Effect of Suckling on the Resumption of Postpartum Ovarian Activity in Buffaloes

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ABSTRACT: Eighteen pluriparous Lanka buffaloes were assigned to one of three treatment groups: restricted suckling (RS), ad libitum suckling (AS) and ad libitum suckling with supplementary feed (AS/S). Blood samples were collected for 8 hours at 20 minute intervals on days 30, 45 and 60 where 2 GnRH injections were given at 0400 and 0600 hours, and on day 90 without GnRH. Rectal examination of ovaries was performed weekly. Plasma concentrations of LH and progestrone were measured by RIA. The mean LH for group RS was higher ($P<0.05$) than those of the other two groups. There was no difference ($P>0.05$) between groups AS and AS/S. In the RS group pulsatile LH secretion was observed by day 60 in all the animals, whereas animals of groups AS and AS/S did not show this. The number of animals which commenced ovarian activity by day 90 was higher ($P<0.05$) in group RS when compared to groups AS and AS/S. The findings of this experiment suggest that ad libitum suckling, a common calf rearing method, can delay the resumption of episodic LH release postpartum which is a prerequisite for the commencement of ovarian activity.

INTRODUCTION

Lanka buffaloes are multipurpose animals and form an integral part of the rural subsistence farming systems in Sri Lanka. Delayed puberty and extended calving intervals were found to be major constraints for economical production (de Silva *et al.*, 1985). On-station and field studies have revealed that long periods of postpartum anoestrus is the primary cause for the extended calving intervals and that conditions such as repeat breeding, postpartum endometritis and pathological conditions

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do not significantly contribute to poor fertility in this species (Perera *et al.*, 1987; Mohan, 1989). This prolonged period of postpartum anoestrus is associated with a delay in the resumption of episodic LH release after calving (Mohan, 1989), which is considered a prerequisite for the resumption of ovarian activity in cattle (Peters and Lamming, 1986).

Suckling and postpartum nutrition have been identified as primary determinants in the process of reinitiation of postpartum ovarian activity in cattle (Peters, 1984). In traditional dry zone farming systems buffalo cows are suckled by calves for extended periods and are maintained on natural herbage, the quantity and quality of which are determined by the seasonal rainfall.

This experiment was undertaken to examine the effect of suckling and improved nutrition on the resumption of ovarian activity, postpartum LH secretion and the response of the pituitary to small doses of exogenous GnRH.

MATERIALS AND METHODS

Animals

Eighteen pluriparous postpartum Lanka buffaloes which calved during the 1986/87 calving period (December 1986 to February 1987) at an experimental farm in the Coconut Triangle were used in this study. These animals were managed semi-extensively on improved pasture.

Experimental protocol

The animals were randomly assigned to one of the following treatment groups (n=6/group) immediately after calving: Group AS – *ad lib.* suckling, where the calves were kept continuously with their mothers. Group AS/S – *ad lib.* suckling and supplementary feeding (the dams were provided with mineral blocks and concentrates). Group RS – Restricted suckling where the calves were separated from dams on the 3rd day after calving and allowed to suckle only for two periods (morning and evening) of 30 minutes each per day. Sequential blood samples were collected through an indwelling jugular venous catheter at 20 min intervals for periods of 8 hours beginning at 0900 hr on day 30,

45 and 60 postpartum. Two doses of 12.5 micrograms of GnRH (Receptal, Hoechst, Germany) were given intravenously at 1300 and 1500 hours. On day 90 postpartum sequential blood samples were collected for 6 hours without GnRH injections. In addition, at weekly intervals blood samples were collected and rectal examination of the ovaries was performed. All the blood samples were centrifuged at 1500 rpm for 10 min and the plasma was separated and stored at -20^{0}C until assayed for hormone concentrations by radioimmunoassay (RIA). Sequential and weekly samples were analysed for LH and progesterone, respectively.

Hormone assays

LH

A heterologous double-antibody RIA for LH was developed by using anti-ovine LH serum (Dr. B.M. Bindon, CSIRO, Australia) and purified bovine LH (USDA-bLH-B-5) with slight modifications to the method described by Niswender *et al.*, (1969). Radiolabelled LH was prepared by Chloramine-T method (IAEA Technical Report Series 233, 1984) using highly purified bovine LH (USDA-bLH-I-1) and ^{125}I (Amersham, UK). This assay was validated in the laboratory for quantification of LH in buffalo plasma. The inter and intra-assay variations were 7.2% and 10.3% respectively. The sensitivity of the assay was 0.38 ng/ml.

