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Research Article

Characterization of Follicular Dynamics in the Kenyan Boran Cow

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ABSTRACT

Follicular dynamics is one of the most important subjects in ovarian physiology. In cows and heifers, it is characterized by waves of follicular growth and regression. This has been largely studied in European breeds while studies on Zebu cattle (*Bos indicus*) are limited. Differences between breeds of cattle exist and this information has been used to establish correct ovulation parameters and management procedures for these breeds. This study aimed at investigating the follicular dynamics of the Kenyan Boran cow (*Bos indicus*) during its estrous cycle.

Follicular developmental characteristics were evaluated using a 5.0-7.5 Megahertz (MHz) linear array portable ultrasound device. Transrectal scanning was done daily for a period of two consecutive estrous cycles in 15 Boran Cows. Predominantly, the cows exhibited three (70.59%) follicular waves per cycles while a smaller population of the cows (25.53%) exhibited two follicular waves per cycle. Of the animals used, (5.88%) had four follicular waves per cycle. The pre-ovulatory dominant follicle size for the Kenyan Boran cow was recorded as 13.56±1.73 mm and was higher than the diameter of all the other dominant follicles. The duration of dominance of wave one was approximately three days longer and the onset of atresia of the first wave occurred approximately two days later in two-wave cycle animals than in three-wave cycle animals ($P<0.05$). There was no significant difference in cows with different waves per cycle.

This study observed that like in other Boran breeds the follicular dynamics in the Kenyan Boran cow are characterized by a higher incidence of cycles that have three follicular waves associated with a short persistence of the dominant follicle of the first wave.

Key words: Boran cow, atresia, folliculogenesis, ultrasonography

INTRODUCTION

Up to 80% of Kenya's landmass is Arid or Semi-Arid Land (ASAL) characterized by high ambient temperature, poor feed quality and high disease challenges (Herlocker, 1999). Since crops do not grow easily in these areas, the inhabitants here are mainly pastoralists who depend on livestock as their most viable source of livelihood. Zebu cattle (*Bos indicus*) are the predominant cattle kept in these ASAL areas. The Zebu cattle are better adapted to survive in these harsh and unfavorable climatic conditions. However, when compared to *Bos taurus* cattle the Zebu cattle have low production and reproductive potential (Lamothe-zavaleta *et al.,* 1991). Among the Zebu breeds, the most commercially viable in Kenya is the Boran (Rewe *et al.,* 2006; Mutembei *et al.,* 2015). Although this seemingly low valued Boran can be uplifted to its optimum reproductive potential using available Assisted Reproductive Technologies (ARTs), basic information that can help this endeavor is lacking. This includes the peculiarity of the reproductive physiology of the Boran cow when compared to the more studied taurine breeds and other Zebu cows in other places in the world.

In *Bos taurus* cattle, more than 95% of all estrous cycles consist of two or three follicular waves (Evans 2003) while Zebu breeds of cattle have shown up to four-wave cycles (Bo' *et al.,* 1995) and even occasionally five-wave cycles (Viana *et al.,* 2000). The zebu cows also differ from these taurine breeds in characteristics such as estradiol profile, dominant follicle diameter (Figueiredo *et al.,* 1997) estrus length (Medrano *et al.,* 1996), ovulation moment (Pinheiro *et al.,* 1998), and a higher number of follicular waves during the estrus cycle (Viana *et al.,* 2000).

This vital information on the peculiarities of follicular dynamics and luteal development of the Kenyan Boran cow is grossly missing and to fill this void this study was developed. By so doing, we aim to enhance effective and efficient utilization of various assisted reproductive technologies in the Boran cow through better understanding of the physiology of the follicular dynamics.

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MATERIALS AND METHODS

The study was carried out in the Kapiti Plains Ranch, in Machakos county Kenya, located at a latitude of 01°30'00" and a longitude of 37°00'00". Fifteen regularly cycling, multiparous, non-lactating Boran cows aged between 5-6 years of body condition score of 2-2.5 were used. Initially, clinical and gynecological examinations were done to rule out any disease or reproductive abnormalities in the selected cows. The cows were maintained under uniform conditions of feeding and management and were not allowed to run with the bull during the study period.

