Proc. Natl. Sci. Counc. ROC(B)
Vol. 24, No. 2, 2000. pp. 70-75

Milk Progesterone Profiles in Various Reproductive States in Dairy Buffaloes under Field Conditions

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(Received December 22, 1998; Accepted August 16, 1999)

ABSTRACT

Fifty-one dairy buffaloes in the last two months of gestation were selected at seven private peri-urban farms in the Peshawar district. Observations were recorded in buffaloes during normal (NBS, August to January) and low breeding seasons (LBS, February to July). After parturition, rectal examination of reproductive organs was carried out. Estrus detection was made through visual observation and the use of intact bul1. Postpartum ovulation was confirmed by ovarian palpation per rectum and milk progesterone levels (MPL), determined through radio-immunoassay. MPL was higher ($p < 0.01$) at various intervals in NBS calves (1.97 ± 0.30 ng/ml) as compared to LBS calves (0.68 ± 0.08 ng/ml). During LBS, MPL remained < 0.30 ng/ml up to the third fortnight and started rising later, reaching a peak of 1.27 ng/ml during the sixth fortnight. During NBS, there was a sharp rise in MPL during the second fortnight. reaching 3.64 ng/ml during the sixth fortnight. MPL was significantly different on different experimental farms ($p < 0.01$). MPL reached the lowest levels on the day of estrus (0.10 ng/ml), reached it's peak on day 7 and started declining on day 17 of estrus. MPL showed two postpartum elevations. In true anestrus buffaloes, MPL remained consistently low. However, in the anestrus period, silent ovu1ations were also noted, as reflected by increasing MPL without estrus signs. In pregnant buffaloes, MPL remained > 1 ng/ml. Results of the study showed that the low postpartum reproductive performance in dairy buffaloes during LBS was primarily due to inadequate functioning of the corpus luteum in secreting optimum concentrations of progesterone. The higher incidence of silent estrus during LBS indicated improved management for the detection of estrus.

Key Words: buffalo, dairy, Pakistan, reproduction, hormone, progesterone

I. Introduction

Progesterone is a steroid hormone, the first biologically active compound in the steroid biosynthesis pathway. This hormone was discovered by Loewe and Lange in 1926 and isolated by Corner and Allen (1929). Its concentration in an animal's body reflects the stage of the reproductive cycle, pregnancy and ovarian disorders. Radioimmunoassay (RIA) was developed to measure progesterone in milk, and the ability to collect a milk sample rather than a blood sample made it possible to monitor the ovarian activity of dairy animals using milk progesterone RIA (Heap, 1974). Milk is preferred over plasma for progesterone assay under field conditions because collection of blood samples from dairy animals is more invasive and disliked by farmers while in milk sampling, no problem of this nature is faced. Lamming and Darwash (1998) concluded that milk progesterone monitoring offers an objective and accurate method for assessing typical and atypical ovarian function in postpartum cows. In buffaloes, progesterone profiles have been studied in postpartum periods (Perera *et al*., 1981; Kamonpatana *et al*., 1983; Jainudeen *et al*., 1983). Following the first postpartum ovulation, plasma progesterone levels were found to increase and remain above 0.7 ng/ml for about 10 days; then, they declined to below 0.25 ng/ml at the next estrus. Progesterone assay was successfully used in estrus detection (Qureshi *et al*., 1989) and in the assessment of ovarian status (Qureshi *et al*., 1992) in Nili-Ravi buffaloes. Usmani *et al*. (1990) found that 86% of Nili-Ravi water buffaloes showed at least 1 short luteal phase (8 to 13 days) before the first estrus. It was reported (Jain and Pandey, 1985) that in buffalo heifers, plasma progesterone concentrations were significantly affected by the season

and weaning, and varied significantly between the puberty and neonatal periods. The present study was conducted to determine milk progesterone profiles under various reproductive states in dairy buffaloes under field conditions.

II. Materials and Methods

Fifty-one dairy buffaloes in their last two months of gestation in the normal (NBS, August to January) and low breeding seasons (LBS, February to July) were selected at seven private farms located within a radius of 70 ki1ometers of Peshawar city.

The buffaloes were ear-tagged and kept under conventional management at the respective farms. Restricted calf suckling at the time of milking was allowed.

