Association of milk yield and body condition score indices with the commencement of luteal activity after parturition in high producing dairy cows

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Summary

The aim of the present study was to investigate the relation between different indices of milk yield and body condition score (BCS) with the commencement of the luteal activity (C-LA) during the postpartum period in high producing dairy cows. Seventy-one multiparous healthy (free of detectable reproductive disorders) Holstein dairy cows (mean peak milk yield = 56.7 ± 7.4 kg) were used in the present study. Transrectal ultrasound scanning was performed twice weekly from the 1st to the 8th week postpartum. Blood samples were also collected twice weekly to measure serum progesterone (P_4) concentrations. The BCS was monitored weekly after calving. Cows with serum P_4 concentrations ≥1 ng/ml on at least 2 consecutive blood samplings were considered to have commenced luteal activity. The C-LA was observed in 51 out of 71 cows (71.8%) earlier than 45 days postpartum, while 20 out of 71 cows (28.2%) showed the C-LA later than 45 days postpartum. Among the indices defined for the milk yield pattern in the present study, difference in milk yield between the 1st week and the peak week and the peak of milk yield were significantly ($P \leq 0.05$) lower in cows that commenced their luteal activity earlier than 45 days postpartum. Cows losing ≥1 unit BCS at the 3rd week postpartum showed their C-LA significantly later than those cows losing less BCS during the same interval ($P \leq 0.05$). In conclusion, among the different indices defined for the milk yield and BCS in the present study, the higher milk yield at the peak and lower A-BCS (Area under the chart of BCS change) were the main factors associated with delayed commencement of luteal activity in clinically healthy high producing dairy cows.

Key words: Commencement of luteal activity, Body condition score, Milk yield, High producing dairy cows

Introduction

During the past decades, milk yield per cow has increased rapidly because of intense genetic selection and improved management and nutrition; however, fertility in high producing dairy cows has declined (Royal *et al.*, 2000; Butler, 2003; Ansari-Lari *et al.*, 2010). The effect of milk yield on fertility has been of considerable interest for bovine nutritionists and reproduction experts. Compared to the past fertility characteristics of dairy cows, today high producing dairy cows have longer intervals to first ovulation, a higher incidence of anestrus and abnormal luteal phases, lower blood progesterone (P_4) concentrations, higher multiple ovulation and twinning rates, and greater embryonic loss (Lucy, 2001). There is a link between increased milk yield and decline per year in conception rates to the first insemination after calving (Lucy, 2001). The rate of decline is reported to be around 0.5 and 1% per year in the USA (Beam and Butler, 1999) and UK (Royal *et al.*, 2000), respectively.

Potential factors such as increased milk yield, that are associated with negative energy balance (NEB), larger herd size, and higher inbreeding percentages have been suggested as reasons for declining fertility in dairy cows (Lucy, 2001). The NEB is considered a physiological phenomenon in high producing dairy cows in early lactation (Goff and Horst, 1997). Evidence suggests that selection for milk yield increases the gap between energy input and output during early lactation (Pryce *et al*., 2001). In high producing dairy cows, usage of fat reserves normally happens during early lactation for producing milk. Subcutaneous fat reserves carried by the dairy cow are clinically reflected by body condition scores (BCS) (Edmonson *et al*., 1989). The importance of the impact of the BCS on reproductive performance in dairy cattle has been reviewed recently in full detail (Roche *et al*., 2009). If the genetic correlation between BCS and fertility is large enough, then BCS could be useful in a selection index to improve fertility, either by restricting BCS to no genetic change (Jones *et al*., 1999) or by using BCS as an indirect selection criterion for fertility. Pryce *et al*. (2000) demonstrated that BCS (adjusted for stage of lactation) has a genetic correlation with a calving interval of -0.40. The relationship between calving interval and BCS was greatest when the BCS measurement was in early lactation (Pryce *et al*., 2000).

Darwash *et al*. (1997) suggested that P_4 profiles could be used to select for the commencement of luteal activity (C-LA), as early establishment of ovarian activity is important for fertility. However, the heritability estimated for C-LA which range from 0.16 to 0.28 (Veerkamp *et al*., 2001) are considerably higher than the heritability estimates for other reproductive traits. Patton *et al*. (2007) found no associations between milk yield and resumption of ovarian activity, but reported a higher NEB to be associated with later C-LA and noted that dry matter intake was the primary component of energy balance affecting reproduction.

