The Indian Journal of Animal Reproduction; **26**(2): 95-98; December 2005

Ultrasonic ovarian status in summer anestrus postpartum Murrah buffaloes

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Received : April 24, 2004
Accepted : July 20, 2005

ABSTRACT

The present investigation was aimed to diagnose the ovarian status in postpartum anestrus buffaloes during summer by ultrasonography. Twelve parous lactating, suckled Murrah buffaloes (*Bubalus bubalis*) 64-180 days postpartum (1st to 3rd lactation) were included in the study. Ovarian follicular changes were monitored with a real time B-mode ultrasound scanner using frequency of 7.0 MHz on day 0 (first day of scanning), 6, 8 ~~and day 10. Ultrasonographic scanning of ovaries at 10 days'~~ interval clearly revealed the presence of CL in three animals (25%). The average number of small (<4mm diameter), medium (4-9 mm diameter), large (>9 mm diameter) and total follicles in both ovaries ranged from 4.8 to 5.3, 2.2 to 2.4, 0.6 to 0.8 and 7.7 to 8.3, respectively. Six of the remaining nine animals had at least one large follicle (> 9 mm diameter) in growing phase from day 6 to day 10. The remaining three animals had the largest follicles of < 9 mm in diameter on day 10. Similarly, overall largest follicle mean diameter ranged from 9.7 to 10.5 mm. In conclusion, anestrus condition occurs due to failure of ovulation, rather than failure of development of dominant follicle, as revealed in summer anestrus postpartum buffaloes except sub estrus animals. Therefore, comprehensive study of ovarian follicular turnover and related endocrine profiles is required in order to understand the physiology of summer acyclicity in buffaloes and in turn, its judicious management.

Key words : Murrah buffaloes, anestrus, postpartum

In spite of being the mainstay of Indian dairy industry buffalo is often considered an inefficient reproducer. The main reasons for low reproductive efficiency of buffalo include weak expression of estrus signs, silent estrus, seasonality of reproduction and long postpartum period of ovarian quiescence. Furthermore, the resumption of ovarian activity after calving is significantly delayed in buffaloes that calved from February to May (116-148 days) compared to rest of the year (18-64 days, Singh and Nanda, 1993). Ultrasonographic studies on ovarian activity during early postpartum period (Murphy *et al.*, 1990; Savio *et al.*, 1990; Roche *et al.*, 1992; Henao *et al.*, 2000) and on the effect of heat stress on follicular dynamics are well documented in the cattle (Wolfeson *et al.*, 1995), but the literature is scanty on ovarian picture in postpartum anestrus buffaloes. Therefore, the present study was undertaken to diagnose the ovarian status in postpartum buffaloes during summer anestrus period.

MATERIALS AND METHODS

The present study was conducted at Central Institute for Research on Buffaloes, Hisar on twelve lactating, suckled Murrah buffaloes (*Bubalus bubalis*), 64-180 days postpartum and in their 1st to 3rd lactations. All the buffaloes were healthy, claved normally and were free from any genital infection. As per farm records the experimental buffaloes were not recorded in estrus after calving and were identified to be anestrus. The investigation was undertaken during summer months (dry hot season) between last week of May and first week of July, 2003 when the ambient temperature ranged from 31.1°C to 46.2°C (average 41.3°C) and the relative humidity ranged from 35-97% (average 66%). Ovarian follicular changes were monitored in all experimental buffaloes with a real time B-mode ultrasound scanner (Just Vision 200 - Model SSA-320A, Toshiba, Japan) equipped with a convex array multi-frequency transducer using frequency of 7.0 MHz on day 0 (first day of scanning), 6, 8 and 10.

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Follicles were appreciated as round non-echogenic cavities in the ovarian stroma with well-defined borders. Recording the position of each follicle, as on a clock dial and following it up at subsequent scannings 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 medical to lateral aspect on any given day. 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 follicular fluid. The follicles were classified as small (<4 mm diameter), medium (4-9 mm diameter) and large (>9 mm diameter). Presence and absence of the corpus luteum (CL) was also recorded. To reduce errors, the same person did all the measurements and ultrasonic monitoring.

