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Ovarian follicular dynamics in 2 to 3 months old Nelore calves (*Bos taurus indicus*)

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Abstract

This study aimed to evaluate reproductive physiology aspects in 2 to 3 months old Nelore (*Bos taurus indicus*) calves. Follicular dynamics was monitored daily by ultrasound in ten calves during 18 consecutive days. Calves younger than 2.25 months old (n = 4) had maximum follicle diameters ≤2.5 mm, so only the other animals (n = 6) continued to be monitored by ultrasound. The mean maximum diameter of the ovaries was 13.6 ± 0.6 mm, which had 31.4 ± 3.45 visible follicles. A successive anovulatory follicular wave-like pattern was identified when each wave showed a dominant follicle, including a variable number of other follicles smaller in size (subordinate follicles), during the observational period. Three consecutive follicular growth waves were detected per animal, during this time interval, when 50% of the animals (n = 3) showed two waves and the other half (n = 3) had three waves. The first day of detection of the dominant follicle, retrospectively identified at a diameter of 2 mm, was defined as the day of wave emergence on each wave (day zero). Considering the grouped data, the follicular wave length was 9.2 ± 2.0 days and the correlated dominant follicle began its regression at 6.33 ± 1.63 days after the day of its emergence. The dominant follicles had a growth rate of 0.23 ± 0.06 mm/day and reached the maximum diameter of 3.03 ± 0.17 mm. Despite the smaller ovarian and follicle diameters in 2 to 3 months old Nelore calves (*Bos taurus indicus*) compared to studies in post pubertal females; the follicular wave-like pattern and the number of recruited follicles were similar to the reported in Zebu females in reproductive activity. These data can characterize an early critical moment on the reproductive development of these animals.

Keywords: prepubertal female, reproductive physiology, ultrasound.

Introduction

The use of ultrasonography has allowed a better understanding of the bovine ovarian follicular dynamics in a real time and noninvasive manner. This tool can aid the understanding of the physiological, morphological and endocrine changes in the estrous cycle of these females, including growth, regression of ovarian follicles and ovulation as well as the changes on the corpus luteum (CL; Ginther *et al*., 1989; Kastelic, 1994; Kulick *et al*., 1999; Ireland *et al*., 2000).

The follicular population in the bovine ovary contains two different groups of follicles, one "with no growth" (which contains primordial follicles) and the "growing" one (primary, secondary and tertiary follicles (Kanitz, 2001). The ovaries of a newborn calf can contain over than 100,000 primordial follicles (Erickson, 1966). The wave pattern of follicular growth occurs from the follicular recruitment, which is the entry of follicles in the "growing" group, starting with the activation of the primordial follicle (Oliveira *et al*., 2011). The development of antral follicles in female cattle occurs in a wave-like pattern of follicular growth, where a follicle (dominant follicle - DF) grows more than the others (subordinate follicles), and may subsequently suffer atresia or ovulate (in post-pubertal females) as seen in European (taurines, *Bos taurus taurus*; Pierson and Ginther, 1988; Driancourt, 2001) and Indian breeds (Zebu cattle, *Bos taurus indicus*; Rhodes *et al*., 1995; Figueiredo *et al*., 1997; Viana *et al*., 2000; Sartori and Barros, 2011). Adams *et al*. (1992) reported that there is a FSH surge preceding the recruitment of follicles on each wave in heifers.

Among the mechanisms described to explain the occurrence of dominant and subordinate follicles is that DF changes its dependence on FSH to LH, continuing to grow even during the FSH deprivation in detriment of the other follicles, which stop growing and undergo atresia (subordinates; Ginther *et al*., 1996). Despite the gonadotropins (FSH and LH) playing a primary endocrine role on follicular development, local factors can also interfere on this process as inhibin and IGF-1 (Insulin Like Growth Factor - 1). Inhibin secretion by the DF, by negative feedback, decreases the FSH secretion, playing an important role on the follicular recruitment and development (Turzillo and Fortune, 1993).

Despite its later puberty (reviewed by Randel, 2005), reports describe a greater follicle recruitment per wave in Zebu cattle than in taurines (33.4 ± 3.3 *vs*. 25.0 ± 2.5 respectively; Carvalho *et al*., 2008 and 39.0 ± 4.0 ***vs.*** 21.0 ± 4.0; Alvarez *et al*., 2000). Additionally, it is described that the maximum diameter of dominant follicles in Zebu cattle (10-13 mm; Figueiredo *et al*., 1997; Sartorelli *et al*., 2005; Castilho *et al*., 2007) is smaller than those reported in taurines (16-20 mm; Ginther *et al*., 1989; Kastelic, 1994;

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Ireland *et al*., 2000), both post-pubertal females.