Although research has been performed on the relationship of milk yield and BCS indices with C-LA in dairy cows, the detailed analysis of this subject has not been sufficiently studied in high producing dairy cows in postpartum. Therefore, mathematically defined indices were designed to characterize the relationship between the milk yield and BCS indices and the commencement of luteal activity in the postpartum period of clinically healthy high producing dairy cows.

Materials and Methods

Animals

The present study was conducted from February 2008 to May 2009 on 81 registered high producing Holstein cows at the Nemoneh farm of Astan e Ghods in Mashhad (latitude of 36° 20′ N and longitude 59° 35′ E, 980 m above sea level), northeast Iran. Throughout the year, cows were kept under roofed structures (free-stall barns) with open sides and washed sand for bedding and fed according to the NRC 2001. The ration (total mixed ration) included mainly alfalfa, corn silage, beet pulp, cotton seed, soybean, corn and barley. The cows under study were non-seasonal with year-round calves. The cows were machine-milked three times daily. The parity of the cows ranged from 3 to 7 years (mean, 3.9). The mean ± SD peak milk yield (9 weeks postpartum) of the cows was 56.7 ± 7.4 kg. Only healthy cows free of detectable reproductive disorders and free of clinical disease during the interval from the 1st to the 9th week after calving were used in the present study. Exclusion criteria were disorders including dystocia, retained placenta (fetal membranes retained longer than 24 h after calving), palpably detectable endometritis, metritis, mastitis, abnormal genital discharges and metabolic diseases such as clinical hypocalcaemia and ketosis.

Indices of milk yield

Milk yield was recorded weekly until the 9th week postpartum. The lactation curve was characterized by 6 indices on the basis of the weekly milk yield as follows:

a) 1st week milk yield;
b) Difference in milk yield between the 1st week and the peak week;
c) Peak week;
d) Ratio of increase in milk yield between the 1st week and the week of peak yield;
e) Peak milk yield (Kawashima *et al*., 2007);
f) Area under the curve of milk yield from 1st to 9th week postpartum (Fig. 1a).

According to the calculation of the area

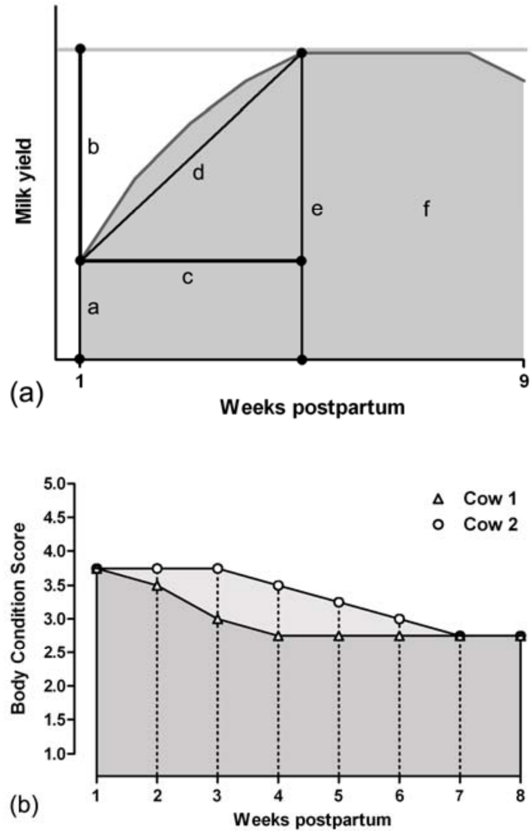

Fig. 1: a, The lactation curve was characterized by 6 indices on the basis of the weekly milk yield: a) 1st week milk yield, b) difference in milk yield between the 1st week and the peak week, c) peak week, d) ratio of increase in milk yield between 1st week and the week of peak yield, e) peak milk yield, and f) area under the curve of milk yield between 1st week and 9th week of postpartum. b, BCS chart of 2 dairy cows with the same loss of BCS (L-BCS), the area under the chart (A-BCS) (cow 1 = 306.25 cm^2, cow 2 = 341.25 cm^2) and variation of BCS (V-BCS) (cow 1 = 0.35, cow 2 = 0.43) are different. The A-BCS calculated by addition of the area of trapezoids and rectangles

of a trapezoid, the amount of area of milk yield (f) was calculated using the formula; where MY is the milk yield and the subscript number is the week postpartum:

$f = [(MY_1+MY_2) + (MY_2+MY_3) + \ldots + (MY_8+MY_9)]/2$

The linearity of index d was calculated as follows:

$d = (MYp - MYf)/PW$

In where,

MYf = the first week milk yield
MYp = the peak week milk yield
PW = the peak week

Indices of body condition score

BCS (scale 1-5 with 0.25 increments) of the cows was taken weekly from the 1st to the 8th week postpartum (Ferguson *et al.*, 1994). Indices of BCS were calculated as follows (Fig. 1b):

- Loss of BCS (L-BCS) of a cow was calculated by subtraction of BCS at the 1st week after calving from BCS at the 3rd, 7th or 8th week postpartum as follows; the subscript number is the week postpartum:

$L\text{-}BCS_8 = BCS_8 - BCS_1$

- Area under the chart of BCS (A-BCS) is the area of the region in the xy-plane bounded by the chart of BCS from the 1st to the 8th week postpartum of a cow. This reflects a combined amount of severity and duration of BCS loss. Amount of A-BCS was calculated using the following formula:

$A\text{-}BCS = [(BCS_1+BCS_2) + (BCS_2+BCS_3) + \ldots + (BCS_7+BCS_8)]/2$

- Variation of BCS (V-BCS) shows the amount of variance of BCS from the 1st to the 8th week postpartum of a cow calculated using the formula of standard deviation of BCS during this period (Petrie and Watson, 2006).
- The slope of the trend line of the chart of BCS (S-BCS) shows that of a straight line in the BCS chart, indicating the general pattern and direction of a time series data of BCS. The lines are drawn and their equation calculated by using statistical software (Microsoft Office Excel, Version 2007).

Reproductive examination

Transrectal ultrasound scanning was performed twice weekly (3 days apart) from the 1st to 8th week postpartum to scan the ovarian structures using a real-time B-mode ultrasound scanner equipped with a 5 MHz liner array transducer (500 V, ami, Canada). The visible structures on the ovaries were recorded. The ovarian stroma was echogenic, being represented by a mottled echotexture. The mature corpus luteum was easily distinguished from the ovarian stroma by its well-defined border and distinctive echotexture (Hanzen *et al.*, 2000). Transrectal palpation of the uterus and visual examination of the vagina was performed to detect the presence of any abnormal discharge.

Serum progesterone determination

Blood samples were collected twice weekly from all cows through the coccygeal vein, from the 1st to 8th week postpartum to measure serum P4 concentrations. The serum was separated by centrifugation (for 10 min at 3000 × g) and stored at -22°C until assayed. Serum P_4 concentrations were determined twice weekly using a validated commercial radioimmunoassay kit (Immunotech kit, France). The intra- and inter-assay coefficients of variation (CVs) of the assays were 5.8, and 9.0%, respectively. The sensitivity of the test was 0.05 ng/ml, and the recovery rate of the assay ranged from 85 to 110%.

Definitions

Cows with serum P_4 concentrations ≥1 ng/ml on at least 2 consecutive blood samplings were considered to have resumed luteal activity (Stevenson, 1997). Resumption of postpartum luteal activity was defined as regular ovarian cycles. Cows were classified into the following groups based on the commencement of luteal activity (C-LA) (Royal *et al*., 2002b; Garmo *et al*., 2009):

1) The C-LA occurring ≤45 days after calving.
2) The C-LA occurring >45 days after calving.

Statistical analysis

Ten cows were excluded from the analysis due to the occurrence of mastitis, metritis, lameness, and abomasal displacement during the study. Weekly changes in milk yield, and BCS in both C-LA groups from the 1st to 9th and 8th week postpartum, respectively, were statistically analyzed with ANOVA for repeated measures using the GLM procedure of SPSS (SPSS for Windows, Version 11.5, SPSS Inc, Chicago, Illinois). Differences between the average of the milk yield indices between the 1st to the 9th week postpartum, and BCS indices between the 1st to the 8th week postpartum between the 1st to the 7th week postpartum of the C-LA groups were analysed by independent t-test. The effects of L-BCS on the C-LA postpartum were further examined. The BCS at the 1st week after calving was classified in three categories (≥3.75, 3.00-3.50 and ≤2.75), and the L-BCS at different postpartum intervals in relation to that at the 1st week after calving, were also classified into three categories (no change to 0.25 decrease, 0.5 to 0.75 decrease and ≥1 decrease). The proportion of cows having C-LA earlier than 45 days among these categories was examined for differences using an χ^2-test. Similarly, the C-LA among the above L-BCS categories was examined using ANOVA (Duncan's multiple range test was used to locate differences). Values of P≤0.05 were considered statistically significant.

