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Research Article

OVARIAN FOLLICULAR DYNAMICS IN RELATION TO PRESENCE OF CORPUS LUTEUM IN BUFFALOES

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ABSTRACT

Transrectal ultrasonography was done every alternate day in nine postpartum buffaloes, starting 2 days post-insemination (1st to 3rd artificial insemination) until day 24. Follicles over 2 mm in diameter were recorded, grouped as small (<5mm) or large (>5mm) and development of each follicle was monitored. Effect of corpus luteum on follicular recruitment and development of dominant follicle (DF) on ipsilateral and contralateral ovaries was studied. Corpus luteum was more commonly present in the right ovary (6/9). Three to four waves of follicular development were detected and a mean of 44.4±3.1 follicles developed during the 24 days' observation period. Overall, significantly ($P<0.5$) more follicles developed in the ovary contralateral (25.2±1.8) to the CL than the ipsilateral (19.2±1.6) ovary and this trend was also evident in individual waves, though the differences were significant only in wave-3. Similarly, dominance was more common for follicles in the contralateral ovary, particularly in wave-3. Growth rate of DFs in the contralateral ovary was marginally higher than that of DFs in the ipsilateral ovary. DF of last wave in the following cycle was located more frequently in the ovary contralateral (6/9) to the existing CL. However, there was no effect of previous wave DF location on the location of DF in the next wave. In conclusion, inhibitory influence of the CL was evident on the follicular development and dominance in the ipsilateral ovary.

Key words: Ovarian follicles, corpus luteum, buffaloes

Ovary is the pivotal organ controlling reproduction. Study of the follicular recruitment/selection and development is essential to understand mechanisms involved in reproductive success/failure. Trans rectal diagnostic ultrasonography has profoundly enhanced ability to evaluate reproductive events and is a reliable technique for identification and measurement of follicles and corpus luteum (CL) in large farm animals (Pierson and Ginther, 1988). Ultrasound studies of buffalo ovarian activity have recorded a 2 or 3 wave pattern of follicular turnover in cyclic buffaloes (Manik *et al*., 1994; Baruselli *et al*., 1997).

During estrous cycle, a majority of dominant follicles in the 1st and 2nd waves appeared in the ovary ipsilateral to the CL, but the ovulatory follicles of the 3rd wave always appeared in the ovary contralateral to the CL (Manabe *et al.*, 1997). Most of the studies have focused on follicular dynamics / ovarian activity during estrous cycles. Follicular dynamics in early pregnancy post insemination has not been studied in buffaloes. Hence, the present study was undertaken to determine the influence of placement of CL on follicular dynamics.

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MATERIALS AND METHODS

The study was conducted on nine parous, lactating, non suckled, inseminated Murrah buffaloes at Buffalo Research Centre, LUVAS, Hisar. The animals were in their 1st to 7th lactations and had been inseminated for 1st to 3rd time at the time of their inclusion in the study. Pregnancy was confirmed by per rectal palpation of genitalia on day 55 post insemination.

All animals were subjected to ultrasonographic monitoring of their ovarian activity every alternate day, starting from day 2 until day 24 post insemination. Follicles were observed as round non-echogenic cavities in the ovarian stroma, with well defined borders. Smallest appreciable and measurable follicles were over 2 mm in diameter. Follicular luteinization was appreciated by appearance of hypo-echoic areas in the follicular cavity. Marking the position of each follicle as on a clock dial and following it up at subsequent scanning made possible sequential monitoring of individual follicles. Total follicular population in an ovary was recorded by noting down each follicle observed during scanning of individual ovary through its entire surface, from medial to lateral aspect. Corpora lutea were appreciated as well defined, granular,

round to oval structures with lower echogenicity than the adjoining ovarian tissue, but greater than that of the follicles.

Follicles observed on ultrasonographic scanning of the ovaries were grouped according to their diameter. Follicles having diameter <5 mm were considered as small and with >5 mm diameter were grouped as large. A wave was considered to emerge when a cohort of 3 or more new small follicles (<5 mm) was detected for the first time and continued on subsequent examination. Individual follicles of each wave were plotted for size against post insemination interval for sequential measurements and the largest follicle for each wave was determined and considered as the dominant follicle (DF) of particular wave. The DF was the one, which grew maximally and exceeded the diameter of all other follicles. The rate of growth of DF was calculated by subtracting the initial diameter from the maximum diameter and dividing by the number of days taken to reach the same. Corpora lutea appeared as slightly (early luteal phase) to moderately (mid luteal phase) or highly echogenic (regressing) structures.

Effect of pregnancy on the number of follicular wave, number of small, large and total follicles in each wave and total ovarian activity was determined. Influence of the side of the ovary bearing a CL or dominant follicles in different waves was assessed with respect to occurrence of dominant follicle and follicle numbers. Data were subjected to statistical analysis using Analysis of Variance t-test and Chi-square test (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

A total of 44.4±3.1 follicles developed in both the ovaries and the development occurred in 3 (n=5) or 4 (n=4) waves during the 24 days post insemination observation period (Table 1). CL was more commonly detected on the right ovary (6/9, Table 1) between 2 to 6 days post insemination and attained maximum diameter (2.16 cm) during mid luteal phase as reported earlier (Rajakoski, 1960; Pierson and Ginther, 1987). There was no difference in CL diameter in relation to pregnancy status.

