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Follicular dynamics in Rathi (*Bos indicus*) cattle

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ABSTRACT

The objective of this study was to investigate follicular dynamics during oestrus cycle in Rathi (*Bos indicus*) breed of cattle. Follicular growth and atresia were evaluated using a portable ultrasound device. Of the 14 interovulatory cycles studied in 7 cows, 11 had two waves and 3 had three waves of follicular growth. There was no difference (P>0.05) in dominant or subordinate follicles growth or atresia rates among follicular waves. The first wave of a two-wave cycle emerged on day 2.10 ± 0.36 and lasted for 13.35 ± 1.72 days, whereas the second wave (ovulatory) emerged on day 10.5 ± 0.6 and lasted for 10.4 ± 0.9 days. During the three-wave cycle the first, second and third (ovulatory) waves emerged on day 0.7 ± 0.5, 7.2 ± 1.0 and 13.2 ± 3.4 of oestrus, respectively. Total number of follicles during the oestrus cycle was in the range of 3.36 ± 0.48 to 7.57 ± 1.01, with only slight variation and without any definite pattern. It was concluded that Rathi cattle have follicular dynamics similar to other breeds of cattle.

Key words: dominant follicle, follicular waves, Rathi cattle, ultrasonography

Introduction

Ovarian follicular dynamics in cows and heifers is characterized by waves of follicular growth and regression during the oestrus cycle (SAVIO et al., 1988; GINTHER et al., 1989; KNOPF et al., 1989; TAYLOR and RAJAMAHENDRAN, 1991b). Waves of follicular growth can also be observed during the pre-pubertal period (MELVIN et al., 1999), in pregnant cattle (TAYLOR and RAJAMAHENDRAN, 1991a) and during the postpartum period (MURPHY et al., 1991). During one inter-ovulatory interval, two (GINTHER et al., 1989; BURKE et al., 2000; BELLMANN, 2001), three (SAVIO et al., 1988; BURKE et al., 2000) or four (RHODES et al., 1995; VIANA et al., 2000) waves have been observed. From the cohort of growing follicles, one follicle is selected for continued growth and becomes dominant.

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If luteolysis occurs during the growth phase of dominant follicles, final maturation and ovulation occurs, but if luteolysis does not occur during the growing and maintenance phase of follicles, their fate is atresia (KANITZ, 2002).

Follicular dynamics is one of the most important subjects in ovarian physiology and has largely been studied in European breeds (SAVIO et al., 1988; SIROIS and FORTUNE, 1988; GINTHER et al., 1989; ROCHE and BOLAND, 1991; BADINGA et al., 1994). However, studies on zebu cattle (*Bos indicus*) are limited (FIGUEIREDO et al., 1997; GAMBINI et al., 1998; VIANA et al., 2000).

Reproductive physiology of zebu cows is not identical to European breeds of cow, and differences are known to be existent in characteristics such as estradiol profile (PUROHIT et al., 2000), dominant follicle diameter (BARROS et al., 1995; FIGUEIREDO et al., 1997), oestrus length (MEDRANO et al., 1996), ovulation moment (PINHEIRO et al., 1998), and a higher number of follicular waves during the oestrus cycle (VIANA et al., 2000). Knowledge of differences between breeds of cattle is important for the establishment of correct ovulation parameters and management procedures for zebu cows (VIANA et al., 2000).

The objective of this study was to evaluate follicular growth and regression characteristics during the oestrus cycle in Rathi cows.

Materials and methods

Regularly cycling Rathi cows (n=7) aged 4-7 years belonging to the college dairy herd were included in the study. Animals were maintained under uniform conditions of feeding and management. Study period was between September to November, 2002 and cows were not inseminated during the study period. Cows were synchronized to oestrus by administration of two injections of a prostaglandin (Inj. Iliren, Hoechst, 5 mL IM) 11 days apart. From the day of synchronized oestrus, ovarian structures were studied by daily real time B-mode ultrasonography for two consecutive oestrus cycles using a transvaginal 5-7.5 MHz dual frequency sector probe (Toshbro Shimadzu, 350 A, Japan). Daily ovarian pictures were recorded on a VCR (BPL-Sanyo) attached to the scanner. Thermal prints were subsequently obtained when required with a video graphic printer (Sony Corp. Tokyo, Japan). The various parameters were determined as per previously described methods (ALVAREZ et al., 2000; BURKE et al., 2000). The tapes were played back, images were projected on a monitor and a diagram depicting the relative location of follicles $\geq$ 2 mm and the *corpus luteum* (CL) were drawn for each ovary. 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 largest follicle present on the ovaries at days 5-7 was assumed to be the first dominant follicle (DF) of the oestrus cycle. A subordinate follicle (SF) was defined as one that originated from the same

