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Follicle deviation and ovulatory capacity in *Bos indicus* heifers

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Abstract

The objectives of Experiment 1 were to determine the interval from ovulation to deviation, and diameter of the dominant follicle (DF) and largest subordinate follicle (SF) at deviation in Nelore (*Bos indicus*) heifers by two methods (observed and calculated). Heifers ($n = 12$) were examined ultrasonographically every 12 h from ovulation (Day 0) to Day 5. The time of deviation and diameter of the DF and largest SF at deviation did not differ ($P > 0.05$) between observed and calculated methods. Overall, deviation occurred 2.5 ± 0.2 d (mean $\pm$ S.E.M.) after ovulation, and diameters for DF and largest SF at deviation were 6.2 ± 0.2 and 5.9 ± 0.2 mm, respectively. Experiment 2 was designed to determine the size at which the DF acquires ovulatory capacity in *B. indicus* heifers. Twenty-nine heifers were monitored every 24 h by ultrasonography, from ovulation until the DF reached diameters of 7.0–8.4 mm ($n = 9$), 8.5–10.0 mm ($n = 10$), or >10.0 mm ($n = 10$). At that time, heifers were treated with 25 mg of pLH and monitored by ultrasonography every 12 h for 48 h. Ovulation occurred in 3 of 9, 8 of 10, and 9 of 10 heifers, respectively ($P < 0.05$). In summary, there was no significant difference between observed and calculated methods of determining the beginning of follicle deviation. Deviation occurred 2.5 d after ovulation when the DF reached 6.2 mm, and ovulatory capacity was acquired by DF as small as 7.0 mm.

Keywords: Follicle deviation; Ovulatory capacity; *Bos indicus*; Nelore; Heifers

1. Introduction

In monovular species, there is a mechanism that selects only one, of a cohort of follicles, for dominance and ovulation [1,2]. During this process, there is a common growth phase subsequent to wave emergence, followed by a change in growth rates between the dominant follicle (DF) and subordinate follicle (SF), which is defined as follicle deviation [1]. Follicle deviation can be determined retrospectively by comparing changes in diameter of the two largest follicles (observed deviation) [1] or by using a segmented, simple linear regression model (calculated deviation) [3].

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In Holstein cattle, follicle deviation occurred 2.8 d after wave emergence at a dominant follicle diameter of 8.5 mm and a largest subordinate follicle diameter of 7.2 mm [4]. Two studies [5,6] suggested that follicle selection occurred when the DF was somewhat smaller in indicus breeds of cattle; those authors reported DF diameters between 5.4 [5] and 5.9 [6] at the time of deviation, 2.7 and 2.6 d after ovulation, respectively.

In Holstein cattle, follicle deviation was associated with a shift in gonadotropin dependency, from FSH to LH [4]. However, the DF following deviation did not acquire ovulatory capacity (in response to exogenously administered pLH) until it reached 10.0 mm in diameter [7]. There is apparently no report regarding when the DF acquires ovulatory capacity in indicus breeds of cattle.

The objectives of the present study were to determine the interval from ovulation to follicle deviation in Nelore (*Bos indicus*) heifers, and the diameter of the largest and second largest follicles at the time of deviation, comparing two methods of evaluation (observed and calculated deviation), and to determine the size at which the DF acquires the capacity to ovulate.

2. Materials and methods

2.1. Experiment 1: follicle deviation

2.1.1. Animals and synchronization protocol

This experiment was conducted on a farm at the University of Sao Paulo (USP, Pirassununga, Sao Paulo, Brazil; latitude: 21°59′46″S, longitude: 47°25′33″O). Eighteen cycling Nelore (*B. indicus*) heifers, ranging from 20 to 24 months of age and weighing more than 325 kg, were maintained on pasture (*Brachiaria decumbens* and *Brachiaria brizantha*), with mineral supplementation and water offered *ad libitum*.

All heifers were treated at random stage of the estrous cycle with 2 mg of estradiol benzoate i.m. (EB; Index Farmaceutica, São Paulo, São Paulo, Brazil) and a norgestomet ear implant (Crestar®, Intervet, São Paulo, São Paulo, Brazil). Eight days later, implants were removed and heifers received 150 μg of D-Cloprostenol i.m. (Preloban®, Intervet). Twenty-four hours later, heifers received 1 mg of EB i.m. [8].

2.1.2. Ultrasonographic examinations

All heifers were evaluated by transrectal ultrasonography (Aloka SSD-500, Tokyo, Japan), every 12 h after implant removal to determine the time of ovulation (Day 0), the time of follicle deviation, and follicle diameters at deviation.