RESULTS AND DISCUSSION

As per the farm records, none of the 12 animals included in the experiment had exhibited postpartum estrus, but ultrasonographic scanning of ovaries at 10 days' interval revealed presence of CL in three animals indicating their cyclicity. Usually, the incidence of silent/unobserved estrus in farm buffaloes is low (10-11%) than in the rural buffaloes (60-70%, Sharma *et al.*, 1967). However, Shah *et al.* (1990) recorded a very high (73%) incidence of silent estrus in postpartum buffaloes under small farm conditions. Observation of luteal progesterone concentrations in milk of buffaloes at an organized farm also revealed a high incidence of silent/unobserved estrus in the early postpartum period even during breeding season (Yashpal *et al.*, 2000). Besides summer stress, inappropriate steroid hormone milieu and lower pituitary stores or responsiveness as well as nutritional deficiencies particularly those of mineral and vitamins, together with some hereditary predisposition could predispose buffaloes to convert estrus. Failure to detect estrus in buffaloes may involve low intensity of estrus signs, shorter duration of estrus and occurrence of estrus particularly during the cooler night hours (Jainudeen and Hafez, 1987). Therefore, the failure to record estrus in 25% of experimental animals of the present study, by routine farm estrus detection practice, could involve both animals as well as human factors. Nevertheless, such animals are presented as anestrus/problem animals both at organized farms as well as under field conditions (Anonymous, 1995), which require veterinary attention.

The assessment of cyclicity by per rectal palpation is rather difficult in buffaloes due to small sized ovaries and usually embedded corpora lutea (Drost *et al.*, 1985; Bahga *et al.*, 1991; Ambrose *et al.*, 1993). Ultrasonography is considered a reliable technique for detection of ovarian structures in buffaloes (Rajamahendran *et al.*, 1994). In the present experiment also, ultrasonographic scanning was not only found useful in diagnosing ovarian structures, but also cyclicity of buffaloes as all the three animals having corpora lutea were easily detected with this technique. In the remaining nine animals, no luteal structures were evident on ultrasonography and were therefore considered true anestrus. Six of these nine animals had at least one large follicle ($\geq$ 9 mm diameter) at the time of ultrasound scanning on day 6, 8 and 10, while the remaining three animals had the largest follicles of less thaqn 9 mm in diameter on these days. The ovarian picture w.r.t. the presence of LF or SF did not differ between different days during pre-treatment period.

In these buffaloes (n =12), the mean number of small, medium, large and total follicles did not differ significantly between days 6, 8 and 10. The average number of small, medium, large and total follicles in both ovaries ranged from 4.8 to 5.3; 2.2 to 2.4; 0.6 to 0.8 and 7.7 to 8.3, respectively (Table 1). In summer anestrus/subestrus buffaloes included in the present study, the population of different sized follicles did not change significantly during serial ultrasonographic examinations and averaged approximately eight follicles on any particular day. Similarly, overall largest follicle average diameter on any particular day of scanning upto day 10 also did not differ considerably and the mean ranged from 9.7 to 10.5 mm. Mean diameter of the largest follicle in anestrus buffaloes reach to about 10 mm, which is close to the pre-ovulatory size of follicle in buffaloes.

Reports on follicular dynamics in postpartum buffaloes during summer anestrus are not available. However, in inseminated cyclic buffaloes during late winter, the mean number of follicles on any particular day was almost double (Kumar, 2001) than that observed in present study. Similarly, the observed largest follicle mean diameter was also smaller than that reported in normal cyclic buffaloes (Taneja *et al.*, 1996). This indicates that although dynamic follicular activity was going on in the ovaries of summer anestrus postpartum buffaloes but it was sub-optimal for cyclicity as the total

Table 1. Ovarian follicular dynamics in postpartum buffaloes during summer anestrus

