ELSEVIER

OVARIAN FOLLICULAR DYNAMICS IN NELORE BREED (Bos indicus) CATTLE

R.A. Figueiredo,[1] C.M. Barros,[1,a] O. L. Pinheiro[1] and J.M.P. Soler[2]

[1]Departamento de Farmacologia, Instituto de Biociências - UNESP
Botucatu-SP, Brazil
[2]Instituto de Matemática e Estatística - USP, São Paulo-SP, Brazil

Received for publication: March 7, 1996
Accepted: June 31, 1996

ABSTRACT

The most common beef cattle raised in Brazil is the Nelore breed (Bos indicus). Information obtained by ultrasonography on follicular growth in Bos taurus cattle has been accumulating rapidly. However, there are few publications to date on follicular development in Bos indicus breeds. The follicular dynamics in Nelore heifers and cows during natural or prostaglandin (PG)-induced estrous cycle were studied. From the detection of estrus onward, all animals were examined daily by ultrasonography for one (n=35) or two (n=10) consecutive estrous cycles. The follicular dynamic in Nelore cattle was characterized by the predominance of 2 follicular waves in the cows (83.3%, n=18, $P<0.05$) and 3 waves in the heifers (64.7%, n=16, $P<0.05$). Most of the cattle observed over 2 consecutive estrous cycles presented the same pattern of follicular waves in the first and second cycle, and only 30% showed variation in the number of waves from one cycle to the other. Most of the follicular parameters analyzed were not affected by PG treatment or age but were altered by follicular waves. Consequently, data on cows and heifers were combined according to the number of follicular waves. The ovulatory follicle was larger than the other dominant follicles ($P<0.05$), and the ovulatory wave was shorter than the preceding waves ($P<0.05$). The interovulatory interval was longer in animals showing 3 waves than those exhibiting 2 waves ($P<0.05$). Maximum diameter of the dominant follicle (around 11 mm) and of the corpus luteum (CL, approximately 17 mm) were smaller than those reported for European breeds. In conclusion, the results demonstrate that although the dominant follicle and corpus luteum are smaller than in European breeds, the follicular dynamics in Nelore cattle were similar to those observed in European breeds and were characterized by 2 or 3 follicular waves for cows and heifers, respectively, during the natural or prostaglandin-induced estrous cycle.

Key words: follicle, corpus luteum, Bos indicus, Nelore, ultrasound

Acknowledgments
The authors wish to thank Dr. Magaly Manzo (Maracay, Venezuela) for providing progesterone antiserum and L. A. Oliveira for technical assistance.
Research was supported by a grant from FAPESP and CNPq of Brazil.
[a]Correspondence and reprint requests: Fax: +55 (014) 821-3744.

0093-691X/97/$17.00
PII S0093-691X(97)00156-8

INTRODUCTION

Zebu cattle (Bos indicus) is predominant in Brazil and in other tropical and subtropical regions. The Nelore breed represents the majority of Zebu beef cattle in Brazil. To date, there is a paucity of information on the reproductive physiology of this breed. In spite of their higher tolerance to heat stress and better resistance to ticks, Bos indicus cattle usually have lower potential for meat and milk production (30) and lower fertility than Bos taurus cattle (10, 24).

It is important to better understand the physiological peculiarities of Nelore and other Zebu breeds in order to develop strategies for improving the reproductive physiology of these cattle. Ultrasonographic information on follicular development in Bos taurus (14,15, 27, 40, 55) breeds has been used to manipulate the estrous cycle in order to improve estrus synchronization (48, 56, 52), timed artificial insemination (35, 36, 43, 44, 52) and embryo transfer (28, 38).

Little has been published on the follicular dynamics of Zebu cattle. In addition to a paper by Rhodes et al. (1995) on Brahman heifers, there has been an initial report on Nelore cows (8) and on prepubertal Brahman heifers (13). Thus, the purpose of the present study was to characterize the follicular dynamics of Nelore cows and heifers during the natural and the PG-induced estrous cycle.

MATERIALS AND METHODS

Location and Experimental Animals

This study was performed at the farm of the Faculdade de Medicina Veterinária da Universidade Estadual Paulista (UNESP) in Botucatu, São Paulo, Brazil (latitude 22° 51'S; longitude 48° 26'W, altitude 786 m). During the past 24 y, the mean annual temperature has been 20.6°C and the mean temperatures for the hottest and coldest months have been 23.6 and 17.4°C, respectively.

Nelore cows were maintained in pasture (Brachiaria decumbens) with 83% corn and 17% soybean supplementation (1 kg/h/d). Body weight ranged from 334 to 487 kg (418 ± 12 kg, mean ± standard error of the mean, SEM), age from 3.6 to 7.8 yr (5.78 ± 0.4), and body score from 2.0 to 3.5 (2.7 ± 0.14) on a 0 to 5 point scale (26). Heifers received a daily ration (28 kg/h/d, consisting of 92% sugar cane and 8% broiler litter; 560 g/h/d consisting of 90% corn and 9% urea); and had access to pasture (Brachiaria decumbens). Body weight ranged from 321 to 439 kg (378 ± 11.2 kg), age from 25 to 36 mo (30.5 ± 0.98 mo) and body score from 2.5 to 3.0 (2.8 ± 0.08).

Estrous cycles of 25 animals (15 cows and 10 heifers) were studied after natural or prostaglandin-induced estrus. Since 10 animals (3 cows and 7 heifers) were monitored during 2 consecutive cycles (first induced and second natural), a total of 35 estrous cycles were monitored and distributed as follows: cows with induced estrus (n=10), cows with natural estrus (n=8), heifers with induced estrus (n=8) and heifers with natural estrus (9).