Ovaries were mapped by recording the diameters of three largest follicles at each examination. Observed deviation was determined retrospectively by tracking the two largest follicles and classifying them as DF and SF [1]. Only follicular waves with a single DF were considered. The beginning of follicle deviation was defined as the end of a common growth phase, when differences in diameters between the two largest follicles were detected [9]. All data were normalized to the day of observed deviation. Calculated deviation was performed with the same observations as used for observed deviation; however, a segmented linear regression model was applied to the data. The segmented regression model consists of two segments (1 and 2) and a Join Point. Basically, Segment 1 represented the common growth phase, Segment 2 represented the period of dominance thereafter, and the Join Point represented the beginning of deviation [3].

2.2. Experiment 2: ovulatory capacity

2.2.1. Animals and synchronization protocol

This experiment was conducted on a farm of Agência Paulista de Tecnologia dos Agronegócios (APTA, Pindamonhangaba, São Paulo, Brazil; latitude: 22°55′26″S, longitude: 45°27′42″O). Thirty-four cycling *Bos indicus* heifers (13 Nelore, 11 Gir, and 10 cross-bred Nelore × Gir), at 24 months of age and weighing more than 350 kg were maintained on pasture (*B. decumbens* and *B. brizantha*) with mineral supplementation and water available *ad libitum*. The synchronization of ovulation protocol was the same as that described in Section 2.1.1

2.2.2. Ultrasonographic examinations and pLH treatment

Ultrasonographic examinations were performed every 12 h from implant removal to ovulation (Day 0). After ovulation, follicle sizes were determined ultrasonically every 24 h until diameters of 7.0–8.4 mm ($n = 9$), 8.5–10.0 mm ($n = 10$), or >10.0 mm ($n = 10$) were recorded. At these diameters, heifers were submitted to a pLH challenge, and then, all heifers were monitored ultrasonographically every 12 h for 48 h. In order to assess ovulatory capacity, heifers received 25 mg of pLH i.m. (Lutropin-V®; Bioniche Animal Health, Inc., Belleville, ON, Canada) at the follicle diameter ranges cited above. The route (i.m.) and anatomical point of injection (*Gluteus medius*) were the same among all animals. The LH was given at the time of the ultrasound examination (between 0600 and 0800 h and between 1800 and 2000 h).

Table 1
Mean (±S.E.M.) characteristics associated with follicle deviation in *Bos indicus* heifers ($n = 12$), using observed and calculated methods to determine deviation (Experiment 1)

Characteristics	Observed method	Calculated method	Combined	Probability
Time of deviation (days after ovulation)	2.5 ± 0.3 (1.5–4.0)	2.4 ± 0.3 (1.2–4.0)	2.5 ± 0.2	0.38
Time of deviation (hours after ovulation)	61.0 ± 6.0 (36.0–96.0)	57.2 ± 6.3 (29.0–96.8)	59.1 ± 5.8	0.38
Dominant follicle at deviation (mm)	6.2 ± 0.2 (5.0–7.1)	6.2 ± 0.3 (4.1–7.8)	6.2 ± 0.2	0.50
Subordinate follicle at deviation (mm)	5.8 ± 0.2 (4.2–7.3)	5.9 ± 0.3 (4.4–7.1)	5.9 ± 0.2	0.62

P-value represents the comparison between observed and calculated methods of deviation. No differences were found, and then data were combined. Numbers within parentheses represent ranges. Day of ovulation = Day 0.

2.3. Statistical analysis

Prior to the analysis, data were tested for normality. As data were normally distributed, no transformation was necessary. Paired *t*-test was used to compare observed and calculated methods of determining the diameter of the DF and largest SF at the time of follicle deviation. The slopes of the regression lines were used to reflect growth rate before (Segment 1) and after (Segment 2) follicle deviation for both the largest and second largest follicles. These growth rates were compared by ANOVA, using a Mixed Model.

Continuous variables, e.g. DF diameter at the time of pLH treatment, follicle diameter at the time of ovulation, and interval from pLH treatment to ovulation were analyzed by ANOVA using the GLM procedure of SAS. Groups were compared by Tukey test. The proportion of animals ovulating within groups was analyzed by ANOVA using GENMOD procedure, and the differences were detected by chi-square test.

All statistical analyses were done with SAS software (SAS, Cary, NC, USA). Significance was indicated by a probability of <0.05 and tendency was indicated by probabilities between 0.05 and 0.10.

3. Results

3.1. Experiment 1: follicle deviation

Fifteen of 18 heifers ovulated with mean (±S.E.M.) diameter of 11.9 ± 0.4 mm at 64.4 ± 1.6 h after norgestomet implant removal. From these 15 heifers, 3 were excluded because of behavioral problems, leaving 12 heifers for the experiment.