To initiate the study, selected animals were restrained in a metallic crush where rectal palpation was done to identify the structures on the ovaries. With the presence of *Corpus Luteum* (CL) noted, the cows received 500µg Cloprostenol (2ml estroplan®) intramuscularly on the side ipsilateral to the CL. Animals that responded by coming of heat 2-3days after this treatment were used for the study.

Ultrasonographic study was then done using a portable ultrasound device equipped with a 5.0/7.5 MHz linear bi-frequential probe (frequency of 7.5 MHz was used as standard). These were done daily for two consecutive estrous cycles. The probe was secured in an examination sleeve to protect the diaphragm and also to hold the acoustic gel in place. The probe was then introduced into the rectum after fecal evacuation was done and moved back and forth over the pelvic area to scan the ovaries from above.

Follicles of different sizes and the CL were identified and their location on the ovaries was noted. The diameters of the three largest follicles and *corpus luteum* (CL) were then measured daily and their location sketched on a research book to ease their identification the following day. These follicles traits were used to track and evaluate for daily follicular dynamics. Various parameters were then determined as previously described by (Alvarez *et al.,* 2000). Follicular populations were obtained by manually counting of all visible follicles. Numbers and sizes of ovarian follicles were determined for each day and follicles were considered small (2 to 5 mm) or medium (6 to 8 mm) (Alvarez *et al.,* 2000).

The dominant follicle (DF) of a wave was defined as the follicle that measured at least 9 mm in diameter and exceeded the diameter of all other follicles in the wave (Ginther *et al.,* 1996). A subordinate follicle (SF) was defined as one that originated from the same follicular pool as the dominant follicle but had reduced or terminated growth after the deviation period. These follicles were the second and third largest follicles subsequently seen and were labeled the first (SF1) or second (SF2) subordinate follicles respectively. Ovulation was determined by the disappearance of the dominant follicle and subsequent formation of a CL at the same location on the ovary and that marked the end of an estrous cycle. The disappearance of a DF marked the beginning of the next cycle which was named Day 0.

Depending on the dominant follicle that ovulated the estrous cycles were described as having two, three or four follicle waves (Burke *et al.,* 2000). The day of wave onset was defined as the day at which the dominant follicle was retrospectively traced to be 4 to 5 mm in diameter (Burke *et al.,* 2000). Atresia onset was considered as the day from which follicles began regressing in size. The growth rate of the dominant follicle was determined as the size difference from the day the dominant follicle was first identified to the day that the diameter of the follicle no longer increased more than 1 mm (Alvarez *et al.,* 2000). Growth and atresia rates were determined by dividing the variation observed on follicular diameter by the number of days of observation (Rhodes *et al.,* 1995).

Data was grouped according to the number of follicle waves present in each estrous cycle. Descriptive statistics was used to calculate the mean, variance and standard error of means. Among animals with the same number of follicular waves per cycle, parameters were evaluated by variance analysis (ANOVA), by a randomized block schedule, in which cow characterized the block. Correlations for parameters of follicle growth rate, estrous cycle length, wave length, dominance of wave one dominant follicle within and among the groups was done using SPSS statistical software and reported as tables.

RESULTS

Of the study animals, 70.59% had three follicular waves per cycle (Fig 1), 23.53% had two follicular waves per cycle while 5.88% had four follicular waves per cycle. For the two consecutive estrous cycles period studied the occurrence of follicular waves revealed 86.7% repeatability of the wave distribution. Two cows (13.3%) had different follicular wave pattern per cycle; one cow had two waves followed by three waves per cycle while the other cow had three waves followed by four waves per cycle. These two animals were grouped according to the wave pattern of the first estrous cycle.

In two-wave cycle animals, the first wave emerged on day 0.61±0.06 attaining a maximum diameter of 13.6±0.92 mm on day 7.20±0.37. The second wave emerged on day 10.40±1.17, with DF maximum diameter of 14.00±0.85mm. Onset of atresia of the first dominant follicle occurred on day 12.20±1.11 which the regressed at a rate of 1.01±0.29mm/day. A higher proportion (73.1%) of follicular growth was seen on the right ovary as compared to the left ovary (26.9%). Table 1 presents the characteristics of follicle dynamics in cows with two waves per cycle.

