b]	20.3 ± 2.5[a]
Number of recruited follicles	17.6 ± 1.2	15.3 ± 0.9
DF diameter (mm)	4.16 ± 0.1	4.17 ± 0.1
SF diameter (mm)	3.84 ± 0.1	3.81 ± 0.1
Beginning of follicular wave divergence		
Interval from ovulation to follicular wave divergence (hours)[c]	52.4 ± 5.2[b]	71.8 ± 4.1[a]
Interval from emergence to follicular wave divergence (hours)	40.7 ± 4.4	50.9 ± 4.2
DF diameter (mm)	7.9 ± 0.2	8.3 ± 0.4
SF diameter (mm)	7.3 ± 0.2	7.7 ± 0.3
Growth rate (from 48 h before divergence to 48 h after divergence)		
DF before follicular wave divergence (mm/8h)	0.63 ± 0.06[A]	0.56 ± 0.03[A]
SF before follicular wave divergence (mm/8h)	0.61 ± 0.05[A]	0.53 ± 0.03[A]
DF after follicular wave divergence (mm/8h)	0.49 ± 0.02[B]	0.47 ± 0.06[B]
SF after follicular wave divergence (mm/8h)	−0.67 ± 0.06[C]	−0.68 ± 0.04[C]

[a,b]Means with different superscripts within the same line differed ($P < 0.05$).
[A,B,C]Means with different superscripts within the same column differed ($P < 0.05$).
Abbreviations: DF, dominant follicle; SF, second largest follicle.
[c] Hour 0 = hour of ovulation.

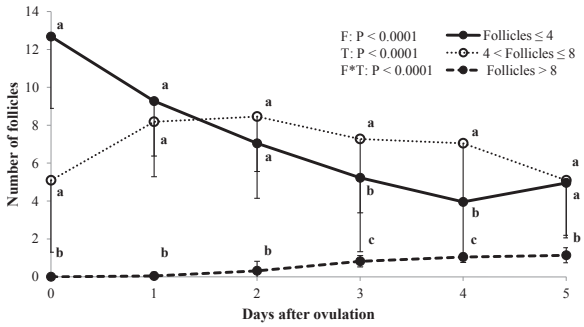

Fig. 1. Number (Means ± SD) of small (≤4 mm), medium (<4 a ≤8 mm), and large (>8 mm) follicles in tropically adapted *Bos taurus* females (n = 22) from the beginning of ovulation (D0) until 5 days after ovulation. F: follicle category effect; T: time effect; F × T: follicle category by time interaction effect. [a,b,c]Values with different superscripts indicate differences (P < 0.05) within follicle category over time.

There were effects corresponding to follicle category, time, and follicle category by time interaction (P < 0.0001) on DF and SF diameters from follicular wave emergence until 104 hours afterward. Figure 2 illustrates DF and SF growth normalized to follicular wave emergence (hour 0). At the time of follicular wave emergence (hour 0), the mean (±SD) diameter of DF (4.1 ± 0.4 mm) was similar to that of the SF (3.9 ± 0.3 mm) and the time of identification of both after ovulation did not differ either for cows (13.8 ± 3.5 hours vs. 16.7 ± 3.5 hours) or for heifers (22.5 ± 2.8 hours vs. 24 ± 3 hours). After the first 24 hours of follicular wave emergence, a gradual divergence in diameters was observed in which the SF ceased to grow, whereas the DF continued to grow. The maximum diameter of DF (11.6 ± 1.1 mm) and SF (7.6 ± 0.8 mm) was reached 4 and 2 days after follicular wave emergence (hour 0), respectively. The beginning of the follicular wave divergence was identified at 45.8 ± 14.8 hours after follicular wave emergence.

When DF and SF growth were normalized to the time of follicular wave divergence (hour 0), differences in development and growth rates were not evident until deviation (Fig. 3A, B). The mean (±SD) DF diameter (8.1 ± 1 mm) did not differ (P = 0.09) from the mean SF diameter (7.6 ± 0.9 mm) at the time of follicular wave divergence. There were effects corresponding to follicle category, time, and category by time interaction (P < 0.0001) in DF and SF diameters when the data were evaluated according to the time of follicular wave divergence.