follicular pool as the dominant follicle. The largest follicles that were subsequently seen were defined as the second or third dominant follicles, as described previously (BURKE et al., 2000). Ovulation was determined by the disappearance of the dominant follicle and subsequent formation of a CL at the same location over the ovary. Maximum diameter and growth rate of the dominant follicle of the first, second or third follicular wave were also estimated. The growth rate of the dominant follicle was determined 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). Gowth rate (mm/day) of follicles was calculated by subtracting the diameter on the day of detection from the maximum diameter and dividing by the interval in days (RHODES et al., 1995). Each oestrus cycle was described as having two or three waves of follicular development if the second or third dominant follicle ovulated (BURKE et al., 2000). The day of wave onset was defined as the day on which the dominant follicle was retrospectively traced to be 4 to 5 mm in diameter (BURKE et al., 2000). Atresia onset was considered the day from which follicles began regressing in size.

Ultrasonographic technique. Cows were restrained in a chute. Faecal material was evacuated from the rectum and the perineum was washed. The probe was inserted into the vagina, enclosed in a disposable sleeve with the jelly applied over the surface of the transducer. The transducer was then moved forward into the vagina and placed lateral to the cervix. The ovary held by the hand in the rectum was brought in front of the transducer face and the entire ovary was scanned. All examinations were made by the same operator. Follicles greater than 2 mm in diameter were recorded. The diameter of follicles and other ovarian structures were measured using inbuilt electronic callipers.

Statistical analysis. Mean ± Standard errors for the numeric values estimated for each parameter were calculated. The various parameters were compared by Chi-squared test as described previously (ALVAREZ et al., 2000).

Results

Oestrus cycles evaluated presented the characteristic pattern of follicular growth waves, with the initial development of a group of 3 to 5 mm follicles (Fig. 1), followed by selection, development and atresia of a dominant follicle (DF). From these oestrus cycles (n=14), 11 (78.57%) presented two follicular waves and 3 (21.42%) presented three follicular waves. There was no cycle with more than three follicular waves.

Table 1 presents the characteristics of follicular dynamics in cows with two follicular waves during the oestrus cycle. The first wave emerged on day 2.10 ± 0.36 with DF maximum diameter of 11.75 ± 1.59 mm on day 5.00 ± 0.63, and onset of atresia on day 10.50 ± 1.11. The second wave emerged on day 10.55 ± 0.62 (Fig. 2) with DF maximum diameter of 14.65 ± 1.24 mm, significantly greater ($P<0.05$) than the dominant follicle

of first wave. This dominant follicle ovulated. Overall, a higher proportion of follicular growth (66.8%) was seen on the right ovary while a lower proportion of follicular growth (33.3%) was seen on the left ovary.

Table 1. Characteristics of follicular development in Rathi cows with two waves of follicular growth

Follicular parameters	Follicular waves	
	Ist	IInd
Wave onset (day)	2.10 ± 0.36	10.55 ± 0.62
Wave duration (day)	13.35 ± 1.72^{a}	10.45 ± 0.98^{b}
DF Max. diameter (mm)	11.75 ± 1.59^{c}	14.65 ± 1.24^{d}
Day of Max. diameter (day)	5.0 ± 0.63	17.5 ± 0.5
Growth rate (mm/day)	1.81 ± 0.32^{e}	1.16 ± 0.22^{e}
Growth period (days)	3.10 ± 0.50^{f}	8.90 ± 0.84^{g}
Onset of atresia (day)	10.5 ± 1.11	-
Length of atresia (days)	6.5 ± 0.81	-
Atresia rate (mm/day)	0.55 ± 0.20	-
Largest SF Max. diameter (mm)	7.42 ± 0.58^{g}	7.60 ± 0.90^{h}

Values followed by different superscripted letters in the same row differ significantly (P<0.0, Values are presented as Mean ± SE