The mean number of small, medium and total number of follicles recorded each day during different days of estrus cycle in the Boran cows are presented in Fig. 2 below. The number of medium-sized follicles was lower throughout the estrus cycle compared to small follicles, although there was no definite pattern. The left ovary was observed to carry a significantly larger number of follicular populations at $P \leq 0.05$ although most of the dominant follicles in the waves emanated from the right ovary.

As referenced in table 2 for animals with three waves per cycle, the first wave emerged on day 0.42 ± 0.16 of the cycle. The selection of the dominant follicle occurred on day 4.13±0.99. The dominant follicle reached a maximum diameter of 10.95 ± 0.34mm on day 6.31±0.44 and atresia commenced on day 10.15±0.34. This was followed by a short period of plateau while the largest

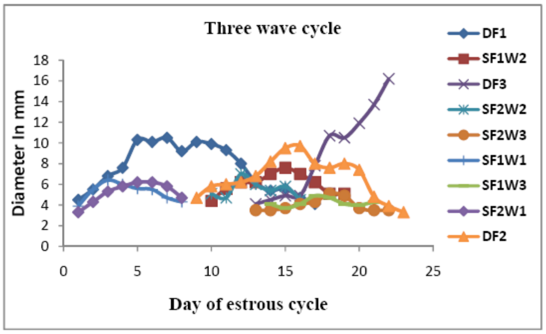

Fig 1: Follicular Dynamics of the Boran Cow with Three Waves per Estrous Cycle (Cow # 54004B)

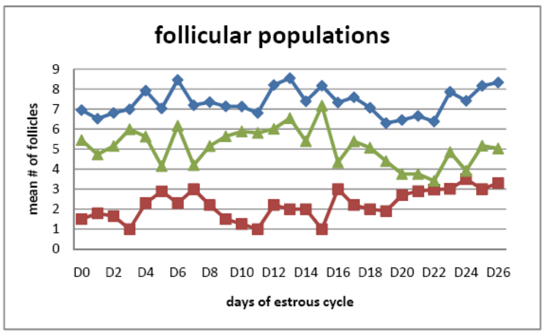

Fig. 2: Plot showing the populations of small and medium sized follicles in an IOI.

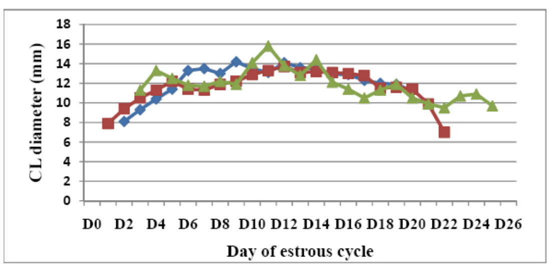

Fig. 3: Plot showing the CL development patterns in 2, 3, and 4-wave patterns.

Table 1: Characteristics of follicular development in Boran cows with two waves per cycle

Characteristic	Follicular wave	
	First	Second
Wave onset (days)	0.61±0.06 [a]	10.40±1.17[b]
Wavelength (days)	16.20±1.24[a]	11.60±1.20[b]
Dominant follicle		
Maximum diameter (mm)	13.6±0.92 [a]	14.00±0.85 [a]
Day of maximum diameter	7.20±0.37 [a]	19.40±2.01[b]
Growth rate (mm/day)	0.92±0.04 [a]	0.78±0.04[b]
Length of growth phase	7.8±0.06 [a]	11.60±1.21[b]
Duration of dominance	12.40±1.12[a]	9.80±1.16 [a]
Onset of atresia(days)	12.20±1.11[a]	
Atresia rate (mm/day)	1.01±0.29 [a]	
Length of atresia (days)	4.00±1.61[a]	
Largest subordinate follicle		
Maximum diameter (mm)	7.40±0.82[a]	7.12±0.42[a]
Growth rate (mm/day)	0.60±0.11[a]	0.52±0.12[a]
Atresia rate (mm/day)	0.74±0.19[a]	0.43±0.92 [a]

[a b] values followed by different letters in superscript in the same line differ significantly P≤0.05; Values presented in means ±SEM

subordinate follicle reached maximum diameter of (6.74±0.42mm in the first wave) and induced its regression.