There were no differences in growth rate between DF and SF before follicular wave divergence, whereas after that point the DF growth rate became greater than that of the SF (P < 0.05). The growth rates of the DF from 32 to 16 hours (1 ± 0.3 mm/16h) and from 16 to 0 hour (0.7 ± 0.3 mm/16h) before follicular wave divergence and from 0 to 16 hours (0.9 ± 0.4 mm/16h) after follicular wave divergence did not differ (Fig. 3B), although they were greater than those seen at 16 to 32 hours after follicular wave divergence (0.4 ± 0.5 mm/16h, P < 0.05). The SF, growth rates from 32 to 16 hours (0.6 ± 0.2 mm/16h) and from 16 to 0 hour (0.7 ± 0.2 mm/16h) before follicular wave divergence also did not differ but were greater (P < 0.05) than those seen from 0 to 16 hours (−0.8 ± 0.5 mm/16h) and 16 to 32 hours (−0.7 ± 0.4 mm/16h) after follicular wave divergence.

Hormonal data normalized to the time of ovulation and the time of follicular wave divergence are shown in Figures 4 and 5, respectively. The concentrations of E_2 decreased (P < 0.05) from 24 hours (3.45 ± 0.53 pg/mL) prior until the time of ovulation (1.28 ± 1.27 pg/mL) in the cows, whereas in the heifers this decrease in concentration was observed from 16 hours (2.32 ± 1.57 pg/mL) before ovulation to 8 hours (0.93 ± 0.71 pg/mL) after ovulation (Fig. 4A). In the cows, FSH concentrations increased (P < 0.05) twice before ovulation (Fig. 4B); from 48 hours (0.29 ± 0.06 ng/mL) to 32 hours prior (0.55 ± 0.08 ng/mL) and from 24 hours (0.44 ± 0.18 ng/mL) to 8 hours (0.63 ± 0.08 ng/mL) before ovulation.

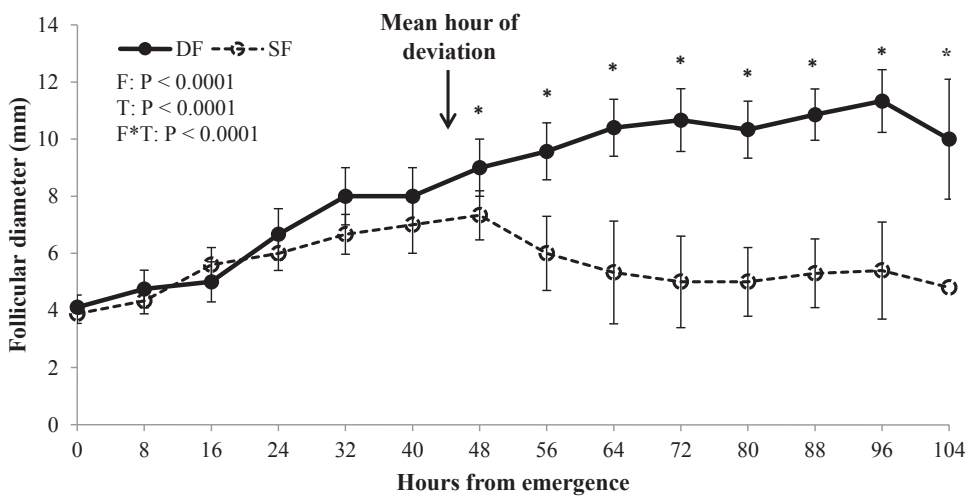

Fig. 2. Comparison of mean (±SD) gradual growth divergence for the dominant (DF, n = 22) and second largest (SF, n = 22) follicles in tropically adapted *Bos taurus* females. The data was normalized to the time of follicular wave emergence (hour 0) during the first follicular wave after ovulation. F: follicle category effect; T: time effect; F × T: follicle category by time interaction effect. *Indicates differences (P < 0.05) between DF and SF over time.