Estrus Detection and Ultrasonography

During the winter (July and August) of 2 consecutive years (1992 and 1993) the estrous cycles of nonlactating Nelore cows (n=24) were synchronized with 2 25-mg injections of PGF2α (5 ml Lutalyse[R], Rhodia-Merieux Veterinária Ltda., São Paulo, Brazil) administered 11 d apart. After the second PGF2α injection, the animals were observed twice daily for estrous behavior. Estrus was detected in 50% of the treated animals (n=12). Estrus was synchronized in Nelore heifers (n=24) the same way as in the cows. However, they were observed continuously (24 h a day) over 5 d in January (n=10) and also 5 d in July (n=14) 1994. Estrus was detected in only 30 and 50% of the heifers treated in January and July, respectively. In addition, follicular growth was monitored in 3 cows and 7 heifers from the above groups, over 2 consecutive estrous cycles; the first cycle was induced with PGF2α, while the second cycle was natural. A total of 35 cycles were monitored in cows (n=15, 18 cycles) and heifers (n=10, 17 cycles).

After estrus detection, follicular and corpus luteum development were assessed via daily ultrasonography using an Scanner 450 VET (Pie Medical, Maastricht, Netherlands) for cows and an Aloka 210 (Aloka Co. Ltd, Tokyo, Japan) for heifers, both with a 5.0 MHz transrectal transducer. During each examination, ovarian maps were drawn to record size and relative position of follicles ≥ 4 mm and the size of CL and ovaries (40).

For individual follicles the time from the first measurable diameter by ultrasonography (≥ 4 mm) and the last day that the maximum diameter was recorded was defined as the growth period, the time from decline in maximum diameter until the last measurable size was defined as the atresia period. Growth rate was calculated by subtracting the minimum diameter (≥ 4 mm) from the maximum diameter and dividing by the growth period.

Blood Sampling and Progesterone Radioimmunoassay (RIA)

Blood samples (10 ml) were collected daily into heparinized vacutainers from the median coccygeal vein for at least 1 interovulatory interval per animal. Following collection, the samples were immediately placed on ice. Plasma was separated by centrifugation within 4 h and then stored at - 20°C until assayed. The progesterone RIA was performed according to the method described by Knickerbocker et al. (20) using the same source of antiserum. Antiserum to bovine progesterone was a gift from Dr. Magaly Manzo (Faculdade de Ciências Veterinárias, Maracay, Venezuela). The intra- and inter-assay coefficients of variation were 5.5 and 11.7%, respectively, and the sensitivity of the assay, as defined by concentration at 90% zero binding, was 60.26 ± 3.54 pg/100 ul.

Statistical Analysis

Age (heifers vs cows) and treatment (natural vs prostaglandin-induced estrus) were considered the primary factors, while year (cows, 1992 and 1993) and season (heifers, summer vs winter) were the secondary factors. Animal variables that were examined were follicular growth and the estrous cycle.

Data were analyzed by generalized least squares analyses of variance using the General Linear Models and CATMOD procedures of the Statistical Analysis System (39). Data for the number of follicular waves were first analyzed with all main effects and interactions for primary and secondary factors using the CATMOD procedure. Main effects included the factors of age, treatment, year and season. The data were subsequently reanalyzed after removing effects from the model that accounted for small and nonsignificant proportions of the total variance. Consequently, in the reduced model, only age as the main effect was considered.

Data on follicular characteristics were analyzed using the GLM procedure. In the adjusted model, the main effects and interactions of age, treatment and number of waves were the factors considered. In the reduced model only the number of follicular waves as the main effect was considered. Additionally, the follicular characteristics of animals with 2 or 3 follicular waves were compared among the different waves.

Data on overall mean progesterone concentration and CL diameter obtained over 1 complete estrous cycle were analyzed by GLM using the main effects and interactions of age, treatment and number of follicular waves. In the reduced model, the number of waves factor was eliminated. Coefficients of correlation for progesterone concentration and CL diameter were calculated on the interactions of the reduced model.

RESULTS

Follicular dynamics in Nelore cows and heifers were characterized by 2 or 3 follicular waves. However, 1 heifer exhibited 4 follicular waves (see bellow) while another presented just 1 follicular wave (preliminary study, data not shown). Although our experiments were not designed to study seasonal influence on follicular growth, the statistical analysis revealed that neither the season nor the year influenced the results significantly. Consequently, the data were analyzed without including these factors. Since prostaglandin treatment did not alter the number of follicular waves, the results from animals with either naturally occurring or induced estrus were combined. There was a predominance of 2 follicular waves (83.3%, $P<0.05$) for cows and 3 waves (64.7%, $P<0.05$) for heifers (Table 1).

Three cows and seven heifers were followed over two consecutive estrous cycles. Most of these animals presented the same pattern of follicular waves in the first and second cycle (3 animals repeated the 2-wave pattern and 4 animals the 3-wave pattern), and only 30% showed variation in the number of waves from one cycle to another (2 animals showed

2 and 3 waves while 1 animal showed 3 and 2 waves, respectively, for the first and second cycle).

The mean diameter profiles of the dominant follicles are illustrated for cows and heifers (Figure 1). Comparing females with the same number of follicular waves, most of the follicular characteristics studied were not different for cows and heifers with natural or prostaglandin-induced estrous cycles. Consequently, data from females with natural and induced estruses were combined, and follicular characteristics were compared between 2 and 3-wave animals (Figure 2, Tables 2 and 3).

Table 1. Number of follicular waves during natural or prostaglandin-induced estrous cycles in Nelore cows and heifers

	2 waves (No. of estrous cycles)	3 waves (No. estrous cycles)
Cows		
Induced estrus	9/10 [a,c]	1/10 [a,d]
Natural estrus	6/8 [a,c]	2/8 [a,c]
Total	15/18 [c]	3/18 [d]
Heifers		
Induced estrus [e]	2/8 [b,c]	5/8 [b,c]
Natural estrus	3/9 [b,c]	6/9 [b,c]
Total [e]	5/17 [c]	11/17 [d]

[a,b] Values with different superscripts within rows differ ($P<0.05$).