Bull No.	Day 6 No. of follicles S	Day 6 No. of follicles M	Day 6 No. of follicles L	Day 6 Total	Day 6 LF Dia. (mm)	Day 6 CL	Day 8 No. of follicles S	Day 8 No. of follicles M	Day 8 No. of follicles L	Day 8 Total	Day 8 LF Dia. (mm)	Day 8 CL	Day 10 No. of follicles S	Day 10 No. of follicles M	Day 10 No. of follicles L	Day 10 Total	Day 10 LF Dia. (mm)	Day 10 CL	St.
1164	3	3	0	6	8.5	P	5	2	0	7	6.7	P	1	4	0	5	7.7	P	G
2032	7	2	1	10	11	P	6	1	1	8	13	P	6	1	1	8	13.4	P	G
1629	5	2	0	7	7.5	P	5	4	0	9	7.9	P	5	2	1	8	12.7	P	G
2215	8	2	1	11	10.5	A	7	3	1	11	11.3	A	3	3	1	7	11.4	A	G
1441	5	0	1	6	14.4	A	2	2	1	5	15	A	4	3	1	8	15.2	A	G
2170	6	1	1	8	11.1	A	6	1	1	8	11.3	A	6	2	1	9	11.5	A	G
2130	3	2	1	6	11.4	A	6	1	1	8	11.8	A	5	1	2	8	11.9	A	G
2169	6	1	1	8	9.2	A	6	2	1	9	9.4	A	6	2	1	9	9.5	A	G
1888	8	1	1	10	9.2	A	7	4	1	12	9.2	A	6	2	1	9	9.3	A	G
2136	4	3	0	7	7.7	A	5	3	0	8	7.5	A	5	2	0	7	8.7	A	G
2216	3	3	0	6	7.7	A	5	2	0	7	7.5	A	6	1	0	7	6.9	A	G
2150	1	8	0	9	8.8	A	3	4	0	7	6.3	A	4	3	0	7	7.9	A	G
Mean	4.9	2.3	0.6	7.8	9.8	3/12	5.3	2.4	0.6	8.3	9.7	3/12	4.8	2.2	0.8	7.7	10.5	3/12	12/12
±SE	0.6	0.6	0.2	0.5	0.6		0.4	0.3	0.1	0.5	0.8		0.4	0.3	0.1	0.3	0.7		

S = Small (2-4 mm), M = Medium (4-9 mm), L = Large (> 9 mm) follicles; CL = Corpus Luteum, LF = Largest follicle; A = CL Absent and P = CL Present; St = Growth status of LF on day 10; G = Growing follicle; R = Regression follicle

follicular population was small and the largest follicles failed to attain pre-ovulatory size and ovulate in a majority of these animals. In cattle too, dynamic follicular activity continued during the anestrus period before first postpartum ovulation (Henao *et al.*, 2000). In early postpartum dairy cows, follicular development was characterized by growth and regression of follicles less than 8mm in diameter until the detection of first postpartum dominant follicle, which subsequently ovulated (Savio *et al.*, 1999). Our findings in summer anestrus buffaloes support the view of Roche *et al.* (1992) that anestrus condition occurs due to failure of ovulation, rather than failure of development of dominant follicle.

Heat stress is also reported to reduce the degree of dominance, together with reduced steroidogenic capacity, leading to lower plasma estradiol concentrations and thus altering the ovulatory mechanism in cattle (Rensis and Scaramuzzi, 2003). Similarly, in early postpartum dairy cows, ovarian follicles fail to produce sufficient concentrations of estradiol to induce pre-ovulatory LH surge and hence, ovulation (Roche and Diskin, 2001). Although it was not in the ambit of present study, but if a similar situation exists in the summer anestrus postpartum buffaloes, needs to be investigated. Low LH concentrations, including peak frequency and amplitude, have been reported in summer anestrus buffaloes (Razdan, 1988). Therefore, comprehensive study of ovarian follicular turnover and related endocrine profiles is required to understand the physiology of summer acyclicity in buffaloes and in turn, its judicious management. Nevertheless, the present study amply demonstrates continued follicular turnover together with the phenomenon of dominance as well as ovulation in a proportion of the summer anestrus buffaloes, but the immediate preovulatory, ovulatory and associated estrus behaviour mechanism may be compromised in the summer season.