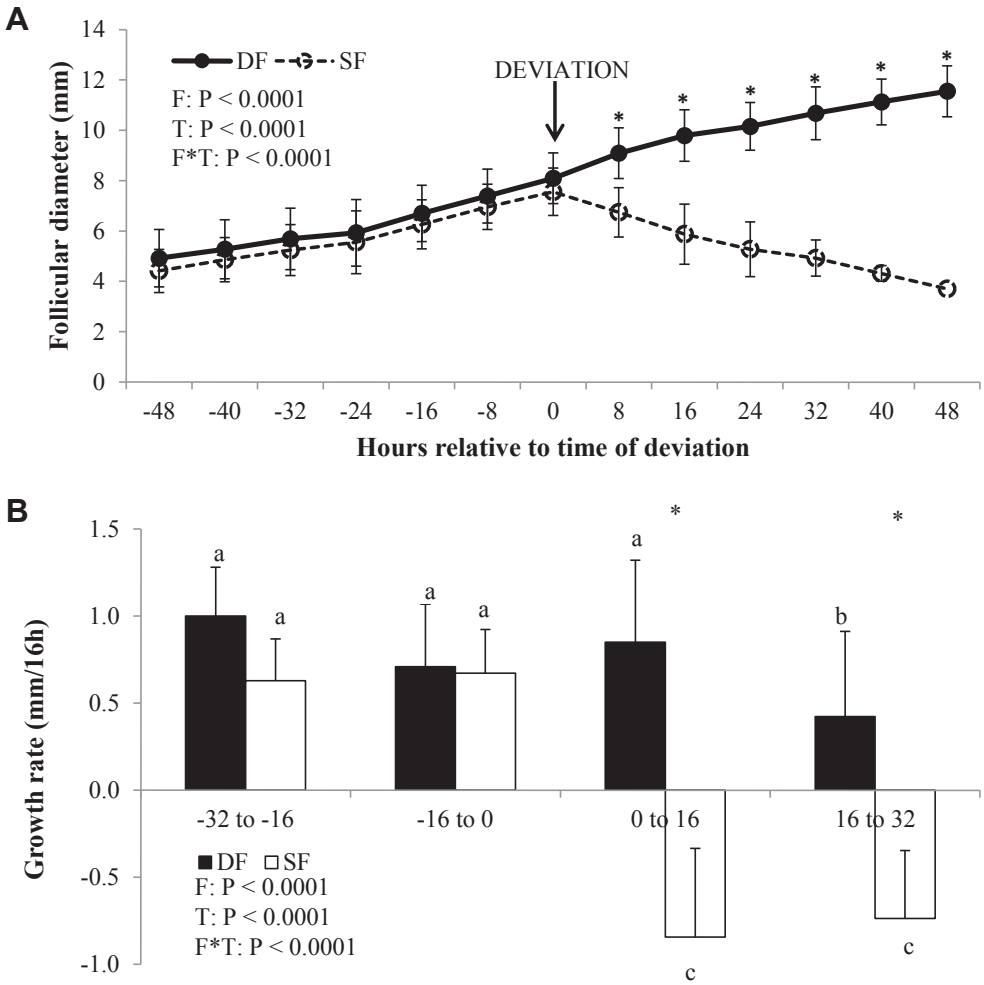

Fig. 3. Follicular diameters (Means ± SD; A) and growth rates (Means ± SD; B) of the dominant follicle (DF, n = 22) and second largest follicle (SF, n = 22) during the first follicular wave after ovulation according to the time of follicular wave divergence (hour 0). F: follicle category effect; T: time effect; F × T: follicle category by time interaction effect. *Indicates differences (P < 0.05) between DF and SF over time. [a,b,c]Values with different superscripts indicate differences (P < 0.05) over time within the same follicle category.

However, FSH concentrations decreased (P < 0.05) in cows from 8 hours (0.63 ± 0.08 ng/mL) before ovulation to 56 hours (0.34 ± 0.14 ng/mL) after ovulation. In the heifers, FSH concentrations increased (P < 0.05) from 48 hours (0.36 ± 0.1 ng/mL) to 16 hours (0.63 ± 0.18 ng/mL) before ovulation. However, FSH concentrations decreased in heifers from 16 hours (0.63 ± 0.18 ng/mL) before ovulation to 48 hours (0.41 ± 0.11 ng/mL) after ovulation.

Hormonal concentrations did not differ between heifers and cows when E_2 and FSH concentrations were normalized to the time of follicular wave divergence (hour = 0). Accordingly, the data were combined (Fig. 5). The concentrations of E_2 did not differ, although there was a time effect (P < 0.0001) on FSH concentrations in relation to the time of follicular wave divergence. The concentrations of FSH decreased (P < 0.05) from 48 hours (0.61 ± 0.24 ng/mL) to 32 hours (0.49 ± 0.13 ng/mL) before follicular wave divergence.

4. Discussion

Identification of the first follicular wave is important to improve the management of the estrous cycle in the use of FTAI, MOET, and IVP. Therefore, this first study on a tropically adapted *B. taurus* breed will be the basis for the development of hormonal protocols that will optimize the use of reproductive biotechnologies for conservation and expansion of these breeds which are considered genetic treasures. The decision to study the first follicular wave was based on the reduced variation in its onset, as compared with the second and/or third waves, and the greater number of follicles recruited [16,17].