[c,d] Values with different superscripts within columns differ($P<0.05$).

[e] One heifer had 4 follicular waves.

Maximum diameters of ovulatory follicles were similar for cows and heifers (approximately 11 mm). Ovulatory follicles were larger than other dominant follicles, and in females with 3 follicular waves the second dominant follicle was smaller than the first (Table 2). Growth and atresia rates did not differ between 2 and 3 wave dominant follicles (Table 2).

In the 2-wave females the first follicular wave tended to be longer ($P<0.10$) than the second ($P<0.05$) compared with that of the 3 wave animals. The ovulatory wave was always the shortest regardless of a 2- or 3-wave pattern. However, the ovulatory wave was longer for females with 2 waves than for those with 3 waves ($P<0.05$, Table 2).

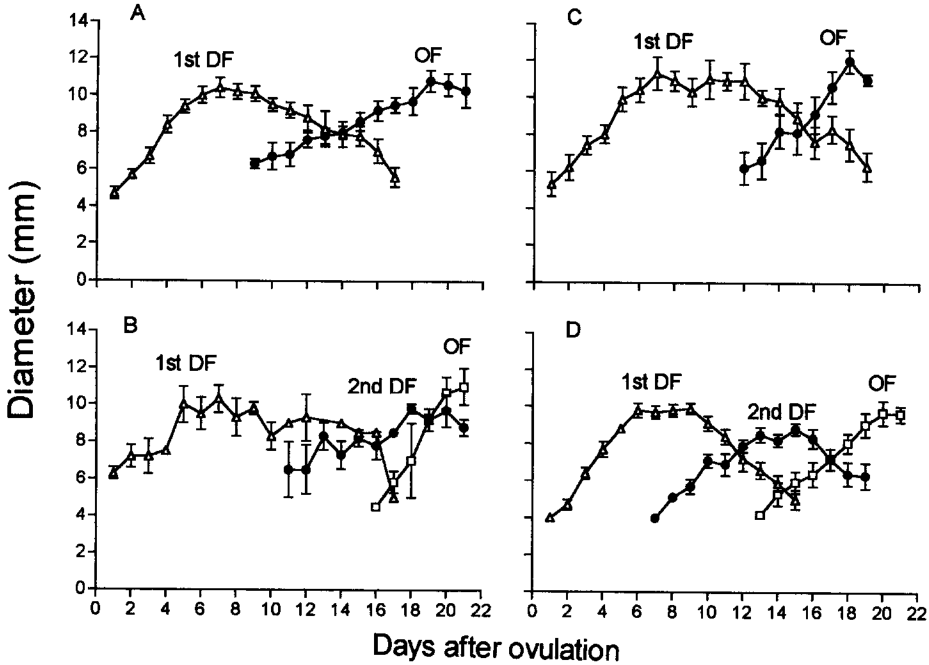

Figure 1. Mean (± SEM) profiles of dominant follicle (DF) diameters during the estrous cycles of Nelore cows (A,B) and heifers (C,D) with either two follicular waves (n=15 cows and 5 heifers) or three follicular waves (n=3 cows and 11 heifers). OF=ovulatory follicle. n = number of estrous cycles.

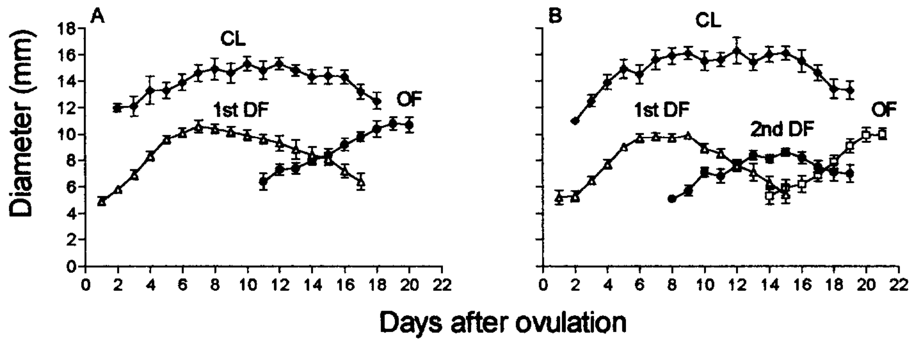

Figure 2. Mean (± SEM) profiles of dominant follicles (DF) and corpus luteum (CL) diameters for Nelore cows and heifers having estrous cycles with two (n=20) or three (n=14) follicular waves. OF = ovulatory follicle.

The ovulatory follicle reached its maximum diameter and ovulated sooner in the 2- vs 3-wave females (P<0.05). As a consequence, the interovulatory interval was shorter for 2-wave animals (P<0.05, Table 2).

Data on the second largest follicle (subordinate follicle) of each follicular wave are shown in Table 3. As expected, subordinate follicles reached a much smaller maximum diameter than dominant follicles. The number of waves did not influence the maximum diameter of the subordinate follicles. On average, the subordinate follicles were detected approximately 2 d after their respective dominant follicles.

For the single heifer that showed 4 follicular waves, the first, second, third and fourth wave lasted 9, 8, 9 and 8 d, respectively. The dominant follicles maximum diameters were 9.5, 6.0, 8.0 and 11.0 mm and were obtained at 5, 11, 18 and 29 d, respectively, after ovulation. The maximum diameter of the corpus luteum was 18.5 mm, and the interovulatory interval for this heifer was 30 d.

Statistical analysis of overall mean progesterone concentrations during the estrous cycles revealed that the number of follicular waves did not influence progesterone levels. Therefore, progesterone profiles for Nelore cows and heifers with 2 or 3 follicular waves are shown separately in Figure 3. In both the cows and heifers progesterone concentrations increased continuously from the second day post ovulation to reach a plateau around Day 8, which was maintained until Day 14 to 16, when progesterone concentrations declined sharply to basal levels.