After parturition, rectal examination of the reproductive organs was carried out on days 14 and 21, and then fortnightly until the occurrence of the first estrus was detected as described by Usmani *et al*. (1985). The positions of the reproductive organs were recorded, and the size of the graafian follicles and corpora lutea or corpora albicancia on the ovaries was measured with the help of fingers. Estrus detection was conducted twice daily, commencing 15 days postpartum. In addition to visual signs of vulvar mucus, frequent micturition and bellowing, an intact bull was also used for the detection of estrus. Standing heat was used as a criterion for estrus confirmation. Postpartum ovulation was confirmed based on the ovarian palpation per rectum and milk progesterone levels (MPL).

Evening milk samples were collected once a week. After removing the fat layer by means of centrifugation (3000 rpm), 100 µl of 0.1% sodium azide was added to 5 ml of milk sample as a preservative. Samples were stored at –20˚C until they were analyzed for MPL, using RIA. The procedure suggested by the FAO/IAEA (1993) was adopted. Milk samples and other assay components were brou-ght to room temperature before the assay was started. Antibody coated polypropylene tubes were labelled for standard, quality control and samples, according to the protocols. Non-coated normal tubes were used for total count. 100 µl of standard, quality control or sample were pipetted into the bottom of the corresponding tube. Then, 1 ml of ^{125}I-progesterone was pipetted into each tube. The tubes were covered with parafilm and incubated at 4˚C over-night. The next morning, the tubes were decanted vigorously except for those used for total count. The radioactivity was counted using a gamma counter (Vegacalc (c) NE Technology Limited). The data were analyzed using the Vegacalc software program. The intra- and inter-assay coefficients of variation were 3.54% and 9.21%, respectively. The sensitivity (detection limit) of the assay was 0.09 ng/ml.

The data were analyzed using analysis of variance and correlation analysis procedures (Steel and Torrie, 1980).

III. Results and Discussion

1. Seasonal Effect on Milk Progesterone Profiles

MPL was higher ($p < 0.01$) in buffaloes during calving NBS (1.97 ± 0.30 ng/ml) than LBS (0.68 ± 0.08 ng/ml, Table 1). The postpartum levels of milk progesterone followed different patterns during the two breeding

Table 1. Mean Values ± Standard Error (SE) and Number of Observations (*n*) of Milk Progesterone Concentrations (ng/ml) during Various Seasons and Reproductive States in Buffaloes

Group	Mean* ± SE	n
Seasons		
Spring	3.00 ± 0.12[a]	53
Winter	1.77 ± 0.32[b]	150
Autumn	0.84 ± 0.72[c]	101
Summer	0.25 ± 0.04[c]	81
Significance level	$p < 0.01$	
Calving periods		
Autumn-Winter (NBS)	1.97 ± 0.30[a]	207
Spring-Summer (LBS)	0.68 ± 0.08[b]	178
Significance level	$p < 0.01$	
Postpartum intervals (days)		
Up to 15	0.26 ± 0.06	20
16 to 30	0.55 ± 0.08	127
31 to 60	1.78 ± 0.26	100
61 to 90	1.84 ± 0.53	69
91 to 120	1.87 ± 0.35	34
121 to 150	2.55 ± 0.59	34
Significance level	Non significant	
Stage of estrus cycle		
During estrus	0.30 ± 0.98[b]	18
Developing corpus luteum	1.43 ± 0.85[a]	14
Developed corpus luteum	3.29 ± 0.84[a]	21
Regressing corpus luteum	0.88 ± 0.15[b]	8
Significance level	$p < 0.01$	

*Means in the same column with different superscripts differ from each other.

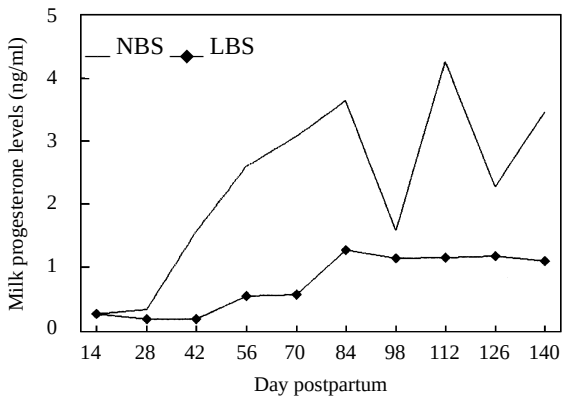

Fig. 1. Milk progesterone levels at various postpartum intervals in normal (NBS) and low breeding season calvers (LBS) (*n* = 384, mean ± SEM = 1.37 ± 0.17 ng/ml).