There was no difference in the estrus-ovulation interval (21 hours) between cows and heifers; however, it was shorter than that found in a preliminary study with Curraleiro Pé-Duro cattle (unpublished results), which was between 24.8 and 26 hours. However, in the preliminary study, estrus detection was undertaken twice a day,

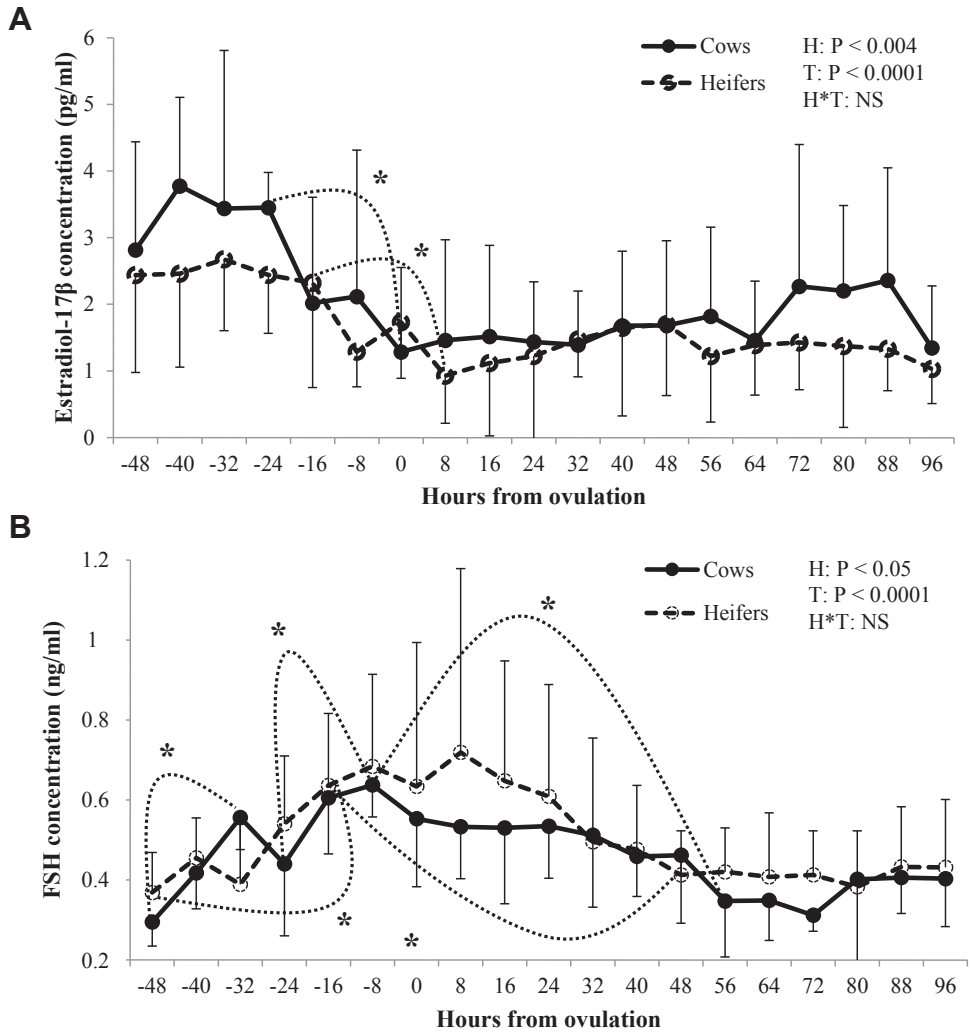

Fig. 4. Estradiol-17β (A) and FSH (B) concentrations (Means ± SD) normalized to ovulation (hour 0) in tropically adapted *Bos taurus* cows (n = 11) and heifers (n = 11). H: hormonal effect; T: time effect; H × T: hormonal by time interaction effect. Stars and dotted lines indicate differences (P < 0.05) between selected means (Means ± SD).

whereas in the present study this was evaluated three times a day. Pinheiro et al. [18], working with Nelore heifers and cows, and Wolfenson et al. [19] working with Holstein heifers and cows also found no difference in the estrus-ovulation interval, 27.7 *vs.* 26.7 hours and 25.4 *vs.* 27.8 hours, respectively. Similar estrus-ovulation intervals (between 22 and 28 hours) were reported by Kornmatitsuk et al. [20] in Holstein cross-bred cows.