These follicular wave patterns appear both in post-pubertal (two or three waves until ovulation) and prepubertal (continuous growth of follicles, without ovulation; Adams *et al*., 2008) females. The reproduction activity constriction by the absence of ovulation in female cattle, sheep and prepubertal female rats is due to this activity inhibition by greater sensitivity to estradiol in the hypothalamus (Ramirez and McCann, 1963; Ramirez and Sawyer, 1965; Day *et al*., 1987) resulting in negative feedback on this structure.

Silva-Santos *et al*. (2011), evaluating *Bos taurus indicus vs. Bos taurus taurus* histological sections of ovaries from fetuses, heifers and cows, concluded that there is much individual variation in the number of ovarian follicles from Zebu cattle compared to taurines. Analyzing these materials in heifer ovaries, Erickson (1966) reported that right after the birth, only few antral follicles can be viewed macroscopically (7 ± 2), but this number starts to increase from two months old calves (49 ± 10). They also observed that this quantity tends to decrease in eight months old animals (33 ± 9). Adams *et al*. (1994) monitored 9 months old prepubertal *Bos taurus taurus* and found that the follicle growth occurs in a wave pattern. They reported that the calves had two follicular waves in an 18 day interval. Evans *et al*. (1994) studying the follicular dynamics and the secretion of gonadotropins in ten Hereford calves (0.5 to 9 months old *Bos taurus taurus*) observed higher maximum diameter of the dominant and subordinate follicles in 0.5 to 2 months old calves. They also found that there is an increase in serum gonadotropin concentrations in 1 to 3.5 months old calves.

This study aimed to evaluate, by ultrassonography, ovarian follicular dynamics in 2 to 3 months old Nelore calves (*Bos taurus indicus*).

Materials and Methods

Localization

The study was conducted at the Embrapa Genetic Resources and Biotechnology Experimental Farm, in Brasilia, Federal District (DF), located at 15º 52' to 15º56' S and 48º00' to 48°02' W, altitudes between 1,050 and 1,250, a tropical climate region. Its area is about 1,800 ha occupied by Cerrado biome native vegetation as well as by agriculture and pasture.

Animals

This study was submitted and approved by *CEUA* (Ethics Committee on Animal Use from Embrapa Genetic Resources and Biotechnology, Brasilia, DF). Ten Nelore calves (2 to 3 months old) were kept with their mothers with water available *ad libitum*. Their body weight ranged from 55 to 100 kg and body condition score was around 3 (range 0 to 5).

Ultrasonographic examinations and record data

This experiment was performed from June to July 2014, during the winter season. The ovaries of each calf were monitored by transrectal ultrasonography using a linear transducer 7.5 MHz (Aloka 500. Aloka CO. Ltd. Tokyo, Japan) connected to a rigid plastic adapter composed of polyvinyl chloride (PVC) approximately 40 cm long, which had a proper diameter to fit the probe.

The ultrasonographic evaluations were performed daily by the same operator up to 18 days (as described by Adams *et al*., 1994; Evans *et al*., 1994). The images of ovaries were identified, frozen when necessary and the diameters on each animal were measured by the mean between the maximum longitudinal and the perpendicular diameters. The overall mean of the maximum diameter of the ovaries was obtained by the sum of the maximum diameter of the ovaries on each animal divided by the total number of animals.

The follicles were then identified, counted and measured in the same manner as described for the ovaries. Data were recorded on diagrams, aiming to register number, size, relative position of the follicles in the ovaries, image quality, the time of examination, animal identification, among others. Only follicle diameters ≥2 mm were measured and recorded. All follicles (≥2 mm in diameter) detected in both ovaries for each day were counted.

The first day of detection of the dominant follicle on each wave, identified at a diameter of 2 mm, was defined as the day of this wave emergence. The follicular waves were monitored since the DF diameter reached ≥2 mm (detection day) until its regression to the initial size and so on for the successive waves. The Wave lengths were obtained by subtracting its detection day from the last day when the DF was detected (≥2 mm) on each wave. The Onset of atresia of each wave was considered the day immediately before the day when the DF diameter was smaller than its previous measurement, counted from the day of detection of each wave. The follicular waves were designated as the first, second and third waves detected during the observational period (as described by Adams *et al*., 1994). The inter-wave interval was defined as the number of days between the emergences of successive dominant follicles. The DF growth rate of each wave was obtained by subtracting the minimum DF diameter from the maximum DF diameter (≥2 mm), divided by the number of days of the growth period.