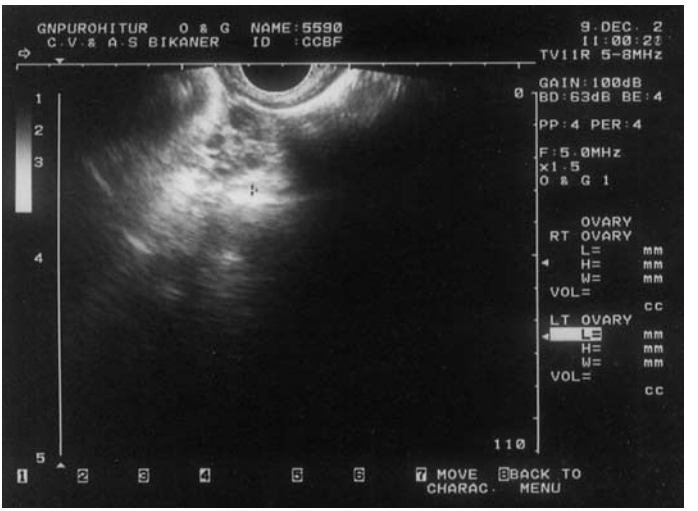

Fig. 1 Sonogram of a cow showing wave emergence. Small follicles are seen.

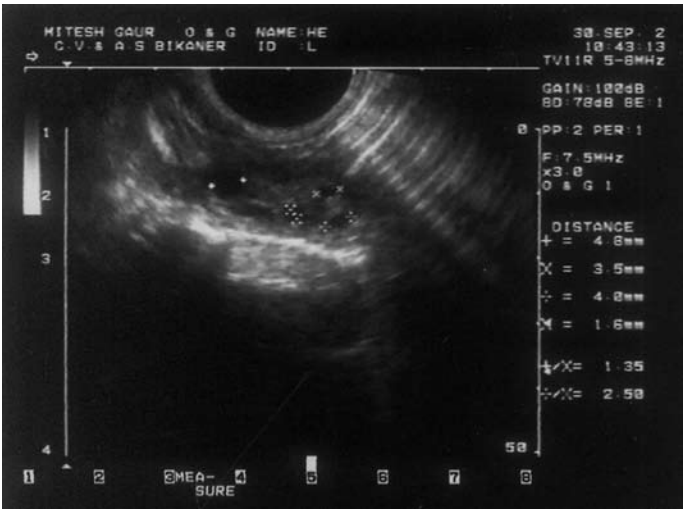

Fig. 2. Sonogram of a cow showing the second follicular wave. The follicular diameters are shown.

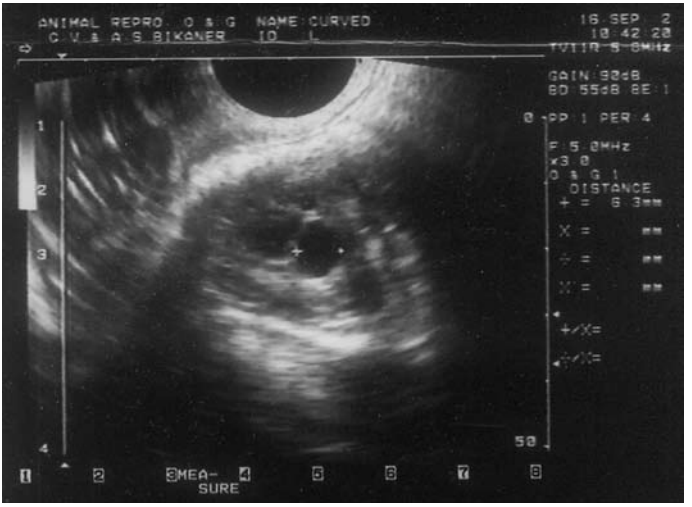

Fig. 3. Sonogram of a cow showing a dominant follicle (6.3 mm) during the third follicular wave