Progesterone

Plasma progesterone concentrations were measured by a conventional single antibody RIA technique with extraction into petroleum ether, using a tritiated label and a charcoal-dextran method as described by Perera et al. (1978). The anti-progesterone antiserum was supplied by IAEA, Vienna, Austria and the tritiated progesterone was obtained from Amersham International, UK. The inter and intra-assay variations were 8.6 and 12.3 respectively.

Characterization of pituitary response to exogenous GnRH

The response of the pituitary gland to exogenous GnRH was characterized by defining the time taken for maximum LH response, maximum LH response following the first and second GnRH challenges and the area under the LH curve transcribed by LH values following the

first and second GnRH challenges. LH pulses in the profiles were detected by the visual appraisal technique described by Mc Leod and Crighton (1985).

Statistical analysis

The data were arranged in two factor nested design (where the treatment effect was considered as the main factor and the effect of time was nested within it) and analysed by ANOVA. Differences between all individual pairs and interactions were checked by Tukey's test (Neeter *et al.*, 1985). Chi – square goodness of fit test was used to estimate the differences in ovarian activity at various stages of the postpartum period between treatment groups as described by Hogg and Tanis (1983).

RESULTS

Ovarian activity

The percentages of animals which resumed ovarian activity at different stages of the postpartum period as detected by the combined use of rectal palpation of ovaries, plasma progesterone levels and oestrus observation are given in Table 1. Resumption of ovarian activity was first noticed in animals from group RS around day 50 as opposed to day 70 and day 90 in groups AS/S and AS, respectively. On day 90 all the animals in group RS resumed ovarian activity while only 1 animal showed ovarian activity in AS group. Three animals resumed ovarian activity in AS/S group by that day. The difference between RS group and AS and AS/S was significant ($P<0.05$) while the difference between AS group and AS/S group was not significant ($P>0.05$).

Mean LH concentrations before GnRH challenge

The mean LH concentrations in blood samples collected during the period preceding GnRH treatment on days 30, 45, 60 and also on day 90 are given in Table 2. The mean LH concentrations for group RS were higher ($P<0.01$) on days 45, 60 and 90 when compared th those of AS/S and AS groups.

Table 1. Percentage of buffaloes which had commenced ovarian activity at successive periods after calving.

Group	Days Postpartum					
	40	50	60	70	80	90
AS	0	0	0	0	0	20
AS/S	0	0	0	33	33	50
RS	0	14	28	57	71	100

Table 2. Mean plasma LH concentrations (pre – GnRH) for different treatment groups on days 30, 45, 60 and 90 postpartum (ng/ml).

Group	Days Postpartum			
	30	45	60	90
AS	**	0.6	0.9	1.3
AS/S	**	0.7	0.9	1.4
RS	0.7	*1.1	*1.4	*1.9

** Below the sensitivity of assay (0.38 ng/ml)
* $P < 0.05$.

LH pulses

The amplitude and frequency of LH pulses detected in these animals during the pre-GnRH treatment period are given in Table 3. The pulsatile LH secretion appeared early in RS group animals. By day 90 all the animals in group RS showed episodic LH release while only 1 from AS group and 3 from AS/S group showed episodic LH release. Further, the appearance of LH was associated with the commencement of ovarian activity in animals from all 3 groups.

Pituitary response to GnRH stimulation

The peak concentrations after the first GnRH challenge were reached within 20 minutes of the GnRH administration in all three treatment groups. The mean peak values after the first and second GnRH injections are shown in Table 4. The peak concentrations following the first and second GnRH injections in RS group was higher ($P<0.05$) than those of AS and AS/S on days 30 and 45. There were no differences ($P>0.01$) between AS and AS/S groups during these 2 treatment periods. The peak concentrations after GnRH injections increased ($P<0.01$) as the days postpartum progressed in all 3 groups. The peak concentration to the second GnRH injection was higher ($P<0.05$) than that to the first GnRH injection in all 3 groups throughout the experiment. The area under the LH curve for the 4 hr period following the GnRH challenges (Table 4) for RS group was greater ($P<0.01$) than those obtained for AS and AS/S groups on days 30, 45 and 60. There was no difference in this parameter ($P>0.01$) between AS and AS/S groups.