There was a discrete positive correlation (varying from 26 to 56%) between progesterone plasma levels and corpus luteum size during the Nelore estrous cycle. Those correlations were 33 and 47 for cows, and 26 and 56% for heifers with natural and induced estrus, respectively. Therefore, at least in the group of heifers with induced estrus the growth and demise of the corpus luteum was significantly correlated (56%) with progesterone blood levels (Figure 3, Panel C).

The maximum CL diameter in Nelore heifers was larger than in cows (17.87 ± 0.35 vs 15.56 ± 0.44 mm; P<0.05). However, the overall mean progesterone concentration was lower in heifers (4.92 ± 0.22 ng/ml; P<0.05) than in cows (6.41± 0.23 ng/ml).

The mean sizes (mm) of the right and left ovaries were similar in cattle with two (23.23 ± 0.44 and 21.94 ± 0.64, respectively) and three follicular wave (22.39 ± 0.47 and 21.47 ± 0.51).

Table 2. Comparison of follicular and luteal characteristics (mean ± SEM) between Nelore cattle with 2 or 3 follicular waves

	Follicular waves	
Characteristics	Two (n=20)*	Three (n=14)*
Estrous detection (day)	19.25 ± 0.48 ^{d}	20.71 ± 0.46 ^{e}
Interovulatory interval (days)	20.65 ± 0.46 ^{d}	22.00 ± 0.44 ^{e}
Wave length (days)		
first wave	14.75 ± 0.70 ^{a,d}	13.00 ± 0.65 ^{a,e}
second wave	9.05 ± 0.69 ^{b,d}	11.50 ± 0.42 ^{a,e}
third wave	-----	6.86 ± 0.51 ^{b}
Maximum diameter (mm)		
corpus luteum	15.90 ± 0.45 ^{d}	17.69 ± 0.40 ^{e}
first dominant follicle	11.30 ± 0.35 ^{a,d}	10.42 ± 0.26 ^{a,e}
second dominant follicle	12.05 ± 0.29 ^{b,d}	9.35 ± 0.30 ^{b,e}
third dominant follicle	------	11.61 ± 0.25 ^{c}
Detection day		
first dominant follicle	1.50 ± 0.15 ^{a}	1.57 ± 0.20 ^{a}
second dominant follicle	12.00 ± 0.91 ^{b,d}	9.14 ± 0.47 ^{b,e}
third dominant follicle	-----	15.14 ± 0.48 ^{c}
Growth rate (mm/day)		
first dominant follicle	0.94 ± 0.10	0.89 ± 0.10
second dominant follicle	0.92 ± 0.08	0.79 ± 0.08
third dominant follicle	-----	1.06 ± 0.07
Onset of atresia (day)		
first dominant follicle	9.15 ± 0.80	8.86 ± 0.50 ^{a}
second dominant follicle	-----	15.71 ± 0.57 ^{b}
third dominant follicle	-----	-----

* Number of estrous cycles.

$^{a, b, c}$ Values followed by different superscripts within rows differ ($P<0.05$).

$^{d, e}$ Values with different superscripts within columns differ ($P<0.05$; $P<0.10$).Table 3. Comparison of largest subordinate follicle characteristics (mean ± SEM) between Nelore cattle with 2 or 3 follicular waves

Table 3. Comparison of largest subordinate follicle characteristics (mean ± SEM) between Nelore cattle with 2 or 3 follicular waves

Characteristics	Follicular waves	
	Two (n=20)*	Three (n=14)*
Maximum diameter (mm)		
first subordinate follicle	7.53 ± 0.40^{d}	6.14 ± 0.37^{e}
second subordinate follicle	7.17 ± 0.36^{d}	5.80 ± 0.45^{e}
third subordinate follicle	------	6.21 ± 0.39
Detection day		
first subordinate follicle	3.85 ± 0.55^{a}	3.79 ± 0.67^{a}
second subordinate follicle	$14.17 \pm 0.77^{b,d}$	$10.80 \pm 0.81^{b,e}$
third subordinate follicle	------	17.21 ± 0.55^{c}
Growth rate (mm/day)		
first subordinate follicle	0.89 ± 0.08	1.08 ± 0.20
second subordinate follicle	1.04 ± 0.18	1.15 ± 0.28
third subordinate follicle	------	1.26 ± 0.23
Onset of atresia (day)		
first subordinate follicle	7.55 ± 0.63^{a}	5.93 ± 0.89^{a}
second subordinate follicle	$17.11 \pm 0.72^{b,d}$	$12.60 \pm 0.90^{b,e}$
third subordinate follicle	------	19.29 ± 0.46^{c}

* Number of estrous cycles.

[a, b, c] Values followed by different superscripts within rows differ ($P<0.05$).

[d,e] Values with different superscripts within columns differ ($P<0.05$).

DISCUSSION

In the present study the follicular dynamics of Nelore cows and heifers were characterized by the presence of 2 or 3 follicular waves. These patterns of follicular growth are similar to those reported for European breeds, in which 2 and (15, 21, 34) 3follicular waves (19, 40, 45) predominate, while 1 or 4 follicular waves are rarely observed (31, 40, 45). For Zebu cattle (Bos indicus), Rhodes et al. (37) reported on 1 to 4 follicular waves in Brahman heifers, with predominance of 3 follicular waves.

Most of the animals followed over 2 consecutive estrous cycles in this study showed the same follicular wave pattern, but only some 30% presented variation in the number of follicular waves from one cycle to another. These results are similar to the results for

Holstein (21) and Brahman heifers (37), and confirm that variation in follicular wave pattern can occur in the same animal.