Results

The commencement of luteal activity

The results of the present study showed that 51 out of 71 (71.8%) cows commenced their luteal activity earlier than 45 days postpartum, while 20 out of 71 (28.2%) cows showed the C-LA later than 45 days. The mean ± SD parity of the cows between the two groups was not significantly different (3.88 ± 1.01 vs 4.05 ± 0.88, P>0.05).

Indices of milk yield and C-LA

The results of the repeated measures ANOVA showed that the increase in milk yield in cows with C-LA before 45 days postpartum was significantly (P=0.01) greater than that in cows with C-LA after 45 days postpartum. Difference in milk yield between the 1st week and the peak week (index b) was considerably higher (P=0.003) in cows that showed the C-LA later than those with earlier C-LA within 45 days postpartum (23.4 ± 6.8 vs 17.2 ± 7.3 kg). In addition, the peak milk yield (index e) was significantly higher (P=0.04) in cows that showed the C-LA later than those which showed the C-LA earlier than 45 days postpartum (59.7 ± 7.3 vs 55.6 ± 6.9 kg). There was no significant difference in the other indices of milk yield defined in the present study between the two groups.

Indices of body condition score and C-LA

The results of the repeated measures

ANOVA showed a significant difference (P=0.04, order 4) in declining BCS between cows with C-LA before and after 45 days postpartum during the period of the study. Further, a significant difference (P=0.05) was observed in declining BCS between the cows with the C-LA before and after 45 days postpartum from the 1st to the 3rd week postpartum (0.3 ± 0.2 vs 0.4 ± 0.4) and from the 1st to the 7th week postpartum (0.7 ± 0.4 vs 0.9 ± 0.3).

The A-BCS of the cows with the earlier C-LA was significantly higher (P=0.004) compared to the cows which showed the C-LA later than 45 days postpartum (163.9 ± 16.8 vs 149.6 ± 17.3 week × BCS). In the same period, the L-BCS of the cows with C-LA before and after 45 days postpartum was not significantly different (P=0.06; 0.66 ± 0.39 vs 0.85 ± 0.27).

A decrease in BCS of ≥1 unit either by the 3rd, 7th or 8th week after calving significantly increased the occurrence of delayed C-LA (P<0.05, Table 1). Calving to C-LA interval was significantly longer in cows that lost greater than 0.5 units BCS within 3 weeks postpartum compared to those that lost less than 0.5 units BCS during the same interval (38.67 ± 13.45 vs 28.79 ± 10.97 days, P=0.02). Also, calving to C-LA interval was longer in cows that lost greater than 0.5 units BCS within 8 weeks postpartum compared to those that lost less than 0.5 units BCS during the same interval (32.71 ± 12.20 vs 27.17 ± 10.60 days, P=0.05).

Discussion

Our study was purposefully carried out using clinically healthy postpartum cows under a well-managed dairy farm to enable us to exclude the detrimental effects of clinical diseases on the commencement of luteal activity. Therefore, the relations of the milk production level and the BCS indices associated with postpartum luteal activity could be investigated. The results of the present study showed that cows losing more

Table 1: Relationship between BCS, loss of BCS (L-BCS), and commencement of luteal activity (C-LA) in postpartum high producing dairy cows

	Number of cows			C-LA days (mean ± SD)
	C-LA >45 days (%)	C-LA ≤45 days (%)	Total	
BCS at 1st week of calving				
≥3.75	7 (16.7)	35 (83.3)	42	29.78 ± 11.30
3.00-3.50	11 (47.8)	12 (52.2)	23	29.95 ± 13.25
≤3	2 (33.4)	4 (66.6)	6	34.00 ± 10.84
L-BCS from 1st week to 3rd week after calving				
≤0.25 decrease	12 (26.1)	34 (73.9)	46	29.14 ± 11.52[a]
0.5-0.75 decrease	4 (19.1)	17 (80.9)	21	28.94 ± 9.36[a]
≥1 decrease	4 (100)	0 (0)	4	52.33 ± 6.35[b]
L-BCS from 1st week to 5th week after calving				
≤0.25 decrease	2 (13.4)	13 (86.6)	15	31.33 ± 11.17
0.5-0.75 decrease	13 (34.2)	25 (65.8)	38	28.12 ± 10.94
≥1 decrease	5 (27.8)	13 (72.2)	18	33.06 ± 13.56
L-BCS from 1st week to 7th week after calving				
≤0.25 decrease	1 (8.4)	11 (91.6)	12	29.92 ± 10.26
0.5-0.75 decrease	9 (27.3)	24 (72.7)	33	28.03 ± 11.42
≥1 decrease	10 (38.5)	16 (61.5)	26	33.52 ± 12.71
L-BCS from 1st week to 8th week after calving				
≤0.25 decrease	1 (9.1)	10 (90.9)	11	29.92 ± 10.26
0.5-0.75 decrease	9 (25.7)	26 (74.3)	35	28.45 ± 11.65
≥1 decrease	10 (40)	15 (60)	25	33.10 ± 12.66