REFERENCES

Ambrose, J.D., Manik, R.S., Singh, S.K. and Madan, M.L. (1993). A simplified laparoscopy technique for repeated ovarian observation in the water buffalo (*Bubalus bubalis*). Theriogenology, **40**: 487-496.

Anonymous (1995). Report of the State Non-plan Scheme on "Investigations into the causes of infertility and sterility in cattle and buffaloes, and prevention thereof, in the state of Haryana", Department of Gynaecology & Obstetrics, CCS Haryana Agricultural University, Hisar.

Bahga, C.S., Singh, N., Matharoo, J.S. and Chaudhary, K.C. (1991). Assessment of rectal palpation compared to milk progesterone kits for detecting ovarian activity in buffaloes (*Bubalus bubalis*). Buffalo J., **1**: 95-99.

Drost, M. and Cripe, W.S. (1985). Superovulation and embryo transfer in buffaloes (*Bubalus bubalis*). In: Proc. First World Buffalo Congress, **3**: 498-505.

Henao, G., Olivera-Angel, M. and Maldonado-Estrada, J.G. (2000). Follicular dynamics during postpartum anestrus and the first oestrus cycle in suckled or non-suckled Brahman (*Bos indicus*) cows. Anim. Reprod. Sci., **63**: 127-136.

Jainudeen, M.R. and Hafez, E.S.E. (1987). In: *Reproduction in Farm Animals*. 5th edn. (Ed.) E.S.E. Hafez, Lea & Febiger, Philadelphia, pp 399-492.

Kumar, D. (2001). Post insemination ultrasonographic monitoring of ovarian activity in Murrah buffaloes. M.V.Sc. thesis, CCS Haryana Agricultural University, Hisar, India.

Rajamahendran, R., Ambrose, D.J. and Burton, B. (1994). Clinical and research application of real-time ultrasonography in bovine reproduction : A review. Can. Vet. J., **35**: 563-572.

Razadan, M.N. (1988). Buffalo performance in relation to climatic environment. Proc. IInd World Buffalo Congress, New Delhi, Vol. II, Part I, pp 173-186.

Rensis, F.D. and Scaramuzzi, R.J. (2003). Heat stress and seasonal effects on reproduction in the dairy cow - A Review. Theriogenology, **60**: 1139-1151.

Roche, J.F. and Diskin, M.G. (2001). Resumption of reproductive activity in the early postpartum period of cows. In: *Fertility in the high producing dairy cow*. (Ed.) M.G. Diskin. Vol. 26, BSAB Edinburgh : Occasional Publication: 2001, pp 31-42.

Roche, J.F., Crowe, M.A. and Boland, M.P. (1992). Postpartum anoestrus in dairy and beef cows. Anim. Reprod. Sci., **28**: 371-378.

Savio, J.D., Boland, M.P., Hynes, N. and Roche, J.F. (1990). Resumption of follicular activity in the early postpartum period of dairy cows. J. Reprod. Fertil., **88**: 569-579.

Shah, S.N.H., Willemse, A.H. and Van Wiel, D.F.M. (1990). Descriptive epidemiology and treatment of postpartum anestrus in dairy buffalo under small farm conditions. Theriogenology, **33**: 1333-1345.

Sharma, O.P., Bhalla, R.C. and Soni, B.K. (1967). Studies on some aspects of ovarian abnormalities as a cuase of infertility in buffalo cow (*Bubalus bubalis*). Ind. Vet. J., **44**: 504-508.

Taneja, M., Ali, A. and Singh, G. (1996). Ovarian follicular dynamics in water buffalo. Theriogenology, **46**: 121-130.

Yashpal, Singh, I., Singh, U. and Phogat, J.B. (2000). Reproductive parameters of postpartum buffaloes. Haryana Vet., **39**: 20-27.

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