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Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows

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Abstract

The objectives of this study were to evaluate factors associated with resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Holstein cows, 6396 from four dairy farms were evaluated to determine the relationships among parity, body condition score (BCS) at calving and at AI, season of year when cows calved, and milk yield on resumption of postpartum estrous cycles by 65 days postpartum, and all the previous variables, estrual or anestrus and AI protocol on conception rates and embryonic survival at the first postpartum insemination. Cows had their estrous cycle pre-synchronized with two $PGF_{2\alpha}$ injections given 14 days apart and were inseminated between 69 and 82 days postpartum following either an estrous or ovulation synchronization protocol initiated 12–14 days after the presynchronization. Blood was sampled and analyzed for progesterone twice, 12–14 days apart, to determine whether cows had initiated onset of estrous cycles after calving. Cows were scored for body condition in the week after calving, and again at AI, between 69 and 82 days postpartum. Pregnancy was diagnosed at 30 ± 3 and 58 ± 3 days after AI. Farm influenced all reproductive outcomes evaluated. More ($P < 0.0001$) multiparous than primiparous cows had initiated estrous cycles. Onset of estrous cycles was also influenced ($P < 0.01$) by BCS at calving and at AI, BCS change, season, and milk yield. More ($P < 0.001$) cows that had initiated estrous cycles than anestrous cows were pregnant at 30 and 58 days after AI, but anestrus did not affect pregnancy loss. Conception rates were also influenced ($P < 0.01$) by parity, BCS at calving and AI, BCS change, and season; however, milk yield and insemination protocol were not associated with conception rates at 30 and 58 days after AI. Factors that reduced conception rate on day 30 after AI also increased pregnancy loss between 30 and 58 days of gestation. Improving BCS at calving and AI, minimizing losses of BCS after calving, and hastening onset of estrous cycles early postpartum are all expected to increase conception because of enhanced embryonic survival.

Keywords: Estrous cycles; Dairy cow; Embryo survival; Reproduction

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1. Introduction

Reproductive efficiency has a major impact on profitability of dairy farms. Recent estimates indicated that the average value of a pregnancy was US $278 in high-producing herds in the USA, whereas the cost of a pregnancy loss was substantially greater (De Vries, 2006). As reproductive efficiency improves, days in milk of a herd are typically reduced and a greater proportion of cows remain in the early stages of lactation, which increases average daily milk production. Furthermore, improvements in reproduction also influence the risk of a cow to be prematurely culled from the herd (De Vries, 2006). Improvement in reproductive performance of dairy cattle encompasses factors associated with resumption of ovarian function, detection of estrus, and establishment and maintenance of pregnancy.

Slow recovery of ovarian activity during the postpartum period is a major impediment to insemination of cows immediately after the end of the voluntary waiting period (Rhodes et al., 2003). Not only did lack of ovarian function reduce detection of estrus, but it also reduced conception rates (Gümen et al., 2003; Cerri et al., 2004; Galvão et al., 2004). Energy balance has been linked with a delay in resumption of postpartum ovulation in dairy cows (Butler, 2003), and cows that have not had ovulations in the first 4–8 weeks after calving are often classified as anestrual or anovular (Gümen et al., 2003). Also, it has been suggested that anestrual cows subjected to insemination following estrous synchronization or timed AI programs have increased late embryonic losses (Santos et al., 2004).

Embryonic mortality in cattle is a major impediment for adequate reproductive performance (Santos et al., 2004). In most high producing dairy herds, pregnancy to an insemination or conception rate ranged from 30 to 50% when diagnosis was performed between 28 and 50 days after AI (Tenhagen et al., 2001, 2004; Chebel et al., 2004), although fertilization rates in lactating dairy cows averaged 76% (Santos et al., 2004). These results have lead to estimates of losses of pregnancy from fertilization to calving summing up to 60% (Santos et al., 2004).

With the advent of ultrasonography, accurate pregnancy diagnosis has been possible as early as 25 days after AI in cattle, thereby facilitating the study of embryonic mortality after the period of maternal recognition of pregnancy. When embryo transfer was compared with timed AI, pregnancy at approximately 60 days for cows that had a synchronized ovulation did not differ between AI and embryo transfer, and averaged 31% (Sartori et al., 2006). These data suggest that factors other than fertilization and early embryonic development up to day 6 have major impact on pregnancy maintenance in lactating dairy cows. Evaluating factors associated with resumption of ovarian activity, conception rate and embryonic survival might improve our understanding of the poor reproductive efficiency in high producing dairy cows.

The objectives of the present study were to evaluate factors associated with resumption of postpartum estrous cycles and embryonic survival in high producing lactating Holstein cows.

2. Materials and methods

The Institutional Animal Care and Use Committee of the University of California Davis approved all procedures involving animals.

2.1. Animals and management

A total of 6396 early lactation Holstein dairy cows from four commercial dairy farms in California were evaluated during a period of 4 years for this study. Information on cow parity,

Table 1
Descriptive statistics by farm

	Farm			
	1	2	3	4
Cows in study, number				
Primiparous	329	413	1161	935
Multiparous	555	606	1388	1009
Milk yield (kg/d)				
Primiparous	33.9	35.4	31.6	36.3
Multiparous	47.2	48.2	45.8	51.3
Cyclic (%)				
Primiparous	77.5	45.5	70.4	78.4
Multiparous	74.6	67.8	85.1	85.0
Median BCS (1–5)				
Calving	3.25	3.25	3.25	3.25
AI	2.75	2.75	3.00	3.00
Timed AI (%)	67.8	63.2	70.5	68.0
Season of calving				
Summer	275	196	430	267
Fall	166	51	417	325
Winter	139	600	926	458
Spring	304	172	776	894

body condition score (BCS) in the week following calving and again at approximately 70 days postpartum, estrual or anestrual status, milk yield in the first 90 days postpartum, season of year when cows calved, method of insemination, and conception rates at 30 and 58 days after first postpartum AI were collected for every cow. Descriptive statistics according to farm are depicted in Table 1.