Different superscript in columns indicate significant difference (P<0.05) than 0.5 units BCS within 3 and 8 weeks postpartum had delayed C-LA compared to the cows that lost less BCS during the same period. Body condition loss during the first and second months after calving was an important risk factor for the occurrence of delayed ovulation in lactating dairy cows (Opsomer *et al*., 2000; Shrestha *et al*., 2005). In addition, the results of the present study showed that cows with delayed C-LA had significantly lower BCS within 3 and 7 weeks after calving compared to those that showed C-LA earlier than 45 days. Consistent with our findings, Shrestha *et al*. (2005) showed that cows with a decrease in BCS of ≥1 unit at 3 and 7 weeks after calving had delayed C-LA. These relations were in accordance with reports showing that NEB during the first 3 to 4 weeks postpartum is correlated with the interval to first ovulation (Lucy *et al*., 1991; Beam and Butler, 1998). Decreasing BCS is a reflection of NEB in postpartum milking dairy cows. Another study showed that cows having delayed C-LA had greater NEB between the 1st and 2nd week postpartum compared with cows having early C-LA (Staples *et al*., 1990). On examining the changes in BCS after calving in both groups of C-LA, it was observed that cows losing ≥1 unit BCS by 7 weeks postpartum and later had a longer interval from calving to C-LA. Similar findings were recently reported by Shrestha *et al*. (2005). The occurrence of C-LA within 3 weeks postpartum was observed in 51 out of 71 (71.8%) cows in the present study, which is higher than what was reported by Darwash *et al*. (1997), Kawashima *et al*. (2006, 2007), and Smith and Wallace (1998). This difference could be attributed to inclusion of cows with postpartum diseases in their studies.

It has been demonstrated that the loss of the BCS and the increased severity and duration of the postpartum NEB adversely affect the reproductive performance in dairy cows (Shrestha *et al*., 2005; Patton *et al*., 2007; Wathes *et al*., 2007; van Straten *et al*., 2009). Measurement of the loss of the BCS (L-BCS) in a specified duration in postpartum cows, albeit a well accepted index to evaluate the energy balance status (Busato *et al*., 2002; Meikle *et al*., 2004), cannot show the depth and severity of the body fat loss during a selected period in postpartum dairy cows. Some cows lose the same amount of BCS with a different pattern in losing their body fat reservations (Fig. 1b). Therefore, measuring the area under the chart of the BCS (A-BCS) seems to represent both the depth and severity of the BCS loss. Using A-BCS, the effects of the NEB on the C-LA can be interpreted better than the L-BCS. Thus, it is proposed that using A-BCS and L-BCS together is more realistic for evaluation of the effect of losing body fat reservations on reproductive parameters.

Cows with a lower slope of the increasing milk yield commenced their luteal activity earlier than 45 days postpartum compared to cows with a higher slope of increasing milk yield in the present study. Similar to our findings, Kawashima *et al*. (2007) showed that the greater increasing ratio of milk yield during early lactation adversely affects the day of the occurrence of the first postpartum ovulation. Among the indices defined for the milk yield pattern in the present study, difference in milk yield between the 1st week and the peak week (index b) and the peak of milk yield (index e) were significantly lower in cows that commenced their luteal activity earlier than 45 days postpartum. Kawashima *et al*. (2007) similarly reported that the ratio of the increase in the milk yield between the 1st week postpartum and the week of peak yield (index b) was greater in cows with delayed ovulation. Royal *et al*. (2002a) showed that the additive genetic correlation between predicted peak milk yield (d 56) and the interval of C-LA is large (0.36) and unfavorable. To summarize, the results of the present study show that the peak of the milk yield, severity, and duration (the area under the chart of the BCS=A-BCS) of BCS loss are the main factors adversely affecting the commencement of luteal activity in clinically normal high producing dairy cows.

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