The second and third wave emerged on day 8.15±0.56 and day 15.00 ± 0.98 respectively. The respective dominant follicles were selected on day 12.53±1.98 and day 17.19±2.75, and they attained a maximum diameter of 10.63±0.58 and 13.52±0.50 on day 14.54±1.22 and day 22.31±1.04 respectively. Atresia of the second wave dominant follicle began on day 16.23±1.18 while the third follicular wave finished with the ovulation of the third wave DF. At P≤0.05 the ovulatory follicle was larger than the other dominant follicles even when they appeared to have had a statistically similar growth rates, length of growth phase and duration of dominance. They all, however, achieved maximum diameters at statistically different days.

The *corpus luteum* developed in a manner that was similar throughout the cycles. Three developmental phases were noted, a growth phase, a lag phase and luteal regression phase (Fig 3). The development was characterized by a steady increase in the diameter of CL then a plateau when the diameter did not change much followed by a decrease in the diameter of the CL. The maximum diameters of the CL among cows with different waves per cycle did not show any significant difference. The CL seemed to regulate the dominance period of the DF in the Boran cow (Table 3).

DISCUSSION

The results of this study shows that follicular dynamics in the Boran cow develop in a wave like fashion. This observation closely compares to that of other Zebu cattle studied elsewhere in the world. None of the cows studied had one or five follicular waves as previously reported in Brazilian Zebu cattle by (Viana *et al.,* 2000). Similarly the Kenyan Boran cow showed a predominance of three follicular waves per cycle. The present findings supported the fact that the number of follicular growth is associated to the duration of estrous cycle and the length of the luteal phase. Animals with four follicular waves presented the longest luteal phase and the longest estrous cycle length. These results agree with those of Sartorelli *et al*. (2005) in *Bos Indicus* cattle. In a two-wave cycle, the first wave emerged on day 0.61±0.06 of the cycle and grew for 7.80±0.60 days, whereas the second wave emerged on day 10.40±1.17 and achieved a maximum diameter of 14.0±0.85 mm. It has been previously seen in studies on cattle that the first and second (ovulatory) waves in a two-wave cycle commence on day 2 to 4 and day 10-11, and the DF reached maximum diameter on days 6 and 19, respectively (Sirois and Fortune, 1988). Findings of this study, however, contrasted with the above recorded findings majorly on the wave onset of the first wave and compared to finding in the Brazilian Zebu cow by Viana *et al*. (2000).

The findings for the Boran cow on wave emergence compared favorably to what has been observed in European cows (Sirois and Fortune, 1988) and also for other Zebu cows in other parts of the world (Sartori *et al.,* 2010). Follicular emergence is usually in reference to the day of ovulation and it is characterized by the sudden growth of 8 to 41 small follicles (Adams *et al.,* 2008;

Table 2: Characteristics of follicular waves, growth and atresia of dominant follicles and of the largest subordinate follicle in cows with three follicular waves during estrous cycle (mean ± standard deviation)

Characteristic	Follicular wave		
	First	Second	Third
Wave onset (days)	0.42±0.16 [a]	8.15±0.56 [b]	15.00±0.98 [c]
Wavelength (days)	15.77±1.03 [a]	13.15±1.48 [a]	8.77±0.74 [b]
Dominant follicle			
Maximum diameter (mm)	10.95±0.34 [a]	10.63±0.58[a]	13.52±0.50[b]
Day of maximum diameter	6.31±0.44 [a]	14.54±1.22 [b]	22.31±1.04 [c]
Growth rate (mm/day)	0.87±0.07[a]	0.73±0.52[a]	0.87±0.81[a]
Length of growth phase	7.08±0.40[a]	7.77±0.77[a]	8.62±0.76[a]
Duration of dominance	9.31±0.60[a]	8.38±0.99[a]	8.77±0.74[a]
Onset of atresia	10.15±0.58[a]	16.23±1.18[b]	
Atresia rate (mm/day)	0.92±0.10[a]	1.02±0.10[a]	
Length of atresia (days)	5.69±0.09[a]	4.46±0.63[a]	
Largest subordinate follicle			
Maximum diameter (mm)	6.74±0.20[a]	6.89±0.37[a]	7.28±0.04[a]
Growth rate (mm/day)	0.60±0.90[a]	0.49±0.07[a]	0.57±0.57[a]
Atresia rate (mm/day)	0.63±0.10[a]	0.69±0.08	0.58±0.11[a]
Persistence (days)	8	9.25	7.75