In the present study, total number of follicles recruited during the observation period which included only the newly detected follicles and excluded repeated subsequent observations on the same follicle are consistent with the findings of Danell (1987). In cattle, visualization of CL with ultrasonography was possible from day 3 to 4 day onwards as a granular echogenic structure with well defined borders (Pierson and Ginther, 1984; Ribadu *et al.*, 1992). In other studies (Pierson and Ginther, 1984; Kastelic *et al.*, 1990), CL was detected from as early as the day of ovulation.

The DF of W1 was more commonly in the left ovary (6/9), however, in subsequent waves no difference in the side of DF location was observed (Table 1). On comparing with respect to the location of CL, the W1 and W2 dominant follicles appeared in the ipsilateral and contralateral ovaries with almost similar frequency.

Table 1

Site of dominant follicle in different waves of follicular development during the first 24 days post insemination in buffaloes

Animal	Pregnancy status	Position of CL	Position of DF of different waves			
			W1	W2	W3	W4
487	P	R	L	L	L	-
617	P	R	L	R	L	L
624	P	R	R	R	L	R
1105	P	L	L	R	-	-
1067	NP	R	L	R	R	-
1089	NP	R	R	L	R	-
1043	NP	R	L	L	L	-
1024	NP	L	L	L	R	R
1171	NP	L	R	R	R	-
Total		L(3) : R(6)	L(6) : R(3)	L(4) : R(5)	L(4) : R(4)	L(1) : R(2)
Ipsilateral (Total 11)	4	4	2	1		
Contralateral (Total 18)	5	5	6	2		

L=Left ovary; R=Right ovary, CL=Corpus luteum; W1=Wave 1; W2=Wave 2; W3=Wave 3; W4=Wave 4; P=Pregnant; NP=Non-pregnant

Table 2
Ovarian follicular development in different waves in relation to presence of CL in buffaloes during the 24 days post-insemination period

Animal No.	Preg. Status	CL site	Follicles ipsilateral to CL in different waves					Follicles contralateral to CL in different waves				
			W1	W2	W3	W4	Total	W1	W2	W3	W4	Total
487	P	R	3	11	2	-	16	4	12	7	-	23
617	P	R	4	5	5	0	14	5	3	8	3	19
624	P	R	6	4	9	4	23	12	10	7	4	33
1105	P	L	14	9	0	-	23	5	7	6	-	18
1067	NP	R	11	4	34	-	18	13	7	9	-	29
1089	NP	R	10	9	5	-	23	12	12	2	-	26
1043	NP	R	6	12	4	-	23	13	9	8	-	30
1024	NP	L	4	8	3	7	23	7	8	6	8	29
1171	NP	L	4	1		2	10	8	4	5	3	20
Mean+S.E.			6.9±1.3	7.0±1.2	3.9±1.9[b]	4.3±0.9	19.2±1.6[b]	8.7±1.2	8.0±1.1	6.4±0.7[a]	4.5±0.8	25.2±1.8[a]

*Different superscripts (a,b) indicate significant difference between ipsilateral and contralateral ovaries (P<0.5). CL=Corpus luteum; W1=Wave 1; W2=Wave 2; W3=Wave 3; W4=Wave 4; P=Pregnant; NP=Non-pregnant

However, in the 3rd wave, which was the last wave of the cycle in several buffaloes, there was a greater incidence of DF appearing in the ovary contralateral to the CL (6/8). Overall, during the 24 days post insemination, total incidence of dominant follicles was greater in the contralateral ovary as compared to the ipsilateral ovary (18 vs.11, Table 1) as reported earlier in cyclic and pregnant cows (Rexroad and Casida, 1975; Pierson and Ginther, 1987; Ginther *et al*., 1989). The influence of location of DF, on the emergence of successive wave DF in the same (11/20) or the opposite ovary (9/20) was not evident. Similar reports viz., no effect (Ginther *et al*., 1989; Baruselli *et al*., 1997), as well as those suggesting altering side of DF (Savio *et al*., 1988) are available in the literature. Ginther *et al*. (1989) reported a higher incidence of ipsilateral location of two successive DFs in pregnant animals which was predominantly an effect of the presence of CL, as most of these DFs were in the ovary contralateral to the CL.

On any particular day of examination, right ovary contained a non-significantly less follicles (4.4±0.16) per ultrasound scan than the left ovary (5.4±0.27). In relation to CL location, significantly more (P<0.05) follicles developed in the ovary contralateral to the CL (25.2±1.8) as compared to the ipsilateral ovary (19.2±1.6) but in different waves the differences were not significant except for W3 in which more follicles (P<0.05) developed in the contralateral (6.4±0.7) ovary than the ipsilateral (3.9±.9; Table 2).

In the present study while the corpora lutea were more often detected in the right ovary, where as total follicular turnover was more in the left ovary. Contrary to the present findings, greater activity of the right ovary has often been reported for cattle and buffaloes with respect to population of various sized follicles, occurrence of DF, as well as incidence of ovulation/CL (Rajakoski, 1960; Pierson and Ginther, 1987). The present findings of greater follicular turnover in the left ovary could possibly be due to the local suppressing effect of CL, which was mostly in the right ovary. In conclusion, some inhibitory influence of the CL was evident on the follicular development and dominance in the ipsilateral ovary.

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