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Research Article

INFLUENCE OF EARLY PREGNANCY ON OVARIAN FOLLICULAR DYNAMICS IN BUFFALOES

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ABSTRACT

Starting on day 2 post-insemination, follicular activity was monitored every alternate day until day 24 by ultrasonography in non-suckled postpartum lactating buffaloes (n=9) during late winter. The follicular development occurred in 3 (n=5) or 4 (n=4) waves during the observation period, corresponding to 2 or 3 waves in a cycle length, with no influence of pregnancy on the numbers of waves or the time when a wave started. Overall, mean numbers of follicles developing during waves 1, 2, 3 and 4 were 15.7, 15, 10.3 and 7.8, respectively, with no significant effect of pregnancy on the follicular population. However, total follicles in waves 1 and 4 were higher in non-pregnant buffaloes than the pregnant ones. Dominant follicles in pregnant buffaloes tended to be smaller in diameter as compared to those in non-pregnant females. In conclusion, follicular recruitment and wave pattern during the first 24 days post insemination was not significantly influenced by the pregnancy status of buffaloes.

Key words: Follicular dynamics, early pregnancy, buffaloes

Transrectal ultrasonography is a reliable technique for identification and measurement of follicles in large farm animals (Pierson and Ginther, 1984). Ultrasonographic monitoring of ovaries in cattle has shown that follicle growth occurs throughout the estrous cycle in 2 or 3, and sometimes 4 waves (Savio *et al.,* 1988, Ginther *et al*., 1989). However, buffalo estrous cycles reportedly have 1, 2 or 3 follicular waves, 2-wave cycles being most common (Baruselli *et al*., 1997). The follicular dynamics in early pregnancy has not been studied in buffaloes. Hence, the present experiment was therefore, designed to study the influence of early pregnancy on ovarian follicular dynamics in buffaloes.

MATERIALS AND METHODS

The study was conducted on nine parous, lactating, non-suckled, inseminated Murrah buffaloes at Buffalo Research Centre of the university at Hisar. The animals were in their 1st to 7th lactations and had been inseminated for the 1st to 3rd time. Pregnancy was confirmed by per rectal palpation of genitalia on day 55 post insemination. All animals were subjected to ultrasonographic monitoring of 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 follicles, measurable with inbuilt calipers, 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. Ovarian follicles observed on ultrasonographic scanning were grouped according to their diameter as small (<5 mm) or large (>5 mm diameter) follicles. 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 monitoring and the largest follicle for each wave was determined and considered as the dominant follicle of particular wave. Rate of growth of the dominant follicle was calculated by subtracting the initial diameter from the maximum diameter and dividing by the number of days taken to reach the same.

Effect of pregnancy on number of follicular waves, numbers of small, large and total follicles in a wave and total ovarian activity, was determined. Data were subjected

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to statistical analysis using analysis of variance, t-test and Chi-square test as appropriate (Snedecor and Cochran, 1968).

RESULTS AND DISCUSSION

Follicular development occurred in 3 (n=5) or 4 (n=4) waves during the observation period of 24 days post-insemination. Restricting to a cycle length duration (approx. 21 days), 2 (n=4), 3 (n=4) or 4 (n=1) waves were recorded. Wave-1 (W1) started on day 2 post-insemination in all animals, while wave-4 (W4) emerged between day 18 to 22 in four buffaloes (Table 1). The last wave was recorded on day 22, except in two animals, irrespective of the number of waves in a buffalo. These results suggested that follicular development in buffaloes occured in a wave pattern, predominantly of 2 or 3 waves in cyclic buffaloes as previously reported (Danell, 1987; Manik *et al.,* 1994; Baruselli *et al.,* 1997). On a small number of observations, Taneja *et al.* (1996) reported a significant incidence of single wave cycles; the observations were based on monitoring of follicles >4 mm diameter, which is rather a large size to be considered for follicle recruitment and hence, the reported day of wave emergence may have been miscalculated. Isolated reports depicting 4 wave cycles are available in cattle (Savio *et al.,* 1988; Rhodes *et al.,* 1995), thus the present study indicated the existence of four waves of follicular development, within 21 days post-insemination in buffaloes.

With alternate day monitoring of ovaries in the present study, first wave was recorded always on day 2 post-insemination which may be a consequence of the transient and small rise in FSH immediately post-ovulation as reported in cattle and sheep (Ware *et al.,* 1988). In addition, pre-ovulatory rise in LH and FSH is also reported to induce large pools of small antral follicles during the post-ovulatory period (Ware *et al.,* 1988). Considering the most common duration of buffalo estrous cycle being 21 days, the last wave recorded around day 22 in all but two buffaloes in the present study could correspond to a new wave on day 2 of the next estrous cycle. However, this was detected both in pregnant as well as non-pregnant animals.