During the daily ultrasound in animals (n = 10) it was found that some (n = 4, age ≤2.25 months) only had follicles ≤2.5 mm. Thus, the other animals (n = 6) had their follicular waves monitored.

Data analysis

For each wave a descriptive analysis (Mean ± Standard Deviation - SD) was performed for the following characteristics: Maximum diameter of the DF (mm), Onset of atresia of the DF (day), Growth rate of the DF (mm/day), Wave length (days) and Inter-wave interval (IWI, days). The Mean ± SD was also obtained for Maximum diameter of the ovaries and Number of follicles detected, completing the descriptive analysis.

The Mann-Whitney test (PROPHET Version 5.0 Program; BBN Systems and Technologies, 1997) was used to compare calves (n = 3) which showed 3 waves for calves which showed two waves during the same observational period.

Results

The 1 to 2.25 months old calves (n = 4) had maximum follicle diameters ≤ 2.5 mm and, consequently, were not used in the records of follicular waves.

A pattern of successive anovulatory follicular waves was observed, including a dominant follicle (DF) and a variable number of other smaller follicles (subordinates) per wave. During 18 consecutive days, 50% of the animals (n = 3) had two waves and the other half had three waves of follicular growth.

The maximum diameter of the ovaries was 13.6 ± 0.6 mm containing 31.4 ± 3.45 visible follicles on ultrasound (Tab.1). The maximum diameter of dominant follicle was 3.03 ± 0.17 mm (n = 15 waves), which had a growth rate of 0.23 ± 0.06 mm/day (n = 15 waves) and its onset of atresia on the 6.33 ± 1.63 th day from the detection day of each wave (n = 15 waves. Tab.1). Wave length was 9.2 ± 2.0 days (n = 15 waves) and the Inter-wave interval (IWI) was 5.55 ± 2.55 (n = 9 waves; Tab.1).

Even the calves (n = 3) which showed a higher number of waves (3 waves) had similar IWIs compared to animals which showed two waves (4.66 ± 2.08 *vs.* 7.0 ± 1.0; respectively for three *vs.* two follicular waves animals; P = 0,795), during the same observational period.

In order to illustrate the dynamics of the dominant and the largest subordinate follicles during the observational period, graphics of 2.7 ± 0.33 months old calves (n = 6) are shown on Fig. 1 to 6.

Table1. Characteristics of ovaries and follicular waves in Zebu calves by ultrasonography.

Ovarian/Follicular characteristics	Value*
Maximum diameter of the ovaries (mm; n = 12 ovaries)	13.6 ± 0.6
Number follicles detected ≥ 2 mm (n = 6 animals)	31.4 ± 3.45
Maximum diameter of the dominant follicle (mm; n = 15 waves)	3.03 ± 0.17
Growth rate of the dominant follicles (mm/day; n = 15 waves)	0.23 ± 0.06
Wave length (days; n = 15 waves)	9.2 ± 2.0
Onset of atresia of dominant follicle (n = 15 waves)	6.33 ± 1.63
Inter-wave Interval (IWI; days; n = 9 waves)	5.55 ± 2.55

*Mean ± SD (standard deviation).