[a b c] values followed by different letters in subscript in the same line differ significantly P≤0.05 Values presented in means ±SEM

Table 3: Luteal characteristics (IOI; mean ± SEM) in Boran cattle

	2-Wave IOI	3-Wave IOI	4-wave IOI	P-value
Inter-ovulatory interval (days)	18.6±1.90	23.6±1.05	27.0±4.0	0.03
Maximum diameter of CL (mm)	17.0±1.29	15.9±0.64	15.1±0.90	0.44
Day of onset of regression of CL after ovulation	10.8±1.74	14.4±0.92	15.4±0.87	0.05
Day of maximum diameter of CL after ovulation	11.2 ± 2.18	12.7 ± 0.61	15.5±4.50	0.21

Muasa *et al.,* 2015; Mutembei *et al.,* 2015). In other breeds wave emergence for two-wave cycle cows occur on day 2-4 and 10-11, which is in the same range with the Kenyan Boran cows. As during recruitment phase the follicles are highly responsive to FSH, this forms the best timing for superovulatory activities as these responsiveness is lost as the follicles get to the next phase of their development (Castilho *et al.,* 2007).

Follicle deviation is characterized by a decrease in the growth rate of the largest SF and an increase in the growth rate of the DF (Ginther *et al.,* 2001). Similar to the other studied Zebu cows, follicle selection in the Boran cow occurred when the DF was somewhat smaller than in the Taurine breeds (Castilho *et al.,* 2007).

The maximum diameters of both the non-ovulatory and ovulatory follicles of the Boran cow seem to be smaller than those reported for Taurine cows (Ginther *et al.,* 1989). However, the Boran cow is similar to other Zebu cows, whose DF diameters have been reported (Figueiredo *et al.,* 1997).

This study notes that in the Boran cows the largest diameter was attained by the ovulatory DF (P<0.05). This was also noted and explained by (Viana *et al.,* 2000) for Gir cows and it is postulated that the status of the ovulatory DF provides the FSH stimulus for stronger growth so as to provide for larger diameter (Perez *et al.,* 2003). The higher incidence of ovulatory DFs in the right ovary is attributed to the fact that the right ovary receives more blood supply compared to the left one and it is clinically observed to be more active than the right ovary.

All the characteristics observed for the DFs of the various waves for the Boran cow compare well with others documented for other breeds of zebu cows (Savio *et al.,* 1988; Taylor and Rajamahendran, 1991).

The study concludes that even though the estrous cycle length of the Boran cows is similar to those of other zebu breeds with predominantly three follicular waves within the estrous cycle, a fourth wave can also occur in this cow. The inter-ovulatory interval is positively correlated to the number of waves exhibited per cycle in this cow. Within the three phases of follicular growth identified, three groups of follicles were also noted; group one measuring less than 3 mm in diameter, group two measuring between 4 and 6 mm in diameter and group three measuring above 7 mm in diameter. Presence of the *corpus luteum* influenced the duration of dominance and ovulation of dominant follicle.

As noted by Mutembei *et al*. (2015) and Muasa *et al*. (2015), this study also recommends that to maximize on superovulatory response using FSH for embryo production, the timing of the first FSH treatment should coincide with first three days of the wave emergence.

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REFERENCES

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

Alvarez P, L Spicer, C Chace jr, M Payton, T Hamilton, R Stewart, R Wettermann, 2000 Ovarian and endocrine characteristics during an estrous cycle in Angus, Brahman, and Senepol cows in a subtropical environment. Journal of Animal Science, 78: 1291-1302.

Bo' G, G Adams, M Caccia, M Martinez, R Pierson, R Mapletotf, 1995 Ovarian follicular wave emergence after treatment with progestogen and estradiol in cattle. Animal Reproduction Science, 39: 193-204.

Burke CR, ML Day, C Bunt, KL Macmillan, 2000 Use of a small dose of estradiol benzoate during diestrus to synchronize development of the ovulatory follicle in cattle . Journal of Animal Science, 76: 145-151.

Castilho C, JM Garcia, A Renesto, GP Nogueira, LFC Brito, 2007 Follicular dynamics and plasma FSH and progesterone concentrations during follicular deviation in the first post-ovulatory wave in Nelore (Bos indicus) heifers. Animal Reproduction Science, 98: 189-96.