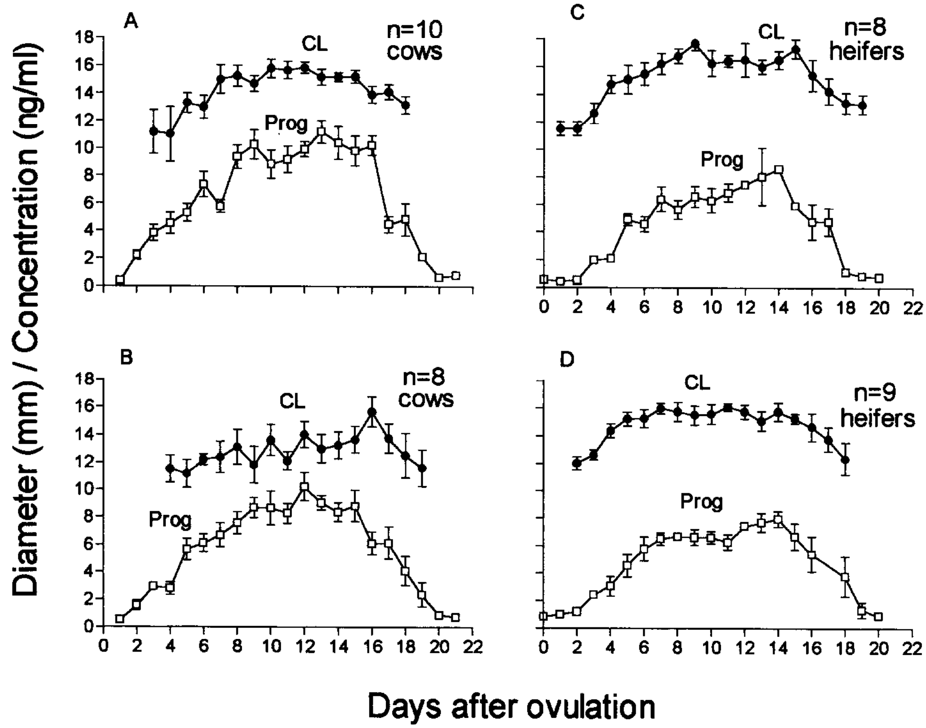

Figure 3. Mean (± SEM) profile of progesterone (Prog) and corpus luteum diameter (CL) for Nelore cows (A and B) and heifers (C and D) with induced (A,C) or natural (B,D) estrous cycles.

Reasons for variations between 2 and 3 follicular wave cattle is not clear. Nevertheless, Ginther et al. (15) compared 2- and 3-wave heifers and observed that the mean length of the 2-wave interovulatory interval (20.4 ± 03 d) was shorter than that for 3-wave intervals (22.8 ± 0.6 d). In addition, luteal regression occurred after emergence of the ovulatory wave, and the next wave did not emerge until near the day of ovulation at the onset of the subsequent interovulatory interval. They concluded that the emergence of a third wave was associated with a longer luteal phase, and the viable dominant follicle present at the time of luteolysis became the ovulatory follicle. Similarly, in the Nelore cattle the mean length of the 2-wave interovulatory interval (20.6 ± 0.5 d) was shorter than of the 3-wave interval (22.0 ± 0.4 d).

This study was not designed to examine the influence of season of the year on follicular development. However, in some experimental groups it was necessary to compare data from different seasons or years. Statistical analysis revealed that those factors did not significantly influence Nelore follicular dynamics, possibly because only a few animals from each experimental group were utilized in the different seasons and years.

Since PGF2α is frequently utilized to synchronize estrus, it was used to test its possible effects on follicular dynamics. In both cows and heifers, the prostaglandin treatment did not significantly modify the follicular characteristics analyzed. Our results indicated that the PFG2α dose (25 mg) administered reproduced the luteolytic effect of endogenous endometrial prostaglandin (18, 20) without causing side effects that could compromise the follicular development.

The detection of the first and second follicular waves in 2-wave Nelore cattle (Days 1.50 ± 0.15 and 12.0 ± 0.91, respectively) occurred a little later compared with those of Holstein heifers (Days 0.2 ± 0.1 and 9.6 ± 0.2, Day 0.2 ± 0.1 and 10.0 ± 0.4, Day -0.4 ± 0.3 and 9.3 ± 0.3, and Day 0.6 ± 0.8 and 11.0 ± 1.1; 1,15, 21, 22, respectively) for the first and second wave. The follicular wave length of Nelore cattle (14.75 ± 0.70 and 9.05 ± 0.69 d) was similar to that of Friesian x Hereford heifers (14.25 ± 2.86 and 9.0 ± 1.08 d; 40). In all the 2-wave cattle the second (ovulatory wave) was shorter than the first.

For 3-wave Nelore cattle detection of the first, second and third wave (Days 1.57 ± 0.20, 9.14 ± 0.47 and 15.14 ± 0.48, respectively) was comparable to that of Holstein heifers (Day 1.90 ± 0.30, 9.40 ± 0.50 and 16.10 ± 0.70, Day -0.50 ± 0.30, 9.0 and 16.0 ± 1.1; 15, 45). The duration of the first and second wave in Nelore cattle (13.0 ± 0.65 and 11.5 ± 0.42 d, respectively) was a little shorter than that reported for Holstein heifers (16.90 ± 0.90 and 13.10 ± 0.89 d) by Sirois and Fortune (45) and a little longer than that described for Friesian x Hereford heifers (11.35 ± 0.86 and 7.35 ± 0.85 d) by Savio et al. (40). The duration of 3 follicular waves in Nelore cattle (6.86 ± 0.51 d) was comparable to that of Bos taurus heifers (6.10 ± 0.72 d, 40; 5.90 ± 0.30; 45). In summary, the follicular waves of Nelore cattle were similar to those observed in European (Bos taurus) breeds.

The maximum diameter of dominant follicles (10 to 12 mm) observed in Nelore heifers was smaller than that reported for Bos taurus heifers (14 to 20 mm, 40; 13 to 18 mm, 15; 14 to 16 mm, 1) and cows (15 to 18 mm [31], 15 to 17 mm [49], approximately 17 mm [38], and approximately 15 mm [5]). However, the diameter was similar to the mean size of first dominant follicle (10.2 mm) as was reported by Rhodes et al. (37) for Australian Brahman heifers.