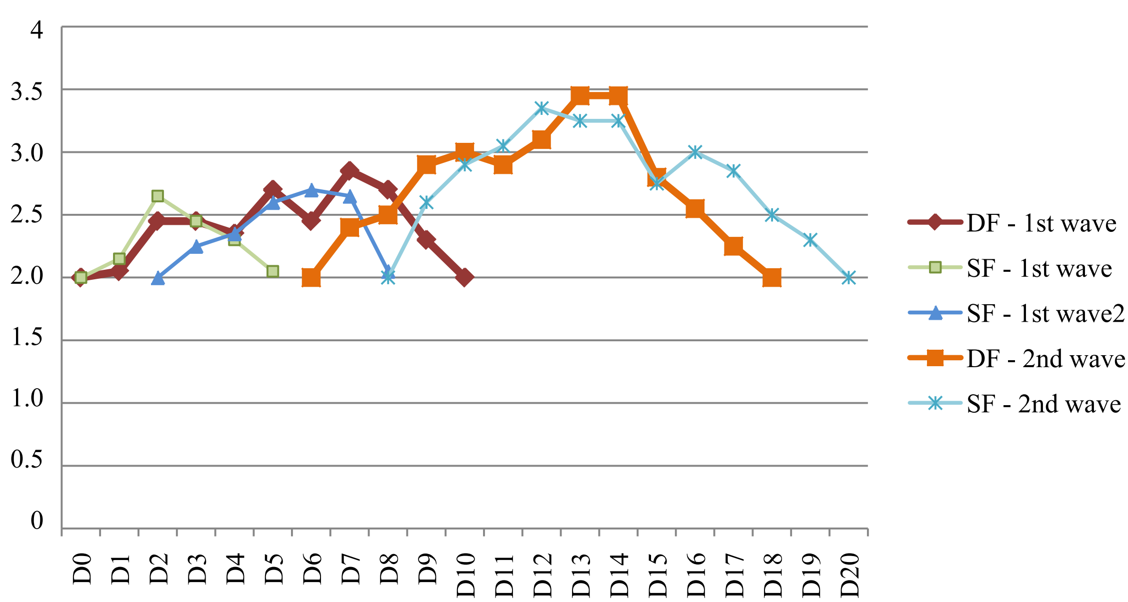

Figure 1. Follicular dynamics in a 3 month old calf.

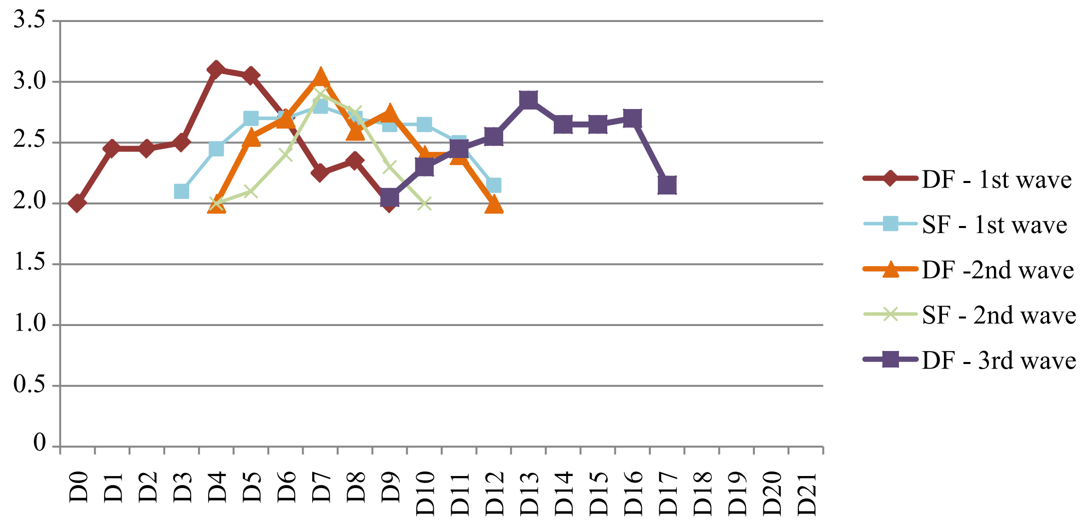

Figure 2. Follicular dynamics in a 3 month old calf.

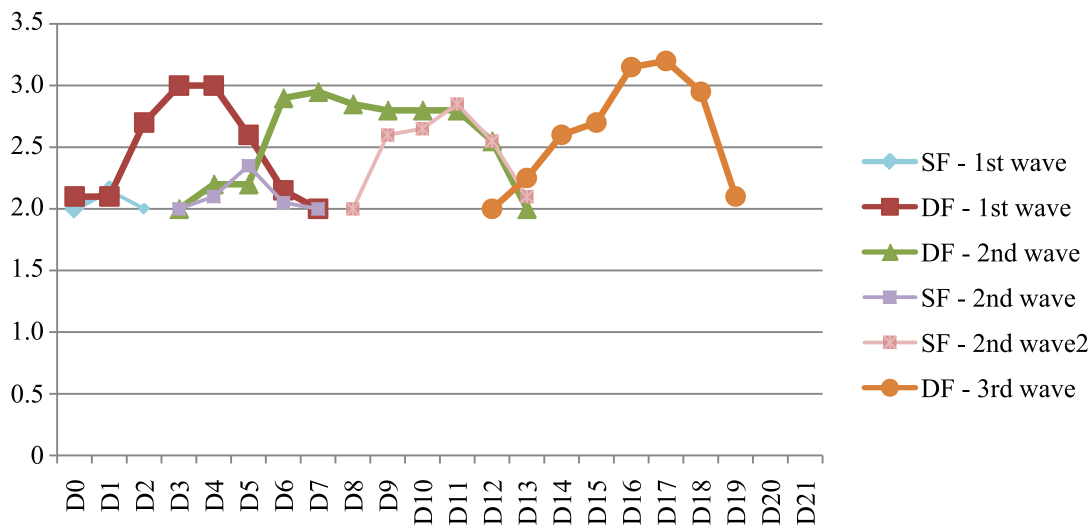

Figure 3. Follicular dynamics in a 3 month old calf.