Evans ACO, 2003. Characteristics of ovarian follicle development in domestic animals. Reproduction in Domestic Animals 38: 1-7.

Figueiredo R, C Barros, O Pinheiro, J Soler, 1997 Ovarian follicular dynamics in Nelore breed (Bos indicus) cattle. Theriogenology, 47: 1489-1505.

Ginther O, M Beg, D Bergfelt, F Donadeu, K Kot, 2001 Follicle Selection in Monovular Species. Biology of Reproduction, 65: 639-647.

Ginther O, J Kastelic, L Knopf, 1989 Intraovarian relationships among dominant and subordinate follicles and the corpus luteum in heifers. Theriogenology, 32: 787-795.

Ginther O, MC Wiltbank, PM Fricke, JR Gibbons, K Kot, 1996 Selection of the dominant follicle in cattle. Biology of Reproduction, 55: 1187-1194.

Herlocker DJ, 1999 Rangeland Resources in Eastern Africa: Their Ecology and Development. GTZ, German Technical Cooperation. Retrieved from http://books.google.co.ke/books?id=YQhJAAAAYAAJ.

Lamothe-zavaleta C, C Fredricksson, H Kindhal, 1991. Reproductive performance of Zebu cattle in Mexico: 1. Sexual behavior and seasonal influence on estrous cyclicity. Theriogenology, 36: 887-896.

Medrano EA, O Hernandez, C Lamothe, CS Galina, 1996 Evidence of asynchrony in the onset of signs of oestrus in zebu cattle treated with a progestogen ear implant. Research in veterinary science, 60: 51-4

Muasa BT, HM Mutembei, LSA Carmago JHM Viana, 2015 Effect of Follicle Size of Boran Cows on Glucose 6 Phosphate Dehydrogenase Activity and Developmental Competence, International Journal of Veterinary Science (http://www.ijvets.com/in-press).

Mutembei HM, VT Tsuma, BT Muasa, J Mraya, ER Mutiga, 2015 Bovine Invitro Embryo Production and its Contribution Towards Improved Food Security in Kenya. African Journal of Food, Agriculture, Nutrition and Development 15: 9722-9743.

Perez CC, I Rodriguez, F Espana, J Dorado, M Hidalgo, J Sanz, 2003 Follicular growth patterns in repeat breeder cows. Veterinary Medicine - Czech, 48: 1-8.

Pinheiro O, C Barros, R Figueiredo, E Do valle, R Encarnacao, C Padovani, 1998 Estrous behavior and the estrus-to-ovulation interval in nelore cattle (with natural estrus or estrus induced with prostaglandin $F2\alpha$ or norgestomet and estradiol valerate. Theriogenology, 49: 667-681.

Rewe TO, D Indetie,JMK Ojango, AK Kahi, 2006 Breeding objectives for the Boran breed in Kenya: Model development and application to pasture-based production systems. Animal Science Journal, 77: 163-177.

Rhodes F, G De'ath, K Entwistle, 1995 Animal and temporal effects on ovarian follicular dynamics in Brahman heifers. Animal reproduction science, 38: 265-277.

Sartorelli E, L Carvalho, D Bergfelt, O Ginther, C Barros, 2005 Morphological characterization of follicle deviation in Nelore (Bos indicus) heifers and cows. Theriogenology, 63: J 2382-2394.

Sartori R, MR Bastos, M Clara, C Mattos, 2010 Physiological Bases for Understanding Estrous Cycle Differences Between Bos taurus and Bos indicus. Acta Scientiae Veterinariae. 38 (Supl 2): 287-295.

Savio JD, L Keenan, MP Boland, JF Roche, 1988. Pattern of growth of dominant follicles during the oestrous cycle of heifers. Journal of Reproduction and Fertility, 83: 663-71.

Sirois J, J Fortune, 1988 Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biology of Reproduction, 39: 308-317.

Taylor C,R Rajamahendran, 1991. Follicular dynamics and corpus luteum growth and function in pregnant versus nonpregnant cows. Canadian Journal of Animal Science, 68: 61-68.

Viana JHM, A Ferreira, W Sá, LSA Camargo, 2000 Follicular dynamics in zebu cattle. Pesq. agropec. bras, 35: 2501-2509.