The duration of growth of ovulatory follicles for 2- (8.65 ± 0.73 d) and 3-wave Nelore cattle (7.0 ± 0.51 d) was comparable to that of Bos taurus heifers with 2 (7.50 ± 1.19 d, 40;

10.9 ± 0.40 d, 15) or 3 waves (5.90 ± 0.30, 45; 6.31 ± 0.69 d, 40). However, the growth rate of the ovulatory and other dominant follicles was slower in Nelore cattle (approximately 0.92 mm/d) than in European breeds (approximately 1.6 mm/d, 45; 3.0 to 5.5 mm/d, 40; 1.4 to 2.0 mm/d, 21; and 1.8 to 2.2 mm/d, 31). Thus, Nelore cattle have smaller dominant follicles than Bos taurus breeds due to their smaller rate of follicular growth.

The ovulatory follicle is larger than the other dominant follicles in 3-wave Nelore cattle, and the second-wave dominant follicles are smaller than first-wave dominant follicles. Similar results were reported previously for 3-wave cattle (45, 49). In addition, 3-wave cattle were found to have a longer estrous cycle (present study, 49) because estrus is delayed when the second dominant follicle fails to ovulate and a third dominant follicle requires additional time to complete development (27). One possible explanation for these differences between dominant follicle sizes is the blood progesterone level. At the beginning of the first wave, progesterone levels are low (Figure 3) because the corpus luteum is still maturing. Therefore, negative progesterone feedback at the hypothalamus and pituitary is not strong enough to prevent LH release (FSH levels are essentially modulated by estradiol and inhibin secreted by the dominant follicle; 2), which increases follicular growth. At the time of the second follicular wave, a mature CL secretes high concentrations of progesterone that inhibit LH release. In the third wave, the CL demise causes a sharp decrease in progesterone levels, which leads to an increase in LH concentration, followed by fast follicular growth and ovulation.

Several studies have reported increased persistence and growth of dominant follicles when low concentrations of progesterone are administered (41, 47, 50). Savio et al. (42) hypothesized that the dominant follicle is maintained after low-level progestogen treatment because of an increased frequency of LH pulses that occurs when cattle are treated with progestogens in the absence of a CL. It is not known if the follicles in the low progesterone environment of Nelore cattle reached sizes as large as those observed in the European breeds.

There is also no data in the literature on LH pulsatility in Nelore cattle. A future study will need to compare LH pulsatility in the Nelore breed with other breeds to verify if possible differences in LH pulsatility could explain the smaller growth rate and size of preovulatory follicles in Nelore cattle. Another approach would be to test the growth of Nelore follicles in a low progesterone environment to see if they would reach sizes as large as those observed in European breeds.

The maximum diameter reached by the second largest follicle (subordinate follicle) of a follicular wave in Nelore (approximately 6 to 7 mm) cattle was similar to that reported for Holstein heifers (6.3 ± 0.8 mm) by Ko et al. (22) and by Fortune (7.4 ± 0.6 mm; 14) and a little smaller (8 to 9 mm) than that observed by Adams et al. (1). In our study, the second largest follicle attained about half the size of the dominant follicle in Nelore cattle.

The means by which a dominant follicle inhibits the growth of subordinate follicles and the recruitment of a new cohort of follicles is not clear. The most promising hypothesis is that the dominant follicle can cause the regression of subordinate follicles indirectly by negative feedback mechanisms. The secretion of feedback regulators such as estradiol and inhibin by the dominant follicle would cause a decrease in FSH to levels which would not support the further growth of subordinate follicles. Those low FSH levels would be insufficient to sustain subordinate follicle growth. However, the low FSH concentrations would not affect the dominant follicle, which would have reached a stage of differentiation that sustains growth even at lower levels of circulating FSH (2, 14, 51).

The average size of CL for 3-wave Nelore cattle (17.69 ± 0.40 mm) was comparable to that obtained by Rhodes et al. (37) for 3-wave Brahman heifers (18.9 ± 0.17 mm). However, the size tended to be smaller than that for Bos taurus (20 to 25 mm; 1, 16, 49) breeds. Thus, the size of dominant follicles and CL in the ovaries of Bos indicus cattle may be smaller than those of Bos taurus breeds, but the pattern of growth and turnover of dominant follicles are similar.

For Nelore cows and heifers, progesterone concentration in the blood increased steadily from the second day post-ovulation and reached a plateau at approximately Days 8 to 16. The sharp decline in progesterone levels suggests that luteolysis occurred between Days 15 and 18 post ovulation. These results are similar to those reported by Mukasa-Mugerwa (30) for other Zebu cattle such as Bunaji (12), Afrikaner (9), Indubrasil (53) and Boran (25). Similar results have been found for Bos taurus (7, 41) breeds. In the present study, the CL of Nelore heifers were larger than those of Nelore cows. This result was unexpected and might be explained by diet. While the heifers received a ration during the experiment, the cows were kept on pasture. Murphy et. al. (32), using circulating progesterone levels, concluded that the effect of alimentation on functional activity and size of the bovine CL has not been fully elucidated since the restriction of food intake may decrease (17, 54), have no effect (6, 46), or increase (11, 29) bovine luteal function.

The mean follicle diameter (approximately 22 mm) of Nelore cows and heifers as determined by ultrasonography of the ovaries was similar to that of Zebu cattle in Nigeria (20.5 mm, data obtained from slaughterhouse animals; 23) and slightly smaller than that reported for European cattle (approximately 25 mm; 33). Other reports, based on ovarian weight (4) or palpation per rectum (3) suggest that Zebu cattle have smaller ovaries than Bos taurus breeds.

