OVARIAN FOLLICULAR DYNAMIC DURING EARLY PREGNANCY IN BUFFALO *Bubalus bubalis* HEIFERS

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ABSTRACT

The aim of the present study was to characterize the follicular dynamics during early pregnancy in Murrah heifers. Ten heifers were submitted to daily ultra-sound examination (USE), from the 18th to 60th day of pregnancy. Three animals presented follicular waves of 9.67±0.58 days in duration and the dominant and largest subordinate follicles measured, respectively, 11.9±1.3 mm and 7.0±1.0 mm. The growth plateau was 6.00±1.00 days in duration. The remaining seven heifers presented an irregular follicular development with three different patterns as follow: the first irregular observed pattern, characterized by waves of 7.40±1.26 days in duration, with the dominant follicle and the largest subordinated follicle reaching maximum diameter of 11.4±1.1 and 7.0±1.6 mm, respectively. The deviation on this pattern happened on day 3.57±0.53 and the plateau had an average duration of 4.67±1.37 days. The second irregular observed pattern presented waves lasting 6.44±1.81 days, with the dominant follicle and the largest subordinated follicle reaching maximum diameter of 9.0±0.6 and 6.8±0.4 mm, respectively. The deviation happened on day 4.00±1.41 after the wave detection. The third irregular pattern observed was characterized by periods of subsequent follicular growth without defined phases as described for the first and second patterns. In conclusion, from the 18th to 60th day of pregnancy, the follicular development is characterized by waves of different patterns in buffalo.

KEY WORDS: Follicle, ovary, pregnancy, ultrasound.

RESUMO

DINÂMICA FOLICULAR DURANTE A GESTAÇÃO INICIAL EM NOVILHAS BÚFALAS *Bubalus bubalis*

O objetivo do presente estudo foi caracterizar a dinâmica folicular durante a gestação inicial em novilhas Murrah. Submeteram-se dez novilhas ao exame ultra-sonográfico transretal diário do 18º ao 60º dia de gestação. Três animais apresentaram ondas foliculares de 9.67±0.58 dias de duração, folículo dominante e maior subordinado com diâmetro de 11.9±1.3 mm e 7.0±1.0 mm, respectivamente, e platô com 6.00±1.00 dias de duração. As sete novilhas restantes apresentaram três padrões de crescimento folicular irregulares: o primeiro padrão irregular observado foi caracterizado por ondas de 7.40±1.26 dias de duração, folículo dominante e maior subordinado alcançando diâmetro máximo de 11.4±1.1 e 7.0±1.6 mm, respectivamente, desvio observado no dia 3.57±0.53 e fase de platô tendo uma duração média de 4.67±1.37 dias. No segundo padrão irregular observado, as ondas foliculares apresentavam duração de 6.44±1.81 dias, com o folículo dominante e maior subordinado alcançando diâmetro de 9.0±0.6 e 6.8±0.4 mm, respectivamente, e com o desvio observado 4.00±1.41 dias após a detecção da onda. O terceiro padrão

irregular observado foi caracterizado por períodos repetitivos de crescimento folicular sem a caracterização de fases definidas como descrito para o primeiro e segundo padrões. Em conclusão, do 18º ao 60º dia de gestação, o desenvolvimento folicular é caracterizado por ondas de diferentes padrões no búfalo.

PALAVRAS-CHAVES: Folículo, ovário, prenhez, ultra-som.

INTRODUCTION

The 2.5 million of Brazilian buffaloes are concentrated mainly in the northern area of the country, where the elevated annual average temperature and high relative humidity make this area a great environment for this species development (MATTOS, 1992; BARUSELLI et al., 1997).

The ever-growing demand from consumers and the increase of the world's buffalo herd have encouraged a number of studies aiming to improve their reproductive efficiency (MIRANDA, 1986; MATTOS, 1992; VILLARES, 1994; ZICARELLI, 1994; BARUSELLI et al., 1997).

Buffaloes present reproductive activity positively influenced by decrease in daylight hours, being classified as polyestrous short days breeders (ZICARELLI, 1990; BARUSELLI, 1994). However, at equatorial zones the females display estrus during all year long (VALE, 1988).