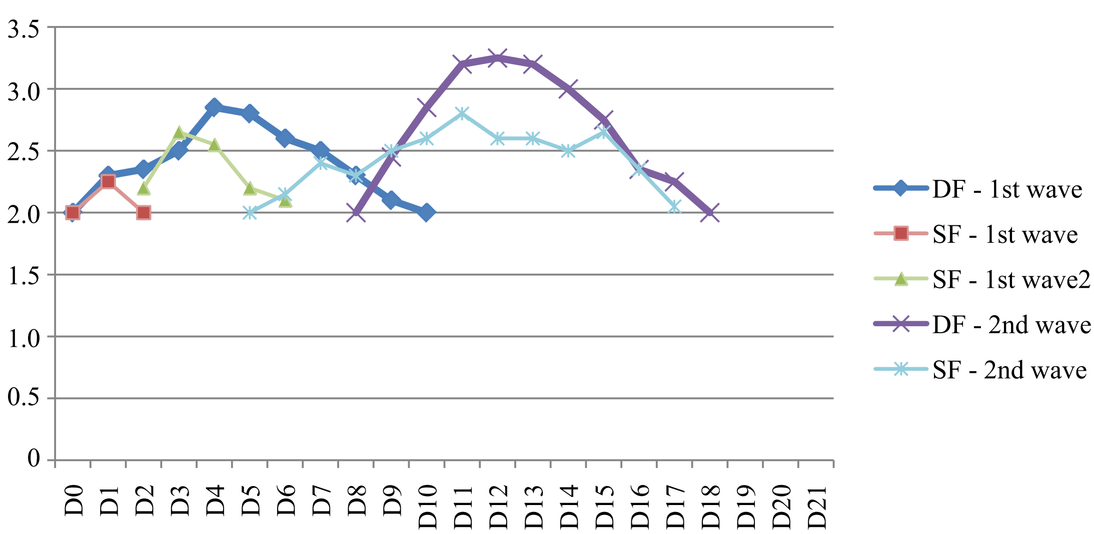

Figure 4. Follicular dynamics in a 2.5 month old calf.

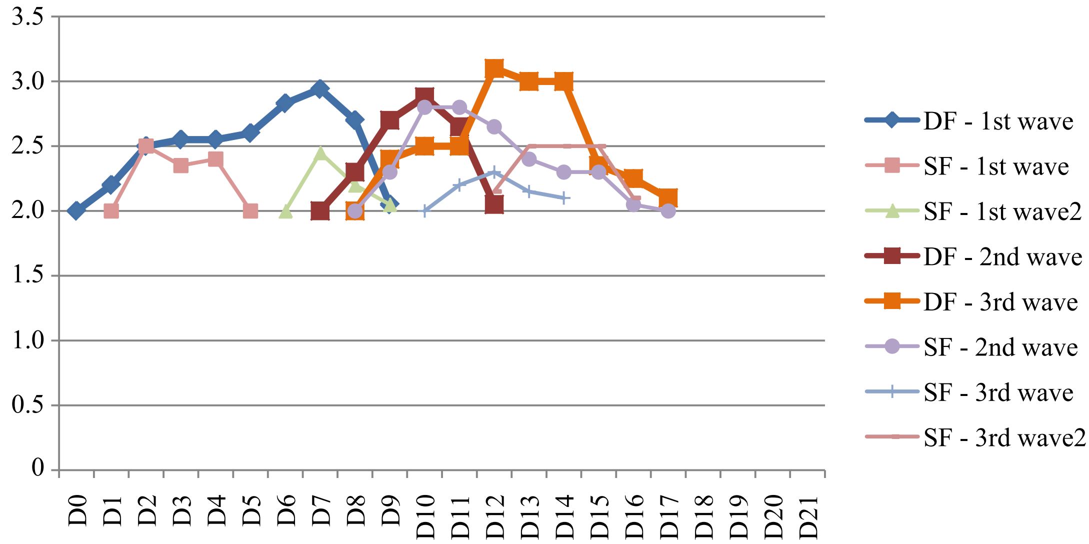

Figure 5. Follicular dynamics in a 2.25 month old calf.