In conclusion, this study has demonstrated that in spite of the smaller size of the dominant follicle and corpus luteum in Nelore cattle (Bos indicus), the follicular dynamic was similar to that observed in European breeds (Bos taurus) and was characterized by either 2 (cows) or 3 (heifers) follicular waves during the natural or prostaglandin-induced estrous cycle. Further experiments based the follicular dynamics of Bos indicus cattle are warranted to test new hormonal treatments for synchronizing estrus with more precision and for enhancing superovulation responses in cattle.

REFERENCES

1. Adams GP, Kot K, Smith CA, Ginther OJ. Effect of the dominant follicle on regression of its subordinates in heifers. Can J Anim Sci 1993;73:267-275.
2. Adams GP, Matteri RL, Kastelic JP, Ko JCH, Ginther OJ. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. J Reprod Fertil 1992;94:177-188.
3. Adeyemo O, Heath E. Plasma progesterone concentrations in Bos taurus and Bos indicus heifers. Theriogenology 1980;14:411-420.
4. Aguilar A, Galina CS, Hummel J. Estudio morfológico comparativo de los ovarios de la vaca cebú y la vaca Holstein. Vet Méx 1983;14:133-136.
5. Ahmad N, Schrick FN, Butcher RL, Inskeep EK. Effect of persistent follicles on embryonic losses in beef cows. Biol Reprod 1995;52:1129-1135.
6. Apgar J, Aspros D, Hixon JE, Saatman RR, Hansel W. Effect of restricted feed intake on the sensitivity of the bovine corpus luteum to LH in vitro. J Anim Sci 1975;41:1120-1123.
7. Assey RJ, Purwantara B, Greve T, Hyttel P, Schimidt MH. Corpus luteum size and plasma progesterone levels in cattle after cloprostenol-induced luteolysis. Theriogenology 1993;39:1321-1330.
8. Barros CM, Figueiredo RA, Papa FO, Rocha G. Follicular growth in Nelore cows (Bos indicus) after PGF2α administration. J Anim Sci 1993;71(Suppl 1):216 abstr.
9. Coetzer WA, Van-Niekerk CH, Morgenthal JC, Van Der Westhuysen JM. Hormone levels in peripheral plasma of the Afrikaner cow. 1. Progesterone and luteinising hormone levels during the estrous cycle. S Afri J Anim Sci 1978;8:1-5.
10. Dobson H, Kamonpatana M. A review of female cattle reproduction with special reference to a comparison between buffaloes, cows and Zebu. J Reprod Fertil 1986;77:1-36.
11. Donaldson LE, Basset JM, Thoburn GD. Peripheral plasma progesterone concentration of cows during puberty, estrous cycles, pregnancy and lactation, and the effect of undernutrition or exogenous oxytocin on progesterone concentrations. J Endocrinol 1970;48:599-614.
12. Eduvie LO, Dawuda PM. Effect of suckling on reproductive activities of Bunaji cows during the postpartum period. J Agric Sci 1986;107:235-238.
13. Fajerson P, Edqvist L-E. Ultrasonographic characterization of the onset of puberty; first ovulation is followed by the development of a corpus luteum and a short luteal phase in brown Swiss and Zebu heifers. J Anim Sci 1993;71 (Suppl 1): 209 abstr.
14. Fortune JE. Ovarian follicular growth and development in mammals. Biol Reprod 1994;50:225-232.
15. Ginther OJ, Knopf L, Kastelic JP. Temporal associations among ovarian events in cattle during estrous cycles with two and three follicular waves. J Reprod Fertil 1989;87:223-230.
16. Hafez ESE. Anatomy of female reproduction. In: Reproduction in Farm Animal. Philadelphia: Lea & Febiger, 1993;23.
17. Hill JR, Lamond DR, Henricks DM, Dickey JF, Niswender GD. The effects of undernutrition on ovarian function and fertility in beef heifers. Biol Reprod 1970;2:78-84.

18. Horton EW, Poyser NL. Uterine luteolytic hormone: a physiological role for prostaglandin F2α. Physiol Rev 1975;56:595 abstr.
19. Ireland JJ, Roche JF. Hypotheses regarding development of dominant follicles during a bovine estrous cycle. In: Roche JF, O'Callaghan D (eds), Follicular Growth and Ovulation Rate in Farm Animals. Boston: Martinus Nijhoff, 1987; 1-18.
20. Knickerbocker JJ, Thatcher WW, Bazer FW, Drost, M, Barron DH, Fincher KB, Roberts RM. Protein secreted by Day 16 to 18 bovine conceptuses extends corpus luteum function in cows. J Reprod Fertil 1986;77:381-391.
21. Knopf L, Kastelic JP, Schallenberger E, Ginther OJ Ovarian follicular dynamics in heifers: test of two-wave hypothesis by ultrasonically monitoring individual follicles. Dom Anim Endocrinol 1989;6:111-119.
22. Ko JCH, Kastelic JP, Del Campo MR, Ginther OJ. Effects of a dominant follicle on ovarian follicular dynamics during the estrous cycle in heifers. J Reprod Fertil 1991;91:511-519.
23. Lamorde AG, Kumar MSA. Observation on the ovaries of Zebu cattle in northern Nigeria. Res Vet Sci 1978;24:305-307.
24. Lamothe-Zavaleta C, Fredriksson G, Kindahl H. Reproductive performance of Zebu cattle in Mexico. 1. Sexual behavior and seasonal influence on estrous cyclicity. Theriogenology 1991;36:887-896.
25. Llewelin CA, Munro CD, Luckins AG, Jordt T, Murray M, Lorenzini E. Behavioral and ovarian changes during the estrous cycle in the Boran (Bos indicus). Br Vet J 1987;143:75-82.
26. Lowman BG, Scott NA, Somerville SH. Condition scoring of cattle. The East of Scotland College of Agriculture, Edinburgh, 1976 (Bulletin 6);1-31.
27. Lucy MC, Savio JD, Badinga L, De La Sota RL, Thatcher WW. Factors that affect ovarian follicular dynamics in cattle. J Anim Sci 1992;70:3615-3626.
28. Mapletoft RJ, Bo GA, Pierson RA. Recruitment of follicles for superovulation. Compend Cont Educ Pract Vet 1994;16:127-141.
29. McCann JP, Hansel W. Relationship between insulin and glucose metabolism and pituitary-ovarian functions in fasted heifers. Biol Reprod 1986;34:630-636.
30. Mukasa-Mugerwa E. A review of reproductive performance of female Bos Indicus (Zebu) cattle. Ethiopia: ILCA, 1989; monograph 6:1-34.
31. Murphy MG, Boland MP, Roche JF. Pattern of follicular growth and resumption of ovarian activity in post-partum beef suckler cows. J Reprod Fertil 1990;90:523-533.
32. Murphy MG, Enright WJ, Crowe MA, Mcconnell K, Spicer LJ, Boland MP, Roche JF. Effect of dietary intake on pattern of growth of dominant follicles during the estrous cycle in beef heifers. J Reprod Fertil 1991;92:333-338.
33. Nickel R, Schummer A, Seiferle E, Sack WO. The Viscera of Domestic Mammals. Berlin: P. Parey, 1973;352.
34. Pierson RA, Ginther OJ. Ultrasonic imaging of the ovaries and uterus in cattle. Theriogenology 1988;29:21-37.
35. Pursley JR, Korosok MR, Wiltbank MC. Reproductive management of lactating dairy cows using synchronization of ovulation. J Anim Sci 1994a;72 (Suppl 1) / J Dairy Sci;77 (Suppl 1):69 abstr.