BARUSELLI et al. (1997) studied the buffalo follicular dynamics and verified that 63%, 33% and 3,3% of animals presented, respectively, two, three and one follicular wave during estrous cycle. The waves were characterized by growth of small follicles (> 3.0 mm) reaching up to 5.0-7.0 mm in diameter. Then, only one follicle continued to grow while the others underwent atresia. Different from which is occasionally observed in cattle, four wave cycles were not detected in buffaloes.

Cows during pregnancy (REXROAD & CASIDA, 1975; PIERSON & GINTHER, 1987; GINTHER at al., 1989; SAVIO et al., 1990; TAYLOR & RAJAMAHENDRAN, 1991) or under exogenous progesterone treatment (BERGFELT et al., 1991) continue to manifest follicular waves at intervals of 8 to 10 days. The understanding of bovine ovarian physiology had an important role for development of reproductive biotechniques and it made possible a better exploitation of the female gametes improving reproductive efficiency.

Despite buffalo importance in animal production industry, there are a relative few number of studies regarding its physiology. The objective of the present experiment was to characterize the follicular dynamics in buffaloes from the day 18 to day 60 of pregnancy.

MATERIALS AND METHODS

Location and experimental animals

The present study was conducted from May to September in Botucatu, Brazil (latitude 22º 52' S, longitude 48º 26' W). Ten Murrah heifers (*Bubalus bubalis*), 2 years old, weighting 360 - 400 Kg were kept on free stall regime with water, food (a balanced ration of concentrate and forage ratio approximately 1:1 on a dry matter basis to allow a daily gain of 1.1 kg) and mineral supplementation.

Reproductive control and ultrasound examination

After an adaptation period of 30 days, the animals were submitted to a gynecological examination assessed by ultra-sound examination (USE) every 8 hours (Pie Medical 100, Maastricht, The Netherlands) equipped with a transrectal 8 MHz linear-array transducer in order to detect ovulation established as the first day of pregnancy and were maintained with a teaser bull for monitoring their estrous behavior.

All heifers were submitted to the following protocol of estrus synchronization: D0: GnRH – 10 μg Buserelina im (Conceptal®); D7: PGF2α - 25 mg Luprostiol im (Prosolvin®); estrus observation from D8 thereafter (twice daily - each observation during 30 minutes). Once estrus was

detected, natural mating with proven fertile males was performed. The ovaries were then scanned every 24 hours from the day 18 to day 60.

Statistical analysis

The follicular development from each animal was graphically represented and the waves grouped in accordance to the following criteria: follicular waves similar to the reported by BARUSELLI et al. (1997) during the estrous cycle – regular pattern; follicular waves with short duration and dominant follicle bigger than 10.00 mm – first irregular pattern; follicular waves with short duration and dominant follicle smaller than 10.00 mm – second irregular pattern; and follicular growth without defined phase – third irregular pattern.

For each follicular development pattern, mean and standard deviation of the following variables (inter wave interval, maximum diameters of the dominant follicles, maximum diameters of largest subordinate follicles, plateau phase and deviation) were calculated.

RESULTS

Follicular growth was observed in all animals during the experimental period.

Three heifers (24 follicular waves) presented inter wave interval lasting for 9.67 ± 0.58 days. The maximum diameters of the dominant and largest subordinate follicles were, respectively, 11.90 ± 1.30 and 7.00 ± 1.00 mm. The plateau phase of the dominant follicle lasting 6.00 ± 1.00 days and two of these animals became pregnant on April and the other on August. The dominant follicle was observed seven times (7 x 3) at the contra-lateral ovary to the corpus luteum and two heifers presented a wave with co-dominant follicles (Table 1).

The remaining animals (seven heifers) that became pregnant between June and August, presented an irregular model of follicular development characterized by three different interchangeable patterns. The regular pattern described above was also present in these animals.