The future DF (4.2 ± 0.2 mm) was larger than the largest SF (3.7 ± 0.2 mm; $P = 0.03$) on the day of ovulation (Day 0). Data concerning time of deviation, and diameter of DF and SF at deviation by observed and calculated methods, are shown in Table 1. The diameters of the two largest follicles normalized to the time of deviation, as determined by the observed method, are summarized in Fig. 1.

The growth rate (mm/12 h) of the DF did not differ before or after follicle deviation (0.50 ± 0.08 and 0.60 ± 0.05, respectively; $P = 0.33$). However, growth rate of the largest SF was higher before than after deviation (0.64 ± 0.08 and −0.06 ± 0.04, respectively; $P < 0.0001$). The growth rate of the DF differed from that of the largest SF after follicle deviation ($P < 0.0001$).

3.2. Experiment 2: ovulatory capacity

There was no effect of breed on the variables analyzed ($P > 0.10$), and so data were combined. Twenty-nine of 34 heifers submitted to the synchronization of ovulation protocol ovulated 67.2 ± 0.8 h after norgestomet implant removal with a mean (±S.E.M.) diameter of the ovulatory follicle of 12.2 ± 0.4 mm. Table 2 summarizes the effect of the pLH challenge

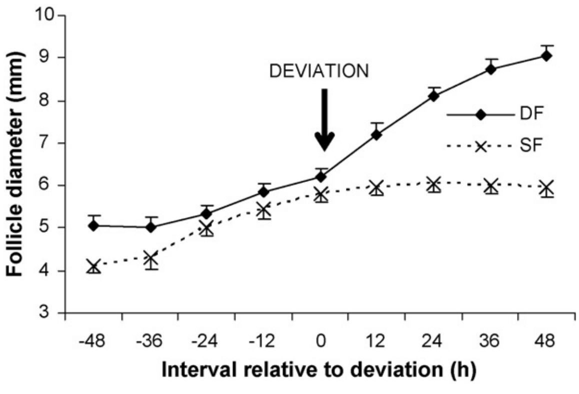

Fig. 1. Mean (±S.E.M.) diameters of the dominant follicle (DF) and largest subordinate follicle (SF) normalized to the day of deviation in 12 Nelore heifers (observed deviation). Heifers were examined ultrasonographically every 12 h from ovulation (Day 0) to Day 5. Overall, deviation occurred 2.5 ± 0.2 d (59.1 ± 5.8 h) after ovulation, and the mean (±S.E.M.) diameters for DF and largest SF at deviation were 6.2 ± 0.2 and 5.9 ± 0.2 mm, respectively. The growth rate of the DF differed from that of the largest SF after follicle deviation ($P < 0.0001$).

Table 2
Mean (±S.E.M.) and proportional characteristics associated with pLH treatment at different follicle-diameter categories in *B. indicus* heifers (Experiment 2)

Characteristics	Follicle-diameter categories (mm)			Probability
	7.0–8.4	8.5–10.0	>10.0	
Number of heifers	9	10	10	–
Dominant follicle (mm) at time of pLH (range)	7.6 c ± 0.1 (7.0–8.0)	9.6 b ± 0.1 (9.2–10.0)	10.9 a ± 0.2 (10.4–12.0)	<0.001
Interval (h) to ovulation after pLH (range)	38.0 ± 4.0 (36.0–48.0)	31.5 ± 2.7 (24.0–48.0)	30.0 ± 2.0 (24.0–48.0)	0.24
Preovulatory follicle (mm; range)	8.1 b ± 0.3 (7.6–8.7)	9.7 a ± 0.3 (9.3–11.0)	10.5 a ± 0.3 (10.5–11.1)	<0.002
Number of animals that ovulated (%)	3/9 b (33.3)	8/10 a (80.0)	9/10 a (90.0)	<0.02

Within a row, means and proportions without a common letter (a–c) differ ($P < 0.05$).

according to follicle diameter ranges at the time of treatment, follicle diameter at the time of ovulation, interval from pLH treatment to ovulation, and ovulation rate. The proportion of heifers that ovulated in the 7.0–8.4 mm group (3/9) was less than in the other two groups (8/10 and 9/10, respectively; $P < 0.02$), but the interval from administration of pLH to ovulation (31.8 ± 1.6 h) did not differ among groups ($P = 0.24$).