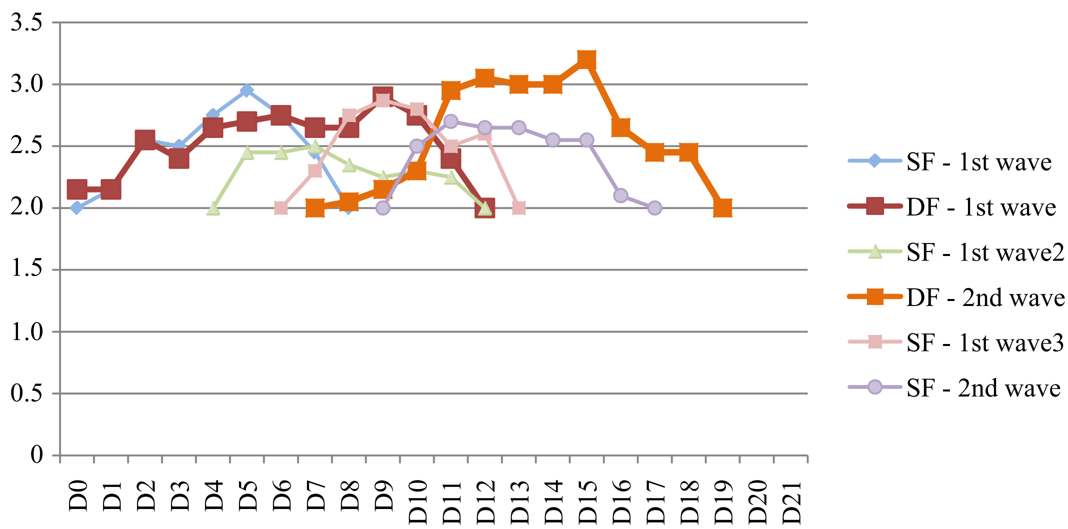

Figure 6. Follicular dynamics in a 2.5 month old calf.

Discussion

The fact that the *Bos taurus indicus* calves younger than 2.25 months old (n = 4) in this study did not present follicles larger than 2.5 mm diameter, corroborates a study by Erickson (1966), who demonstrated that calves (*Bos taurus taurus*) began to present a greater number of antral follicles from 2 months old on. However, Kauffold *et al*. (2005) conducting follicular aspiration in *Bos taurus taurus* calves (<2 months old) demonstrated that those animals had follicle diameters >8 mm. The onset of reproductive activity or even the steps before this stage can be influenced by several factors (*e.g*., climate, nutrition, handling, breed). Additionally, there is a particular distinction between Zebu cattle (*Bos taurus indicus*), which is usually begins reproductive activity later than the taurine (Randel, 2005). Therefore, besides subspecies issues, other factors may influence the follicular population (Ireland *et al*., 2008, 2011). Variations within the same breed may occur, for example: in bovine of the same breed, there are animals with different follicular populations according to the farm. Despite this variation, it is known that the repeatability in the antral follicle count is maintained in the same individual. So, some of these factors could have determined the moment that the evaluated calves began to show larger follicles.

The successive follicular wave-like pattern observed in 2.7 ± 0.33 months old calves corroborates reported data in *Bos taurus taurus* and *Bos taurus indicus* as in post-pubertal (Pierson and Ginther, 1988, Rhodes *et al*., 1995; Figueiredo *et al*., 1997; Viana *et al*., 2000; Sartori and Barros, 2011) and in prepubertal females follicular dynamics (Evans *et al*., 1994; Reis *et al*., 2005; Adams *et al*., 2008). The same was observed for the presence of a dominant follicle and a variable number of subordinate follicles, which could be explained by the presumed secretion of inhibin by DF, which would decrease the serum concentration of FSH (Turzillo and Fortune, 1993) beyond changing dependence of the dominant follicle FSH to LH, which allows it to continue to grow even during the deprivation of FSH in the detriment of other follicles (Ginther *et al*., 1996).

It was also expected that the observed waves in this study were anovulatory due to specific blockade peaks in the secretion of GnRH in the hypothalamus of prepubertal females, which consequently limits peaks of FSH and LH sufficient to promote the development of follicles to the preovulatory phase and to the resulting ovulation (Ramirez and McCann, 1963; Ramirez and Sawyer, 1965; Day *et al*., 1987; Adams *et al*., 2008). The same reason can also explain the fact that the maximum diameter of the dominant follicles of these calves (3.03 ± 0.17 mm) was smaller than those reported in *Bos taurus indicus* post-pubertal females (10 to 13 mm, Figueiredo *et al*., 1997; Sartorelli *et al*., 2005; Castilho *et al*., 2007) and, consequently, the maximum diameter of the ovaries was lower too (13.6 *vs*. 27.2 mm, on this study *vs*. Carvalho *et al*., 2008 reported data; respectively) due to containing smaller follicles, as well as not having any CL.

