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

T.A. Zacarias¹, S.B. Sena-Netto², A.S. Mendonça¹, M.M. Franco¹,³, R.A. Figueiredo³,⁴

¹Universidade Federal de Uberlândia (UFU), Uberlândia, MG, Brazil.
²Universidade de Brasília (UnB), Brasília, DF, Brazil.
³Embrapa Recursos Genéticos e Biotecnologia (CENARGEN), Brasília, DF, Brazil.

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 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;
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 ultrasonography, 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 15°52’ to 15°56’ S and 48°00’ 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). The 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 ≥2.55 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.

Table 1. Characteristics of ovaries and follicular waves in Zebu calves by ultrasonography.

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

*Mean ± SD (standard deviation).
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, 1989).
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 (3.0 ± 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 ± 3.4, 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 length of 13 to 17 days. Similar wave length reported in 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.

Acknowledgments

This study was supported by EMBRAPA, CAPES and FAPEMIG.

References