4. Discussion

In *Bos taurus* breeds of cattle, the time of deviation after wave emergence, and the diameter of DF at the time of deviation did not differ between observed (62 h and 8.4 mm) and calculated (61 h and 8.8 mm) methods [3]. In *B. indicus* cattle, differences between methods have not been described in either heifers (observed: 2.9 d after ovulation and 5.7 mm; calculated: 2.7 d and 5.7 mm) or cows (observed: 2.3 d after ovulation and 6.0 mm; calculated: 2.4 d and 6.1 mm) [6]. In the present study, when observed or calculated methods of determining follicle deviation were compared, both were efficacious and did not differ statistically, which is in agreement with previous results in *B. taurus* [3] and *B. indicus* [6] cattle. Either can be used effectively in *B. indicus* cattle.

The future DF was larger than the future largest SF on the day of first detection (day of ovulation), consistent with previous studies in Holstein heifers, in which the selected DF had a size advantage at the time of wave emergence [10] or at follicle deviation [4]. We inferred that follicle selection may have started before wave emergence was detected ultrasonographically, perhaps at the early antral or even preantral phases, although this hypothesis has yet to be tested.

The time of follicle deviation after ovulation was similar in the present study to recent reports describing follicular dynamics in Nelore heifers (2.8 d [6] and 2.7 d [5]). However, follicle diameters at deviation in Nelore heifers in this study and in the previous reports [5,6] were somewhat smaller than that reported for Holstein heifers [4]. We speculate that this finding could be an inherent trait of Nelore cattle, which may be due to differences in metabolism, or a lower follicular daily growth rate of this breed as compared to Holsteins.

Following ovulation, the two largest follicles in the wave had a common growth phase, but following follicle deviation, the growth rate of the DF was maintained, whereas there was a decrease of the growth rate of the largest SF, as previously described for Holstein heifers [1]. However, our results differed from that reported by Castilho et al. [5] in Nelore heifers. These authors reported that deviation was characterized by a decrease in the growth rate of the largest SF and an increase in the growth rate of the DF. This difference may be attributable to the low growth rate of the DF (0.9 mm/d) relative to that of SF (1.8 mm/d) before the deviation in that study [5].

There would appear to be a difference in growth rate of follicles within the wave between *B. indicus* and *B. taurus* cattle, which may affect the diameter of the DF at deviation. In Holstein cattle, growth rate of the DF ranged from 1.5 to 2.1 mm/d prior to deviation [10–12], whereas the growth rate of the DF in Nelore heifers ranged from 0.9 to 1.4 mm/d [5,6].

Based on a study in suckled Zebu cows in which follicles did not reach diameters larger than 6 mm during the postpartum period [13], Wiltbank et al. [14] hypothesized that these animals had a deficiency in FSH. However, based on the present study and at least two others [5,6], follicular diameters at the time of follicle deviation in Nelore cattle seemed generally smaller than those described in Holstein cattle. Therefore, the postpartum anovulatory condition described in lactating Zebu cattle [13] may not be associated with FSH deficiency, since follicles grew to 6 mm, which corresponds to a shift in gonadotropin dependency from FSH to LH, and this may be inherent to indicus breeds of cattle. Therefore, the anovulatory condition in lactating Zebu cattle seems more likely to be related to deficiency of LH release.

A possible explanation for the smaller diameter attained by the DF at the time of deviation in *B. indicus* as compared to *B. taurus* cattle could be the difference in levels of IGF-1 between these two genetic groups [15]. Although controversial and not clearly understood, the IGF system has been associated with follicle selection [1]. In that regard, IGF-1 has been reported to stimulate *in vitro* steroidogenesis and proliferation of bovine thecal and granulosa cells [16,17], acting synergistically with gonadotropins. Even when FSH begins to decline at the time of follicle selection, IGF-1 is apparently responsible for enhancing follicle responsiveness to FSH (reviewed in [6,15,18,19]). This observation may be even more pertinent in *B. indicus* cattle, since the mean concentrations of FSH were lower (0.45 ng/mL vs. 0.6 ng/mL), whereas mean circulating concentrations of IGF-1 were greater (22.9 ng/mL vs. 10.0 ng/mL) in Brahman than in Angus cows throughout the estrous cycle [20]. However, IGF-1 also increased the sensitivity of follicular cells to LH [21]. We hypothesize that the higher responsiveness of *B. indicus* cattle to FSH and LH when compared to *B. taurus* cattle may be attributed to higher IGF-1 concentrations. As a consequence of the acquisition of LH receptors when follicles are at a smaller diameter in *B. indicus* cattle, deviation and ovulatory capacity would logically occur at a smaller follicle diameter. Although this hypothesis needs to be critically tested, data from the current experiments provide rationale.