Regarding the initial objective of comparing the first follicular wave in cows and heifers, a difference was ascertained only in the period of follicular wave emergence and follicular wave divergence in relation to ovulation. Follicular wave emergence and divergence occurred approximately 10 and 20 hours earlier, respectively, in the cows compared with the heifers (Table 1). The E_2 and FSH concentrations from 48 hours prior until 96 hours after ovulation corroborate this finding (Fig. 4). The concentration of E_2 in the cows showed a reduction about 8 hours before that detected in the heifers, whereas FSH concentration in the cows increased 16 hours before a similar increase was detected in the heifers. Another difference that may have anticipated follicular wave emergence in the cows was the pattern of FSH secretion observed with two abrupt increases in concentration. Research with Holstein heifers also found this pattern of FSH secretion, in which the first increase was related to the LH surge and the second to a decrease in the concentration of LH and E_2 to minimal levels [21,22]. Similar FSH patterns were observed by Kaneko et al. [23] in Japanese brown cattle.

The concentration of FSH (0.2–0.6 ng/mL) around the time of ovulation in the Curraleiro Pé-Duro females corroborates other research with *B. taurus* and *B. indicus* females [7,21]. However, E_2 concentration (2–3.8 pg/mL) before ovulation was less than that seen in Senepol cows (8 pg/mL) [11] and Holstein heifers (7 pg/mL) [21]. Follicle diameter is related to the production of E_2 [24]. In

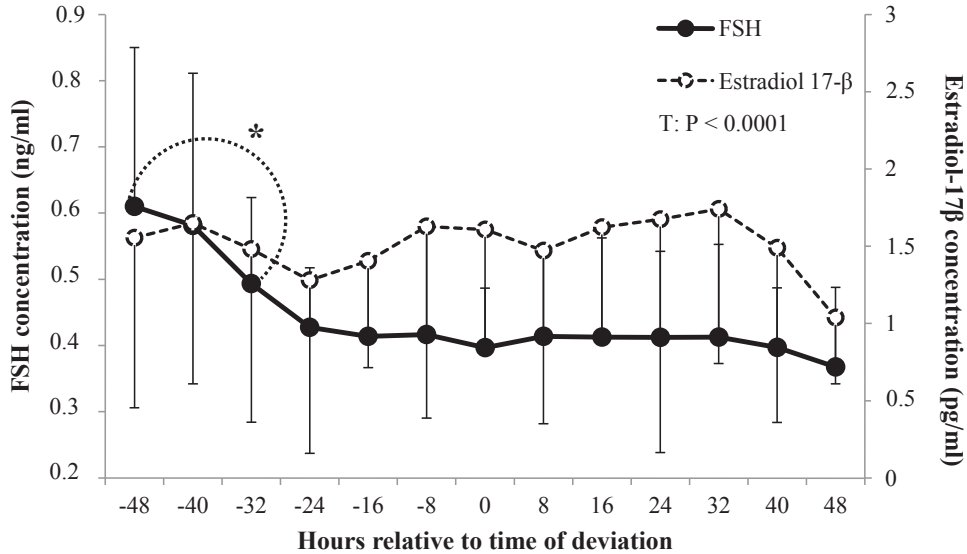

Fig. 5. Estradiol-17β and FSH concentrations normalized to the time of follicular wave divergence (hour 0) during the first follicular wave after ovulation in tropically adapted *Bos taurus* females (n = 22). T: time effect. Stars and dotted lines indicate differences ($P < 0.05$) between selected means (Means ± SD).

Curraleiro Pé-Duro females, the ovulatory follicle (12–13 mm; unpublished data) appears to be smaller than what was reported in other studies with *B. taurus* females (16–17 mm and 13–16.5 mm) [19,25]. The difference observed between the tropically adapted *B. taurus* (Curraleiro Pé-Duro) animals and the research cited with pure *B. taurus* animals may be related to the adaptive characteristics that these animals acquired throughout 500 years of natural selection in a tropical environment and the possible introgression of genes into the breed due to crossbreeding with Indian animals over the years [2].