The main characteristics of the follicular dynamics in the cows with three follicular waves are presented in Table 2. The first wave emerged on day 0.78 ± 0.44 of the cycle. Selection of the dominant follicle, characterized by the divergence among the dominant and subordinate follicle growth rate occurred on day 4.13 ± 0.99. The dominant follicle reached maximum diameter on day 6.45 ± 0.88 and entered atresia on day 7.89 ± 0.78, showing a brief period of stabilization. The second wave emerged on day 7.11 ± 1.05, with the respective dominant follicle being selected on day 11.33 ± 2.06, reaching its maximum diameter on day 12.89 ± 3.06 and beginning atresia on day 15.55 ± 3.36. The third wave (ovulatory) emerged on day 13.22 ± 2.44, with the dominant follicle being selected on day 16.89 ± 2.85 (Fig. 3) and reaching its maximum diameter on day 20.44 ± 1.42. The difference between dominant or subordinate follicle growth rate or atresia rate was not significant, but the DF maximum diameter of second wave was significantly smaller (10.44 ± 2.13 mm) than the diameter of other DF_S (11.78 ± 1.20 and 12.44 ± 1.59 mm). The third follicular wave finished with ovulation of the dominant follicle.

Table 2. Characteristics of follicular development in Rathi cows with three waves of follicular growth

	Follicular waves		
Folicular parameters	I	II	III
Wave onset (day)	0.78 ± 0.44[a]	7.11 ± 1.05[b]	13.22 ± 2.44[c]
Wave duration (day)	13.00 ± 1.58[a]	11.44 ± 2.19[a]	7.67 ± 1.80[a]
DF max. diameter (mm)	11.78 ± 1.20[a]	10.44 ± 2.13[a]	12.44 ± 1.59[b]
Day of max. diameter	6.45 ± 0.88	12.89 ± 3.06	20.44 ± 1.42
Growth rate (mm/day)	1.03 ± 0.24[a]	1.07 ± 0.23[a]	1.08 ± 0.26[a]
Growth period (days)	5.67 ± 0.71[a]	5.22 ± 1.72[a]	7.00 ± 1.50[a]
Onset of atresia (day)	7.89 ± 0.78	15.55 ± 3.36	-
Length of atresia (days)	7.00 ± 1.32[a]	4.78 ± 1.30[b]	-
Atresia rate (mm/day)	0.97 ± 0.26[a]	0.96 ± 0.33[a]	-
Largest SF max. diameter (mm)	7.25 ± 0.71[a]	7.22 ± 0.1.39[a]	7.00 ± 1.32[a]

Values followed by different letters within row differ significantly ($P<0.05$). Values are presented as Mean ± SE.

The mean number of small, medium and total number of follicles recorded each day during different days of oestrus cycle in Rathi cows are presented in Fig. 4. The number of medium-sized follicles was lower throughout the oestrus cycle compared to small follicles, although there was no definite pattern.

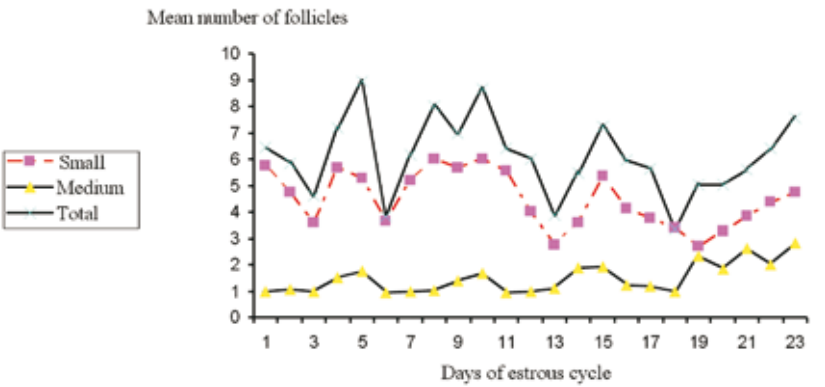

Fig. 4. Overall mean number of small, medium and total follicles at different days of oestrus cycle in Rathi cattle

Discussion

The oestrus cycles evaluated evidenced a higher proportion of cycles (78.57%) with two follicular waves compared to three follicular waves (21.42%). ADAMS (1999) concluded from the available data that greater than 95% of oestrus cycles in cattle are composed of either two or three follicular waves.

However, a higher prevalence of cycles with three follicular waves was observed by many authors in European (SAVIO et al., 1988; SIROIS and FORTUNE, 1988) and Zebu (GAMBINI et al., 1998; VIANA et al., 2000) cows. No cycle was seen with four or more follicular waves during the present study. A previous study in Gir cows (VIANA et al., 2000) recorded a small proportion of cows showing four or more follicular waves during the oestrus cycle.