DISCUSSION

Appearance of pulsatile LH secretion was associated with the resumption of ovarian activity in postpartum buffaloes. Studies in cattle had shown an association between the appearance of pulsatile LH release postpartum and resumption of ovarian activity (Peters and Lamming, 1986). Findings of our study provide further confirmatory evidence in this regard and also suggest a similarity between cattle and buffaloes in the endocrine control of postpartum ovarian activity.

Table 3. Frequency and amplitude of LH pulses in treatment groups.

Days Postpartum	Group	% of Animals which had pulses	Number of pulses (mean)	Mean Amplitude of peak ng/ml
30	AS	0	0/4 hr	–
	AS/S	0	0/4 hr	–
	RS	0	0/4 hr	–
45	AS	0	0/4 hr	–
	AS/S	0	0/4 hr	–
	RS	40	1/4 hr	1.8
60	AS	20	0/4 hr	–
	AS/S	33	1/4 hr	1.65
	RS	57	1.25/4 hr	2.4
90	AS	20	1.5/6 hr	1.9
	AS/S	50	1.5/6 hr	2.0
	RS	100	2.6/6 hr	2.4

Table 4. Responsiveness of the pituitary glands to GnRH.

Days pp	Group	Area under curve ng/mlxhr	1st peak ng/ml	2nd peak ng/ml
30	AS	14.4 ± 4.6	2.3 ± 0.92	2.5 ± 1.10
	AS/S	16.3 ± 3.7	1.8 ± 0.70	3.2 ± 0.98
	RS	*34.5 ± 8.2	*3.1 ± 1.40	*4.7 ± 1.64
45	AS	37.1 ± 11.2	4.1 ± 0.96	4.4 ± 1.20
	AS/S	34.0 ± 9.3	4.1 ± 0.67	5.8 ± 1.52
	RS	*49.0 ± 13.2	*5.3 ± 0.78	*8.4 ± 1.74
60	AS	66.9 ± 14.7	5.7 ± 1.13	7.9 ± 1.42
	AS/S	64.3 ± 11.9	6.5 ± 1.23	8.6 ± 1.67
	RS	*90.4 ± 16.6	7.4 ± 1.61	*11.9 ± 1.91

* $P < 0.05$

Episodic LH secretion is driven by episodic release of GnRH from the hypothalamus (Baird, 1984). Absence of pulsatile LH release postpartum suggests that the secretion of GnRH is deficient in the early postpartum period. Absence of pulsatile LH release in the free suckled groups (AS & AS/S) tend to suggest that effects of suckling by the calf and perhaps the behavioural interactions between the calf and cow, may be inhibitory to GnRH release from the hypothalamus. The responsiveness of the pituitary gland of postpartum buffalo cows to exogenous GnRH increased as the postpartum period progressed. A similar increase in the responsiveness of the pituitary gland to exogenous GnRH during the postpartum period has also been demonstrated in cattle (Fernandes *et al.*, 1978; Williams *et al.*, 1982) and it is regarded as an endocrinological event which precedes the resumption of ovarian activity in this species. Reduction in pituitary responsiveness to exogenous GnRH in the *ad lib.* suckled buffaloes suggests that the suckling stimulus exerts inhibition at the pituitary level as well. This inhibition appears to be more marked during the early postpartum period.

Supplementary feeding in the presence of the *ad lib.* suckling stimulus did not facilitate the process of reinitiation of ovarian activity. This lack of facilitatory effect may suggest that the suckling stimulus is more inhibitory to the gradual recovery process of the hypothalamo–hypophyseal–gonadal axis than the deficiencies of nutrients. Another possible explanation is that the animals in this experiment may have been in an adequate level of nutrition and supplementation of nutrients over and above the requirements may not have an overriding effect on the resumption of ovarian activity.

CONCLUSION

Postpartum endocrine events which precede the resumption of ovarian activity in buffaloes appear to be similar to those in cattle. Suckling appears to inhibit this process both at hypothalamic and hypophyseal levels. The inhibitory effects of suckling by the calf may be reduced by the practice of restricted suckling. This can be adopted as an effective management practice during the postpartum period to induce early resumption of ovarian activity.

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