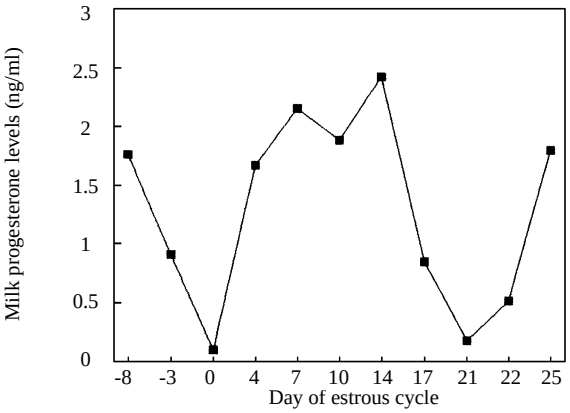

Fig. 2. Milk progesterone levels on different days of the estrus cycle in Nili-Ravi buffaloes (n = 61, mean ± SEM = 1.48 ± 0.71 ng/ml).

seasons as shown in Fig. 1. During NBS, MPL remained at the lowest level until day 28 postpartum, followed by a sharp increase to a maximum concentration of 3.64 ng/ml on day 84. During LBS, the changes in MPL were less marked and were characterized by a minimum level of 0.30 ng/ml until day 42, followed by a peak level of 1.27 ng/ml on day 84. The earlier rise in MPL during NBS suggested earlier resumption of estrus activity. In other studies (Bahga and Ganwar, 1988), the progesterone levels remained at basal levels from day 5 to day 30 postpartum and started rising thereafter. An increase in progesterone levels beyond day 30 was correlated with luteal function and cyclic activity (Madan, 1984).

The results of the present study suggest that during LBS, MPL did not reach the optimum levels until day 84 postpartum, indicating that the corpus luteum did not function efficiently so as to maintain sufficiently high progesterone levels to maintain reproductive cyclicity. This confirms the findings of Madan (1984), who attributed the low reproductive efficiency of buffaloes in summer to 1ow luteal activities, indicated by low progesterone levels. You and Chen (1992) reported that a cow experienced four estrus cycles, three of which were behavioral, during a 2-months period. The missing cycle and the low values of plasma and fecal progesterone during the luteal phase from May to June implied a weak seasonal effect in Taiwan. In the present study, fodder scarcity during May to July of LBS, which was associated with a minimum body condition score of 2.2 and low energy intake, could have adversely affected ovarian activity through nutritional deficiency (Qureshi, 1998).

2. Milk Progesterone Levels during Postpartum Intervals

The milk progesterone concentration varied during various postpartum months ($p < 0.08$). The lowest concentrations were recorded 15 days postpartum, followed by the second fortnight and the second, third, fourth and fifth months postpartum (0.26, 0.55, 1.78, 1.84, 1.87, 2.55 ng/ml, Table 1). The present findings are in agreement with those of Perera *et al*. (1981) and Jainudeen *et al*. (1983), who reported that elevated progesterone levels in the serum of buffaloes declined rapidly following parturition to undetectable levels by day 3 or 4 postpartum and remained low thereafter for different periods until ovarian cyclicity was restored. In this study, MPL correlated negatively with suckling duration ($r = –0.13$, $p < 0.05$) and was significantly different on different experimental farms ($p < 0.01$).

3. Estrous Cycle Pattern

The changes in the ovarian structure and concurrent MPL are presented in Table 1. MPL varied during various stages of the estrus cycle. Observations of twenty-four first postpartum estrus cycles showed an average length of 21.0 days. The estrus cycle pattern of MPL is shown in Fig. 2. MPL started falling eight days prior to the commencement of the estrus cycle and reached the lowest levels on day 0 (the day of estrus, 0.10 ng/ml). After ovulation, MPL increased, indicating a developing corpus luteum. MPL remained > 1.5 ng/ml from day 4 to 14 of the estrus cycle, followed by a rapid decrease, indicating developed and regressing luteal tissues, respectively.