36. Pursley JR, Mee MO, Brown MD, Wiltbank MC. Synchronization of ovulation in dairy cattle using GnRH and PGF2alfa. J Anim Sci 1994b;72(Suppl 1) / J Dairy Sci; 77 (Suppl 1):230 abstr.
37. Rhodes FM, De'ath G, Entwistle KW. Animal and temporal effects on ovarian follicular dynamics in Brahman heifers. Anim Reprod Sci 1995;38:265-277.
38. Roberts AJ, Grizzle JM, Echternkamp SE. Follicular development and superovulation response in cows administered multiple FSH injections early in the estrous cycle. Theriogenology 1994;42:917-929.
39. SAS Institute Inc. SAS/Stat Guide for Personal Computers: Version 6. Cary, NC, SAS Institute, 1987.
40. Savio JD, Keenan L, Boland MP, Roche JF. Pattern of growth of dominant follicles during the estrous cycle of heifers. J Reprod Fertil 1988;83:663-671.
41. Savio JD, Thatcher WW, Badinga L, De La Sota RL, Wolfenson D. Regulation of dominant follicle turnover during the estrous cycle in cows. J Reprod Fertil 1993;97:197-203.
42. Savio JD, Thatcher WW, Badinga L, De La Sota RL. Turnover of dominant ovarian follicles as regulated by protestins and dynamics of LH secretion in cattle. J Reprod Fertil 1990;Ser 6:23 abstr.
43. Schmitt EJP, Diaz TC, Drost M, Thatcher WW. Use of a GnRH-agonist for a timed-insemination protocol in cattle. J Anim Sci 1994a;72 (Suppl 1) / J Dairy Sci;77 (Suppl 1):292 abstr.
44. Schimitt EJP, Drost M, Diaz TC, Roomes C, Thatcher WW. Effect of a GnRH agonist on follicle recruitment and pregnancy rate in cattle. J Anim Sci 1994b;72 (Suppl 1) / J Dairy Sci 77 (Suppl 1):230 abstr.
45. Sirois J, Fortune JE. Ovarian follicular dynamics during the estrous cycle in heifers monitored by Real-Time Ultrasonography. Biol Reprod 1988;39:308-317.
46. Spitzer JC, Neiswender GD, Seidel GE, Wiltbank JN. Fertilization and blood levels of progesterone and LH in beef heifers on a restricted energy diet. J Anim Sci 1978;46:1071-1077.
47. Stock AE, Fortune JE. Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology 1993;132:1108-1114.
48. Thatcher WW, Drost M, Savio JD, Macmillan KL, Entwistle KW, Schmitt EJ, De La Sota RL, Morris GR. New clinical uses of GnRH and its analogues in cattle. Anim Reprod Sci 1993; 33:27-49.
49. Taylor C, Rajamahendran R. Follicular dynamics and corpus luteum growth and function in pregnant versus nonpregnant dairy cows. J Dairy Sci 1991;74:115-123.
50. Taylor C, Rajamahendran R, Walton JS. Ovarian follicular dynamics and plasma luteinizing hormone concentrations in norgestomet-treated heifers. Anim Reprod Sci 1993;32:173-184.
51. Turzillo AM, Fortune JE. Suppression of the secondary FSH surge with bovine follicular fluid is associated with delayed ovarian follicular development in heifers. J Reprod Fertil 1990;89:643 abstr.

52. Twagiramungu H, Guilbault LA, Dufour J. Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: a review. J Anim Sci 1995,73:3141-3151.
53. Vaca LA, Galina C, Fernandez Baca S, Escobar J, Ramirez B. Progesterone levels and relationships with the diagnosis of a corpus luteum by rectal palpation during estrous cycles in Zebu cows. Theriogenology 1983;2067-2076.
54. Villa-Godoy A, Hughes TL, Emery RS, Enright WJ, Ealy AD, Zinn SA, Fogwell RL. Energy balance and body condition influences luteal function in Holstein heifers. Dom Anim Endocrinol 1990;7:135-148.
55. Wolfenson D, Thatcher WW, Badinga L, Savio JD, Meidan R, Lew BJ, Braw Tal-R, Berman A. Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. Biol Reprod 1995;52:1106-1113.
56. Wolfenson D, Thatcher WW, Savio JD, Badinga L, Lucy MC. The effect of a GnRH analogue on the dynamics of follicular development and synchronization of estrus in lactating cyclic dairy cows. Theriogenology 1994;42:633-644.