The number of follicles detected in the calves' ovaries (≥2 mm; 31.4 ± 3.45) was similar to that reported in post-pubertal Zebu cattle (33.4 ± 3.3, Carvalho *et al*., 2008 and 39.0 ± 4.0, Alvarez *et al*., 2000) and was higher than those reported for post-pubertal taurine females (25.0 ± 2.5, Carvalho *et al*., 2008 and 21.0 ± 4.0, Alvarez *et al*., 2000) or 3.5 months old prepubertal calves (19.4 ± 2.1, Evans *et al*., 1994). This occurrence can be explained by the fact that the evaluated calves were Zebu cattle (*Bos taurus indicus*), thus it can develop a greater number of follicles per wave than taurines.

The growth rate of the dominant follicle was up to 0.23 ± 0.06 mm/day; that is lower than that reported in cows (0.92 mm/day in Nelore breed, Figueiredo *et al*., 1997, and 1.6 mm/day in taurine, Sirois and Fortune, 1988).

The wave length in the evaluated calves (9.2 ± 2.0 days) was similar to that reported by Evans *et al*. (1994, 11.6 ± 1.3 days) in 2 months old *Bos taurus taurus* calves (Hereford) and that reported by Reis *et al*. (2005), in 22 months old prepubertal Zebu heifers (Gir), who reported that 12% of the animals showed follicular wave of up to 12 days and about 88% of the animals had wave lenght of 13 to 17 days. Similar wave length reported on this study was also described by other authors in Zebu females (Sartorelli *et al*., 2005; Castilho *et al*., 2007; Carvalho *et al*., 2008).

The Onset of atresia of the dominant follicles in monitored calves occurred from the 6.33 ± 1.63th day on, that is similar to that reported in 22 months old prepubertal heifers by Reis *et al*. (2005; 7.4 ± 0.3 days; Gir), Adams *et al*. (1994; around day 8, 8 months old Hereford heifers) and Evans *et al*. (1994; around day 5 in 1 months old Hereford calves) and post-pubertal Nelore females by Figueiredo *et al*. (1997; 8.86 ± 0.5 days).

In conclusion, despite the smaller ovarian and follicle diameters in 2 to 3 months old Nelore calves (*Bos taurus indicus*) compared to studies in post pubertal females; the follicular wave-like pattern and the number of recruited follicles were similar to the reported in Zebu females at reproductive activity. These data can characterize an early critical moment on reproductive development of these animals.

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References

Adams GP, Matteri RL, Kastelic JP, Ko JCH, Ginther OJ. 1992. Association between surges of follicle stimulating hormone and the emergence of follicular waves in heifers. *J Reprod Fertil*, 94:177-188.

Adams GP, Evans ACO, Rawlings NC. 1994. Follicular waves and circulating gonadotrophins in 8-months-old prepubertal heifers. *J Reprod Fertil*, 100:27-33.

Adams GP, Jaiswal R, Singh J, Malhi P. 2008. Progress in understanding ovarian follicular dynamics in cattle. *Theriogenology*, 69:72-80.

Alvarez P, Spicer LJ, Chase Jr CC, Payton ME, Hamilton TD, Stewart RE, Hammond AC, Olson TA, Wettemann RP. 2000. Ovarian and endocrine characteristics during an estrous cycle in Angus, Brahman and Senepol cows in a subtropical enviroment. *J Anim Sci*, 78:1291-1302.

Carvalho JB, Carvalho NA, Reis EL, Nichi M, Souza AH, Baruselli PS. 2008. Effect of early luteolysis in progesterone-based timed AI protocols in *Bos indicus*, *Bos indicus* x *Bos taurus*, and *Bos taurus* heifers. *Theriogenology*, 69:167-175.

Castilho C, Garcia JM, Renesto A, Nogueira GP, Brito LF. 2007. Follicular dynamics and plasma FSH and progesterone concentrations during follicular deviation in the first post-ovulatory wave in Nelore (*Bos indicus*) heifers. *Anim Reprod Sci*, 98:189-196.

Day ML, Imakawa K, Wolfe PL, Kittok RJ, Kinder JE. 1987. Endocrine mechanisms of puberty in heifers. Role of hypothalamo-pituitary estradiol receptors in the negative feedback of estradiol on luteinizing hormone secretion. *Biol Reprod*, 37:1054-1065.

Driancourt MA. 2001. Regulation of ovarian follicular dynamics in farm animals. Implications for manipulation of reproduction. *Theriogenology*, 55:1211-1239.

Erickson BH. 1966. Development and senescence of the postnatal bovine ovary. *J Anim Sci*, 25:800-805.

Evans ACO, Adams GP, Rawlings NC. 1994. Follicular and hormonal development in prepubertal heifers from 2 to 36 weeks of age. *J Reprod Fertil*, 102:463-470.