In previous studies (Perera *et al*., 1981; Jainudeen *et al*., 1983), following the first postpartum ovulation, the plasma progesterone level increased and remained above 0.70 ng/ml for about 10 days, and then declined to below 0.25 ng/ml at the next estrus. In agreement with the findings of this study, Kaur and Arora (1984) reported that the plasma progesterone level was low on the day of oestrus and then increased progressively, reaching a peak value between days 14 and 18, depending upon the estrous cycle length. Similarly, in Surti buffaloes, serum progesterone levels were higher ($p < 0.05$) at the diestrus phase, followed by the proestrus and estrus stages (2.78, 2.19 and 0.64 ng/ml) (Sarvaiya and Pathak, 1992). Kamonpatana (1982) found that progesterone levels declined on days 19-21 of the estrous cycle in swamp buffaloes.

In the present study, MPL showed two postpartum elevations. The first rise was noted at a postpartum interval of 34.3 days and the second rise at 64.0 days. In previous studies, based on rectal palpation, the interval from parturition to first ovulation was found to be 38 days in milked river buffaloes in India (Singh *et al*., 1979) and to be 69 days in suckled buffaloes in Egypt (El-Fouly *et al*., 1976).

4. Anestrus and Silent Ovulation

MPL remained consistently lower (< 0.1 ng/ml) in

anestrus than in cyclic buffaloes (0.1 to 30.7 ng/ml). Among the anestrus buffaloes, different progesterone patterns were recorded. Anestrus was associated with either consistently low or oscillating MPL. Fig. 3 shows the MPL of buffaloes number A005, A255, B113 and B300, which did not show estrus symptoms during the study period. Buffalo number A005 showed true anestrus, with neither estrus signs nor elevated milk progesterone concentrations. However, in the remaining four buffaloes, during the anestrous period, silent ovulations were also noted, reflected by increasing progesterone levels. Silent ovulations (SO) were indicated by SO. Interestingly, the incidence of silent ovulation in the buffaloes was higher in LBS than in NBS (70.6 vs 29.4%, respectively). Similarly, in a study on 17 complete postpartum periods in Murrah buffaloes in Sri Lanka, plasma progesterone concentrations remained basal (< 0.25 ng/ml) for a period ranging from 92-210 days (Perera *et al.*, 1984). In Swamp buffaloes (Perera, 1982), it was found that postpartum ane-strus was due to a failure in the resumption of ovarian cyclicity in the suckled buffaloes. Kaur and Arora (1984) concluded that malnutrition coupled with high environmental temperature stress was responsible for long anestrous periods in buffaloes.