The first irregular observed pattern (ten follicular waves) was characterized by the presence of follicular waves lasting 7.40 ± 1.26 days. The maximum diameters of the dominant and largest subordinate follicles were, respectively, of 11.40 ± 1.1 and 7.00 ± 1.60 mm. The difference in growth rate between the dominant and largest subordinate follicle occurred at 3.57 ± 0.53 days after wave detection which characterized deviation. The plateau phase of the dominant follicle last 4.67 ± 1.37 days and one wave with co-dominant follicles was also observed (Table 1).

TABLE 1. Patterns of follicular growth according to the inter wave interval, maximum diameters of dominant and largest subordinate follicles, plateau phase and deviation observed during early pregnancy in buffalo heifers

	Inter wave interval	Maximum diameters of the dominant follicles	Maximum diameters of largest subordinate follicles	Plateau Phase	Deviation
Regular pattern	9.67 ± 0.58 days	11.90 ± 1.30 mm	7.00 ± 1.00 mm	6.00 ± 1.00 days	-
First irregular pattern	7.40 ± 1.26 days	11.40 ± 1.10 mm	7.00 ± 1.60 mm	4.67 ± 1.37 days	3.57 ± 0.53 days
Second irregular Pattern	6.44 ± 1.81 days	9.00 ± 0.60 mm	6.80 ± 0.40 mm	-	4.00 ± 1.41 days
Third irregular pattern	5.67 ± 2.08 days	This pattern was characterized by periods of subsequent follicular growth without defined phase (emergence, selection and dominance).			

The second irregular observed pattern (nine follicular waves) was characterized by short follicular waves lasting 6.44 ± 1.81 days. The maximum diameters of dominant and largest subordinate follicles were, respectively, 9.00 ± 0.60 and 6.80 ± 0.40 mm. The difference in growth rate between the dominant and largest subordinate follicle occurred at 4.00 ± 1.41 days after wave detection. One heifer presented co-dominance in two waves, and the same event was observed in another heifer, however occurring only in one wave at this time (Table 1).

The third irregular observed pattern (seven follicular growths) was characterized by periods of subsequent follicular growth without defined phases (emergence, selection and dominance). The largest follicle observed in this case has not exceeded 6.00 mm in diameter. Three heifers presented the described pattern, lasting 5.67 ± 2.08 days in each animal (Table 1).

DISCUSSION

The wave phenomenon is also observed in bovine during early pregnancy (PIERSON & GINTHER, 1986; GINTHER et al., 1989) and in progesterone-treated nonpregnant heifers (BERGFELT et al., 1991). Each wave begins with a cohort of growing follicles (4.0 mm in diameter) from which a single follicle continues to grow and becomes dominant; the other follicles in the cohort cease growth and regress (ADAMS et al., 1993). Selection of the dominant follicle is associated with a deviation in growth rate between the dominant and the largest subordinate follicle, an event termed deviation (GINTHER et al., 1996a; GINTHER et al., 1997; GINTHER, 2000). Three of our animals presented the above described pattern of follicular growth.

These follicular waves lasting 9.67 ± 0.58 days had similar duration compared to those waves observed by BARUSELLI et al. (1997), which were 9.67 and 11.17 days in average, respectively, for the first and the second waves, in two wave cycles. They also observed that in the three-wave cycles, the dominant follicles that were undergoing emergence and atresia during progesterone phase (second follicular wave) were smaller than those follicles selected when plasma progesterone concentrations were characterized by a gradual increase, showing low concentrations initially, followed by a rise (first follicular wave). The influence of high luteal progesterone levels reducing the diameter of second dominant follicle in three-wave cycles is well documented in bovine by BERGFELT et al. (1991) and FORTUNE (1993). The dominant follicles observed during buffalo pregnancy (11.9 ± 1.3 mm) were smaller than those described for the first dominant follicle (15.1 ± 2.4 and 13.3 ± 1.8 mm) and the ovulatory follicle (15.5 ± 1.6 and 13.4 ± 1.3 mm), respectively, in two and three waves estrous cycles in buffaloes (BARUSELLI et al., 1997). Probably, the high levels of progesterone reduced the maximum size of the dominant follicle during buffalo pregnancy.