The present study provided novel information regarding the acquisition of the capacity of the DF to ovulate in *B. indicus* cattle. It is well known that LH binds to its receptor on granulosa cells, triggering several events that culminate in the ovulation. However, the key event is the acquisition of LH receptors (reviewed in [22,23]), which based on the current experiment, apparently occurred at a smaller diameter in *B. indicus* than in *B. taurus* cattle.

The mean ($\pm$S.E.M.) interval from endogenous LH release to ovulation was 25.9 ± 0.6 h in *B. indicus* cattle [24] and 29.4 ± 1.5 h in *B. taurus* cattle [25]. In the present experiment in *B. indicus* heifers, the interval from the administration of pLH at pre-determined follicle sizes to ovulation was 31.8 ± 1.6 h. Similarly, in a previous study in *B. taurus* heifers [26], the interval from the administration of pLH on pre-determined days of the follicular wave to ovulation was 33.6 ± 4.8 h. However, the size at which follicles responded by ovulating in the present study seemed smaller than has been reported in *B. taurus* heifers [7,26].

All follicle diameter ranges chosen for pLH challenge in the present study were larger than the DF diameter at the time of deviation. Therefore, ovulation could be expected to occur even in follicles in the 7.0–8.4 mm group. In *B. taurus* breeds, the acquisition of the capacity to ovulate in response to exogenously administered ovulation inducers seemed to differ according to the breed of cattle. In Holstein cows, ovulation in response to the administration of LH has been reported to occur at a size of, or greater than, 10 mm [7]; no ovulations occurred when LH was administered when follicles were 7 or 8.5 mm in diameter. Conversely, treatment with GnRH or pLH on Day 3 of first follicular wave in *B. taurus* beef heifers, resulted in ovulation in 78% of the animals, at mean follicular diameter of 9.6 mm [26]. However, when the incidence of ovulation was assigned to follicular diameter, 100% of heifers with 9 or 10 mm follicles ovulated in response to the treatment, whereas only 50% of heifers with an 8 mm follicle ovulated [26]. We inferred that ovulatory capacity in beef heifers may occur at a smaller diameter than in Holstein cows, suggesting differences within breeds of *B. taurus* cattle. Based on these studies, we purpose that ovulatory capacity can be defined as the minimum diameter in which a dominant follicle is able to respond by ovulating after treatment with an ovulation inducer. In the present study, ovulatory capacity was acquired by follicles between 7.0 and 8.4 mm in *B. indicus* heifers (mean of 7.6 mm), which seemed somewhat smaller than in Holstein cows, but may not be different from *B. taurus* beef heifers.

Although there were some differences in follicle sizes between superstimulated and single-ovulating cattle, recent data on the synchronization of ovulation for fixed-time AI in superstimulated Nelore [27] and Holstein [28–30] donors were consistent with results of the present experiment. In the previous studies, donors were assigned to receive 25 mg of pLH 12 or 24 h after progesterone device removal and submitted to fixed-time AI 12 and 24 h later. In Holstein donors, the pLH treatment given at 24 h after the progesterone device removal improved superovulatory response and the number of transferable embryos [28–30]. Conversely, delaying pLH treatment from 12 to 24 h in Nelore donors increased the number of degenerated embryos, and a decrease in the number of transferable and freezable embryos [27]. Therefore, we inferred that ovulation in superstimulation protocols must be induced earlier in *B. indicus* donors, when follicles are at a smaller size, whereas in *B. taurus* donors, it seems necessary to delay treatment with an ovulation inducer, thereby allowing an increase in follicle size, and the acquisition of LH receptors.

Due to these physiological differences between *indicus* and *taurus* breeds, distinct reproductive management protocols must be considered, especially for timed-AI and superstimulation at a self-appointed times. Further studies are required to determine the consequences of inducing ovulation of small follicles on oocyte competence and conception and pregnancy rates in *B. indicus* cattle. In this way, the relationship between diameter of the ovulatory follicle and fertility can be determined.

In summary, the diameter of dominant (6.2 mm) and largest subordinate follicle (5.9 mm) at the time of deviation (2.5 d after ovulation) in Nelore (*B. indicus*) heifers seemed to be smaller than that previously reported for Holstein (*B. taurus*) cattle. There was no significant difference between observed and calculated methods of deviation analysis. Follicles of *B. indicus* heifers seemed to respond to exogenous LH at smaller diameters than that previously reported in *B. taurus* (Holstein) cattle. In *B. indicus* heifers, ovulatory capacity was acquired when the dominant follicle was between 7.0 and 8.4 mm (mean of 7.6 mm) in diameter; thereafter, responsiveness increased significantly after the dominant follicle reached a diameter of 8.5 mm.

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