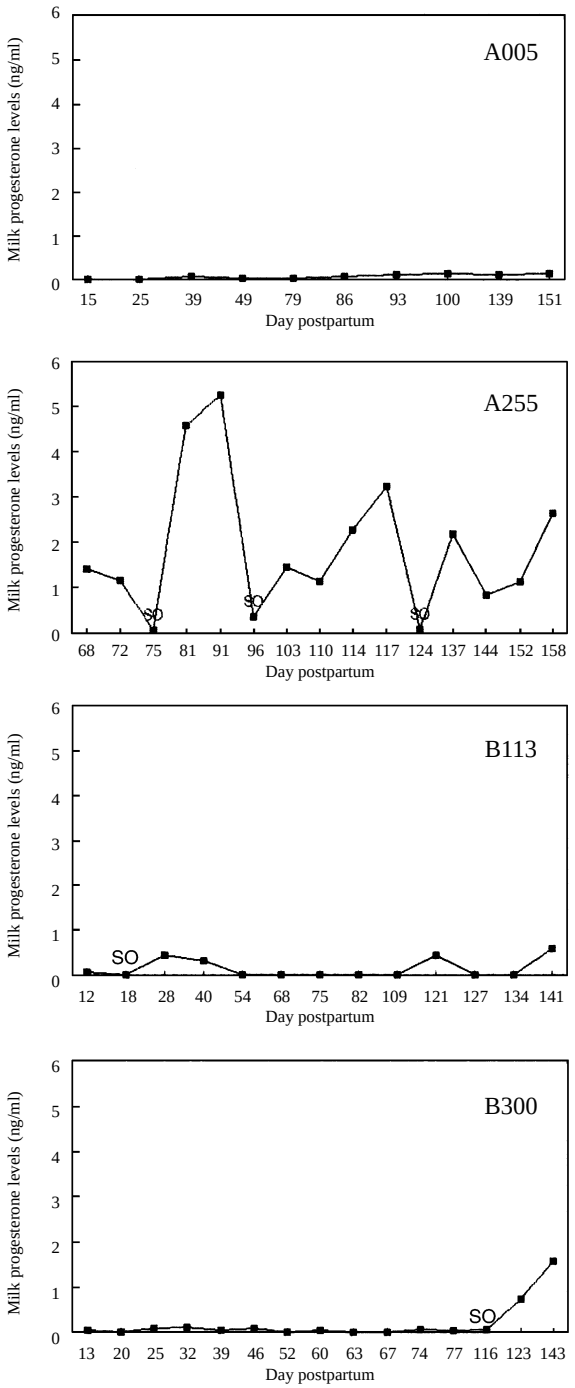

Fig. 3. Milk progesterone levels in buffaloes during anestrus (A005) and silent ovulation (SO) (A255, B113, B300).

Lamming and Darwash (1998) found that 10.94% of dairy cows showed delayed type I ovulation (P_4 levels < 3 ng/ml for > 45 days postpartum), and 12.85% showed type II (P_4 levels < 3 ng/ml for > 12 days between two luteal phases). 31.7% of the animals had at least one atypical ovarian pattern before insemination that contributed to delayed conception, a higher number of services per conception, and a lower first service conception rate.

In pregnant buffaloes, MPL remained > 1 ng/ml (1.1 to 30.69 ng/ml). In a previous study (McCool *et al.*, 1987), plasma progesterone levels were found to be > 1 ng/ml for pregnant, 0.4-4.0 ng/ml for cycling and < 1 ng/ml for anestrous Swamp buffaloes.

Results of the present study lead us to the conclusion that low postpartum reproductive performance in dairy buffaloes during LBS was primarily due to inadequate functioning of the corpus luteum in secreting optimum concentrations of progesterone. The higher incidence of silent estrus during LBS indicated improved management for the detection of estrus.

Acknowledgment

Financial sponsorship by the National Coordinated Buffalo Research Program/ARP-II/PARC, Islamabad, and the donation of Progesterone RIA kits by the Animal Production and Health Section, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA Vienna, Austria, are acknowledged.

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Milk Progesterone Profiles in Dairy Buffaloes

不同生殖季節乳用水牛乳中助孕酮含量之田間試驗

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摘　　要

從巴基斯坦Peshawar 地區之七個私人水牛場，選取泌乳末期最後2 個月之51 頭乳用水牛進行試驗。將正常配種季節 (NBS, 8 月至1 月) 及低配種季節 (LBS, 2 月至7 月)之觀測資料詳予記錄。牛隻在分娩後以直腸觸診法，檢查生殖器官之正常否，也利用公牛及人們觀察牛隻發情。而排卵之情形，亦以卵巢觸診法及利用放射免疫法以乳中助孕酮含量 (MPL) 偵測確定之。在NBS 及LBS 比較MPL 之含量，兩者差異極顯著 (1.97 ± 0.30 VS. 0.68 ± 0.08 ng/ml)。在LBS 組，MPL 維持在< 0.30 ng/ml ，直到6 週，此後隨即升高，達到12 週後之1.27 ng/ml 。在NBS 組第4 週之MPL 即快速上升，達到12 週後之3.64 ng/ml 濃度。而MPL 含量在不同之試驗農戶差異極顯著 ($p < 0.01$)。MPL 在發情當天之含量最低 (0.10 ng/ml) ，到第7 天最高，直到17 天才下降。MPL 顯示出2 個產後高峰期。在無發情之水牛，MPL 維持一定，但在不發情期，從無發情現象之MPL 增高，可看出靜默排卵現象。在有孕水牛，MPL 維持 > 1 ng/ml 。研究結果顯示，乳用水牛在LBS 季節，產後低生殖現象之表現，主要由於黃體功能不足，無法分泌出適當的助孕酮濃度。在LBS 期間，靜默發情之高發生率亦顯示飼養管理之改善有其必要性。