In a two-wave cycle, the first wave emerged on day 2.10 ± 0.36 of the cycle and was followed by selection of DF on day 3.25 ± 0.89 and growth for 5.18 ± 1.71 days, whereas the second wave emerged on day 10.55 ± 0.62 with a maximum diameter of 14.65 ± 1.24 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 (SAVIO et al., 1988; SIROIS and FORTUNE, 1988). Findings of a higher number of cows showing a two-wave cycle are similar to the previous findings of PIERSON and GINTHER (1988) and TAYLOR and RAJAMAHENDRAN (1991a, b).

The dominant follicle of the first wave in the three-wave cycle emerged on day 4.13±0.99 and reached maximum diameter on day 6.5 ± 0.88, showing a short period of stabilization. This growth pattern differs from that reported for two-wave cows, in which

the first DF regression occurs only after days 11 or 13 (GINTHER et al., 1989; KNOPF et al., 1989; TAYLOR and RAJAMAHENDRAN, 1991b), but was similar to that observed in cycles with three follicular waves in European (SAVIO et al., 1988; SIROIS and FORTUNE, 1988; GAMBINI et al., 1998) or Zebu (VIANA et al., 2000) cows. Other studies that evaluated cows with three follicular waves (SIROIS and FORTUNE, 1988; GINTHER et al., 1989) agree with the appearance of waves at intervals of approximately seven days.

There was no difference ($P>0.05$) in dominant or subordinate follicle growth or atresia rates among the three follicular waves, but the DF maximum diameter of the second wave was smaller ($P<0.05$) than the diameter of the other DFs, similar to a previous study in Zebu (Gir) cows (VIANA et al., 2000). This difference has been postulated to be due to the fact that the second wave emerges during the period of higher progesterone production by the corpus luteum, whereas the first and third waves emerge, respectively, during the luteogenic and luteolytic periods (VIANA et al., 2000). Mean diameter of the DFs was lower than that reported for cows of European breeds, as observed by FIGUEIREDO et al. (1997), GAMBINI et al. (1998), and VIANA et al. (2000).

The total number of medium-sized follicles was lower throughout the oestrus cycle compared to small follicles (Table 3). This is contrary to the findings of LUCY et al., (1992) who observed that early during the oestrus cycle (days 1-5), the number of small follicles (3-5 mm) decreases, while the number of medium follicles (6-9 mm) increases. ALVAREZ et al. (2000) had recorded a greater number of small and medium follicles in Brahman (*Bos indicus*) cows adapted to the tropics. The pronounced effect of dominance by the preovulatory follicle on other follicles could be one probable reason for this. The increased number of small-sized follicles detected on days 1, 7 and 14 might have resulted from the stimulatory effects of peri-ovulatory discharge of FSH.

In conclusion, Rathi cattle have follicular dynamics similar to other cattle, with a preponderance of two-wave cycles.

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SAŽETAK

Svrha rada bila je istražiti folikularnu dinamiku u tijeku spolnoga ciklusa u Rathi (*Bos indicus*) krava. Pri tome je rabljen prijenosni ultrazvuk kojim je praćen razvoj i atreziju folikula. Od ukupno 14 interovulatornih ciklusa, praćenih u 7 krava, ustanovljeno je da je 11 imalo 2 vala dok su 3 ciklusa imala 3 vala folikularnoga rasta. Nije bilo značajne razlike između rasta dominantnih ili subordinantnih folikula kao ni pojave atrezije između promatranih valova rasta folikula (P>0,05). Prvi val zrenja kod ciklusa s 2 vala zrenja počeo je nakon 2,10 ± 0,36 i trajao 13,35 ± 1,72 dana, dok je ovulatorni odnosno drugi val počeo rasti 10,5 ± 0,6 i trajao 10,4 ± 0,9 dana. Kod ciklusa s tri vala valovi su počeli rasti nakon 0,7 ± 0,5 odnosno 7,2 ± 1,0 i 13,2 ± 3,4. Ukupan broj folikula u tijeku ciklusa iznosio je u rasponu od 3,36 ± 0,48 do 7,57 ± 1,01 s laganom varijacijom i bez konačnoga specifičnoga obrasca. Zaključeno je da Rathi krave imaju sličnu folikularnu dinamiku kao i ostale pasmine krava.

Ključne riječi: dominanti folikul, folikularni valovi, govedo Rathi, ultrazvuk