In the present study, day of start of the 2nd wave was widely variable: day 6-12; at an interval of 4-10 days from the 1st wave (average 6.4 days). The interval between emergence of two consecutive waves varied from 4 to 14 days, being significantly ($P<0.01$) shorter in animals with 4 waves (6.3±0.7 days) than those with 3 waves (9.6±0.5 days). Similarly, Baruselli *et al.* (1997) reported that though there was no difference with regard to the day of emergence of the first wave (day 1-2), but interval to wave-2 emergence was shorter in buffaloes having 3-wave cycles than those with 2-wave cycles. Manik *et al.* (1994) reported an interval of 9.6±1.16 days between two consecutive waves in two-wave cycles of buffaloes. The short interval between the two waves in the present study could be attributed to more number of waves.

Consistently, one or more waves were recorded during the mid-cycle in all buffaloes as reported in cattle (Savio *et al.,* 1988). In the cycle length duration, 3 non-pregnant buffaloes had a new wave emerging between days 16 to 18, which can be correlated with luteal demise. The last wave of the first 21 days post-insemination, also emerged as early as day 8 to 12, though attainment of the preovulatory size and ovulation could not be ascertained because either the females became pregnant or failed to return to estrus after normal cycle duration.

The mean number of follicles developing in W1, W2, W3 and W4 were 15.7, 15.0, 10.3 and 7.8, respectively (Table 1), being maximum in W1 and gradually declining to W4. When individual waves were compared, it was found that W1 had significantly ($P<0.05$) more number of follicles developing as compared to W3 and W4. The number of follicles developing per wave was significantly higher ($P<0.05$) in 3 waves animals (15.3±1.7, n=5) as compared to 4 wave animals (10.7±1.1, n=4). Plasma progesterone regulates terminal follicular development; higher progesterone arrests follicular development (Savio *et al.,* 1988) whereas low progesterone levels increase LH pulse frequency with extended periods of follicular dominance. A dip in circulating progesterone concentrations towards the end of cyclic luteal length is also observed in pregnant animals, triggering more release of FSH, thereby causing a wave to emerge towards the end of the normal cyclic luteal lifespan. Similarly in the present study, not only the total follicular turnover (16.1±1.6), but also the proportion of large follicles was greater during the last wave in a cycle length, probably coinciding with low progesterone regime. Overall, the greater number of follicles developing in 3 wave buffaloes than 4 waves buffaloes in the present study could be an effect of lower turnover in the 4th wave, which had mostly started on day 22, either due to pregnancy or lack of continued cyclicity in non-pregnant buffaloes.

Similar to the finding of greater number of follicles

Table 1
Number of follicles recruited and developing in different waves during 24 days post insemination in buffaloes

Animal No.	Pregnancy status	Day of start of the wave				Number of follicles in the wave			
		W1	W2	W3	W4	W1	W2	W3	W4
487	P	2	10	22	-	7	23	9	-
617	P	2	6	10	22	9	8	13	3
624	P	2	6	14	22	18	14	16	8
1105	P	2	12	22	-	19	16	6	-
1067	NP	2	10	18	-	24	11	12	-
1089	NP	2	8	22	-	22	21	6	-
1043	NP	2	10	22	-	19	21	13	-
1024	NP	2	6	16	18	11	16	10	15
1171	NP	2	10	16	22	12	5	8	5
Mean±S.E.						15.7±2.0[a]	15.0±2.1[a]	10.3±1.1[b]	7.8±2.7[b]

Means with different superscripts differ significantly (P<0.05

recruited and developing during the 1st wave, Baruselli *et al.* (1997) also found lower average follicular recruitment during the 2 wave or 3 wave estrous cycles of buffaloes. However, mean values per wave were greater in the present study, possibly due to the size of follicles (2 mm vs 3 mm).

The follicular turnover continued uninterrupted even in buffaloes which conceived (n=4) and was almost similar to that of buffaloes which failed to conceive (n=5). However, there was some lower follicular recruitment in pregnant buffaloes. Previous studies have indicated the follicular development in wave patterns during bovine pregnancy (Rexroad and Casida, 1975; Pierson and Ginther, 1987). The present study reveals that the follicular recruitment, development and dominance occur during the initial stages of pregnancy, as well as in inseminated non-pregnant buffaloes.

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