The number of small follicles (7.2 ± 5.1) was lower than what was reported by Alvarez et al. [11] with Angus, Brahman, and Senepol cows (tropical *B. taurus*). Reasons for this discrepancy may be (1) the fact that the present study was conducted during the dry season, whereas Alvarez et al. [11] conducted their study during the rainy season; and/or (2) the present study classified follicles less than 4 mm as small, whereas Alvarez et al. [11] classified follicles between 2 and 5 mm as small. However, the number of medium (6.8 ± 3.5) and large (0.6 ± 0.5) follicles was similar. The interval between ovulation and follicular wave emergence in the cows was similar to that found in Holstein heifers (0.3 days) [6], whereas the interval between ovulation and follicular wave emergence in heifers was similar to that observed in Nelore cows and heifers (0.9 days) [26]. In contrast, Figueiredo et al. [27] reported emergence around 1.5 days after ovulation in Nelore females. This greater interval may be due to only one follicular observation being conducted per day instead of conducting two to three follicular observations/day. Research with Nelore and Holstein females reported intervals between ovulation and follicular wave divergence of between 2.4 and 2.8 days [14,26,28,29]. These results are similar to those observed in tropically adapted *B. taurus* Curraleiro Pé-Duro cows and heifers (2.1 and 2.9 days, respectively).

The time of identification of future DF and larger SF relative to ovulation did not differ, and neither did the proportions. Castilho et al. [7] studied Nelore heifers and also found no differences in the time of identification of the DF and SF (approximately 30 hours after ovulation) and their respective sizes (4.2 mm vs. 3.7 mm). In contrast, Ginther et al. [14] and Kullick et al. [30] reported that the DF was identified before the SF, and thus would be further developed in the follicular wave emergence. In this study it was decided to identify the DF at around 4 mm (emergence), since Ginther et al. [14] ascertained the early onset of DF beginning with 3 mm.

The size of the DF (8.1 ± 1 mm) and SF (7.6 ± 0.9 mm) at the time of follicular wave divergence (Fig. 3) did not differ ($P = 0.09$), as was the case in the research with Holstein heifers (7.7–8.5 mm vs. 7.2–7.3 mm) [8,21] and Nelore heifers (5.4–6.2 mm vs. 5.4–5.9 mm) [7,26,29]. The *B. taurus* animals, including the tropically adapted animals such as the Curraleiro Pé-Duro, have a larger DF at divergence than the *B. indicus* animals. This difference may be partially explained by FSH levels and a growth factor similar to insulin (IGF-1) in the different genetic groups [11,31]. The IGF-1 appears to be involved in follicular selection [24], because when it is abundantly present in follicular fluid there is an increase in the responsiveness of the DF to FSH [19,32]. Similarly, IGF-1 increases the sensitivity of follicular cells to LH [33]. Based on this, Gimenes et al. [29] reported the hypothesis that increased sensitivity to LH in *B. indicus* cows, due to higher follicular fluid concentrations of IGF-1, anticipates the acquisition of LH receptors in the DF. Consequently, the time of follicular wave divergence is anticipated, and *B. indicus* cows ovulate smaller follicles than *B. taurus cows*.

There was no difference in the growth rate between the DF and SF (Fig. 3A, B) before the follicular wave divergence, whereas afterward the DF maintained a similar growth rate and that of the SF regressed. This finding corroborates with the data produced by Ginther et al. [24] in Holstein heifers. The DF growth rate seems to be higher in Holstein cows (1.5–2.1 mm/day) [21,24] than in *B. indicus* cows (0.9–1.8 mm/12h) [7,29]. This difference is reflected in the DF diameter at the time of follicular wave divergence, which is greater in *B. taurus* animals, as was already mentioned.

As in other studies [8,21,24,30], FSH concentration declined around 48 to 24 hours before follicular wave divergence and remained low up to 48 hours after (Fig. 5). The low FSH concentrations during and after follicular wave divergence were expected due to the higher production of E_2 and inhibin by the DF leading to a negative feedback on FSH [14]. However, in this study, no increase in E_2 concentration after follicular wave divergence was detected, which was not expected. The information of which we are currently aware contradicts other studies that report an increase in E_2 concentration from the time of follicular wave divergence until 24 hours afterward [21,24,30]. Perhaps, the greater assay sensitivity of these studies could explain this difference.

To conclude, follicular wave emergence and divergence occurred earlier in the cows, which can be confirmed by the different patterns of FSH and E_2 concentrations. Dominant follicle size at follicular divergence was similar to that found in *B. taurus* females, and follicular growth rate in these animals was higher than that found in *B. indicus* females. There were no variations in E_2 concentration during and after follicular wave divergence within 8-hour intervals, despite the identification of a growing DF. The data obtained from this research may provide the basis for future scientific research with this breed and for hormonal manipulations to preserve and expand the numbers of tropically adapted breeds such as the Curraleiro Pé-Duro.

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Competing interests

The authors have declared no conflicts of interest.

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