The irregular model of follicular development observed in our study was characterized by short waves with (second irregular pattern) or without (first irregular pattern) decrease in dominant follicle diameter. GINTHER et al. (1996b) also observed the decrease in dominant follicle diameter in waves detected after day 90 of bovine pregnancy and attributed this to a decrease in LH pulse frequency and/or average LH concentrations, or to a poor number of LH receptors in follicular granulosa cells.

Studying the role of LH in follicle development GINTHER et al. (2001) related that progesterone treatment beginning before deviation did not affect diameter at deviation. If therapy is maintained during the period after deviation it can also reduce the diameter of the largest follicle of the wave. This reduction is associated with low level of estradiol and free IGF-1 in follicular-fluid. Their results indicated that LH has a role in the growth and function of the largest follicles after the beginning of deviation.

FIKE et al. (1997) treated heifers during estrous cycle with LHRH-ant promoted a dramatic reduction in LH. The treatment accomplished from emergence to post deviation phase of follicular wave decreases the diameter of dominant follicle and shortened the wave.

CROWE et al. (2001) observed in anestrous heifers, which were GnRH-immunized, that treatment with pFSH alone allows growth of a large number of medium-sized follicles but few large follicles were observed, which were all estrogen-inactive. This shows that in cattle, follicles above 8-9 mm in diameter require pulsatile LH to grow and to develop their normal steroidogenic capacity.

Another reason for an inadequate growth of dominant follicle is a poor development of gonadotrophin receptors in the granulosa cells. XU et al. (1995) reported that mRNA for LH receptors appeared in the granulosa cells between second and fourth days after wave emergence, which encompasses the reported day of the beginning of deviation. In the same way FIKE et al. (1997) related that heifers treated with LHRH-ant from day 2 to 7 of the oestrous cycle had a decrease in FSH over time on day 2 and had a diminished ability to attain maximal production of estradiol. They both hypothesized that the dominant follicle is selected because it acquires LH receptors on its granulosa cells and that this allows the cells to synthesize estradiol in response to LH.

Then, dominant follicles with smaller diameters and short waves, observed in our study in the first and second irregular patterns may be due to a deficient LH support or an inadequate supply of LH receptors.

The third irregular observed pattern was periods of follicular growth not exceeding 6.0 mm in diameter observed in our study were also described by RUIZ-CORTÉS et al. (1999) when studying the ovarian follicular dynamics in suckled zebu (*Bos indicus*) cows. They called this phenomenon interdominance interval and, although, doesn't have a clear explanation for it, tropical conditions and nutritional factors have been mentioned. A similar pattern of follicle development was observed by GINTHER et al. (1996b) during the last 21 days of cattle pregnancy.

CROWE et al. (2001) evaluate the roles of FSH and LH in follicular growth using GnRH-immunized anestrous heifers and concluded that treatment of these animals with pLH alone did not induce growth of follicles > 5.0 mm in diameter. All these findings support the hypothesis that the periods of follicular growth not exceeding 6.0 mm in diameter (third irregular pattern) observed during buffalo pregnancy could be consequence of an inadequate FSH support.

Buffaloes are classified as polyestrous short day's breeders (ZICARELLI, 1990; BARUSELLI, 1994). The irregular model of follicular development observed in our study occurred in heifers that became pregnant between June to August, 2000. Therefore, the experiment was conducted under increasing number of daylight hours. Under these conditions the animals could have a lower plasmatic concentration of FSH and/or LH. This seasonal condition may be the cause of the observed different patterns in follicular development. ROY et al. (1968) and SINGH et al. (1988) considered nutrition as the principal factor influencing the seasonal character of buffalo reproduction. In our experiment this influence can be excluded once the animals were raised under balanced nourishment management.

A study of buffalo pregnancy follicular development during breeding and non-breeding seasons involving measurement of plasmatic levels of gonadotrophins could better explain the several patterns of follicular development.

CONCLUSION

The follicular growth patterns during early pregnancy (day 18 to day 60) observed in this study in Murrah heifers occurred in waves. However the irregular patterns of follicular growth observed could be influenced by the seasonal condition of these species. New studies with hormone assays could be more elucidative.

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