Figueiredo RA, Barros CM, Pinheiro OL, Soler JMP. 1997. Ovarian follicular dynamics in Nelore breed (*Bos indicus*) cattle. *Theriogenology*, 47:1489-1505.

Ginther OJ, Knopf L, Kastelic JP. 1989. Temporal associations among ovarian events in cattle during oestrous cycles with two or three follicular waves. *J Reprod Fertil*, v.87, p.223-230.

Ginther OJ, Wiltbank MC, Fricke PM, Gibbons JR, Kot K. 1996. Selection of the dominant follicle in cattle. *Biol Reprod*, 55:1187-1194.

Ireland JJ, Mihm M, Austin E, Diskin MG, Roche JF. 2000. Historical perspective of turnover of dominant follicles during the bovine estrous cycle: key concepts, studies, advancements, and terms. *J Dairy Sci*, 83:1648-1658.

Ireland JL, Scheetz D, Jimenez-Krassel F, Themmen AP, Ward F, Lonergan P, Smith GW, Perez GI, Evans AC, Ireland JJ. 2008. Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. *Biol Reprod*, 79:1219-1225.

Ireland JJ, Smith GW, Scheetz D, Jimenez-Krassel F, Folger JK, Ireland JL, Mossa F, Lonergan P, Evans AC. 2011. Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Müllerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. *Reprod Fertil Dev*, 23:1-14.

Kanitz W. 2001. Comparative aspects of folicular development, follicular and oocyte maturation and ovulation in cattle and pigs. *Arch Anim Breed*, 44:p.9-23.

Kastelic JP. 1994. Understanding ovarian follicular development in cattle. *Vet Med*, 6:64-71.

Kauffold J, Amer HAH, Bergfeld U, Muller F, Weber W, Sobiraj A. 2005. Offspring from non-stimulated calves at an age younger than two months: a preliminary report. *J Reprod Dev*, v.51:427-432.

Kulick LJ, Kot K, Wiltbank MC. 1999. Follicular and hormonal dynamics during the first follicular wave in heifers. *Theriogenology*, 52:913-921.

Oliveira MEF, Ferreira RM, Mingoti GZ. 2011. Controle do crescimento e da seleção follicular por fatores locais e sistêmicos na espécie bovina. *Rev Bras Reprod Anim*, 35:418-432.

Pierson RA, Ginther OJ. 1988. Ultrasonic imaging of the ovaries and uterus in cattle. *Theriogenology*, 29:21-37.

Ramirez VD, Mccann SM. 1963. Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult. *Endocrinology*, 72:452-464.

Ramirez VD, Sawyer CH. 1965. Advancement of puberty in the female rat by estrogen. *Endocrinology*, 76:1158-1168.

Randel RD. 2005. Reproduction of *Bos indicus* breeds and crosses. *In*: Proceedings. Applied Reproductive Strategies in Beef Cattle, 2005, College Station, TX. College Station, TX: Texas A&M University. 20 pp.

Reis AR, Reyes A, Gambarini ML, Rumpf R, Oliveira CC, Oliveira Filho BD. 2005. Dinâmica follicular por ultrassonografia em novilhas pré-púberes da raça Gir. *Arch Latinoam Prod Anim*, 13:51-55.

Rhodes FM, Fitzpatrick LA, Entwistle KW, De´Ath G. 1995. Sequential changes in ovarian follicular dynamics in *Bos indicus* heifers before and after nutritional anoestrous. *J Reprod Fertil*, 104:41-49.

Sartorelli ES, Carvalho LM, Bergfelt DR, Ginther OJ, Barros CM. 2005. Morphological characterization of follicle deviation in Nelore (*Bos indicus*) heifers and cows. *Theriogenology*, 63:2382-2394

Sartori R, Barros CM. 2011. Reproductive cycles in *Bos indicus* cattle. *Anim Reprod Sci*, 124:244-250.

Silva-Santos KC, Santos GMG, Siloto LS, Hertel MF, Andrade ER, Rubin MIB, Sturio L, Melo-Sterza FA, Seneda MM. 2011. Estimate of the population of preantral follicles in the ovaries of *Bos taurus indicus* and *Bos taurus taurus* cattle. *Theriogenology*, 76:1051-1057.

Sirois J, Fortune JE. 1988. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. *Biol Reprod*, 39:308-317.

Turzillo AM, Fortune JE. 1993. Effects of suppressing plasma FSH on ovarian follicular dominance in cattle. *J Reprod Fertil*, 98:113-119.

Viana JHM, Ferreira AM, Sá WF, Camargo LSA. 2000. Follicular dynamics in zebu cattle. *Pesq Agrop Bras*, 35:2501-2509.