Cows were scored for body condition in a 1–5 scale (1 = emaciated, 5 = obese) in the first week after calving and again at AI, at approximately 70 days postpartum, as described by Ferguson et al. (1994). For purpose of analyses of the relationships between BCS and BCS changes on initiation of estrous cycles, conception rates, and pregnancy loss, cows were classified according to BCS at calving or AI as thin if BCS was equal to or less than 2.75, moderate if BCS was between 3.00 and 3.50, or overconditioned if BCS was equal to or greater than 3.75. Cows were also classified according to BCS change from calving to AI, as having excessive loss of BCS (≥1 unit), moderate loss (<1 unit of BCS) or no change and gain of BCS.

Yields of milk were recorded for the first 90 days postpartum for individual cows to determine the relationships between milk yield and time of initiation of estrous cycles following calving, conception rates, and pregnancy loss. This period corresponded to the time when cows were evaluated for time of initiation of estrous cycles and pregnancy. The mean yields of milk (± S.E.M.) for primiparous and multiparous cows in the first 90 days postpartum during the study were 48.0 ± 0.13 and 33.9 ± 0.11 kg/d.

2.2. Reproductive management

In all farms, stage of cows estrous cycle was pre-synchronized (Moreira et al., 2001) with two injections of 25 mg of $PGF_{2\alpha}$ (Lutalyse, 5 mg/mL dinoprost tromethamine sterile solution, Pfizer Animal Health, New York, NY), and either 12 or 14 days later cows were enrolled in an estrous or ovulation synchronization protocol. For both synchronizations, the initial portion of the protocols were similar and consisted of 100 μg injection of GnRH (Cystorelin, 50 μg/mL gonadorelin diacetate tetrahydrate, Merial Ltd., Iselin, NJ) followed 7 days later by an injection of 25 mg of $PGF_{2\alpha}$. Cows subjected to insemination upon detection of estrus did not receive any further treatment. These cows were observed for signs of estrus once daily, in the morning, by tail chalking using paintsticks (All-weather Paintstik, LA-CO Industries, Chicago, IL) and those observed in estrus based on rubbed chalk were inseminated in the same morning. In three farms, days postpartum when cows were inseminated at detected estrus ranged from 69 to 82, and on one farm it ranged from 66 to 79. Median and mean (± S.E.M.) days postpartum at AI for cows inseminated at estrus were 71 and 70.3 ± 0.1. Cows receiving timed AI received an injection of 100 μg of GnRH 48 h after $PGF_{2\alpha}$ and were timed AI 12 to 24 h later. In three farms, days postpartum when cows received timed AI ranged from 72 to 78, and in 1 farm it ranged from 69 to 75. The proportion of cows inseminated following timed AI was similar across farms (Table 1).

2.3. Blood sample collection and analyses

Cows were classified as having a functional CL or anestrual based on two blood samples collected 12–14 days apart for analysis of progesterone by ELISA as described previously (Cerri et al., 2004). Approximately 7 mL of blood was collected by puncture of the median coccygeal vein or artery using evacuated tubes (Vacutainer system, Becton; Dickinson and Company, Franklin Lakes, NJ) containing K_2 EDTA. The samples were immediately placed in ice, and later centrifuged at $2000 \times g$ for 15 min for separation of plasma. Plasma samples were frozen at −25 °C until later analysis.

Samples were collected during the synchronization program, at the time of the second $PGF_{2\alpha}$ injection of the presynchronization protocol and again, 12–14 days later, at the time of the initiation of the estrous or ovulation synchronization programs. In three farms, these samples were collected at 51 ± 3 and 65 ± 3 days postpartum, and in one farm, the samples were collected at 51 ± 3 and 63 ± 3 days postpartum. Cows were classified as having initiated estrous cycles if progesterone concentration was ≥1.0 ng/mL in one of the two samples, or anestrual, when both samples were <1.0 ng/mL.

2.4. Pregnancy diagnosis

All cows were examined for pregnancy by ultrasonography on day 30 ± 3 after AI. The detection of an embryonic vesicle with a viable embryo based on presence of heartbeat was used as an indicator of pregnancy. Cows diagnosed as pregnant were palpated per rectum for detection of an embryonic vesicle to confirm pregnancy and determine late embryonic loss 4 weeks later, on gestation day 58 ± 3. Conception rate was calculated as the proportion of inseminated cows pregnant either at 30 or 58 days after AI. Pregnancy loss was calculated using the number of pregnant cows on day 30 that were not pregnant on day 58 divided by the number of pregnant cows on day 30.

2.5. Statistical analysis

Information from individual cows from each farm was collated into a single data set for statistical analysis. Data were analyzed by a multivariate logistic regression using the LOGISTIC procedure of SAS (SAS version 9.1, SAS Inst. Inc., Cary, NC). A backward stepwise regression model was utilized, and explanatory variables were sequentially removed from the model by the Wald statistic criterion if $P > 0.15$. The effects of milk production on outcome variables of interest were analyzed either as continuous variables or categorized into quartiles for primiparous and multiparous. Because no difference in the results of statistical analyses was observed with either method of inclusion of milk yield, the categorized values were used to generate frequency tables.

For analysis of whether onset of estrous cycles had occurred by 65 days postpartum, the model included farm (1–4), parity (primiparous and multiparous), BCS at calving categorized, BCS at AI categorized, BCS change from calving to AI categorized, season of calving (summer, fall, winter and spring), and milk yield in the first 90 days postpartum categorized as quartiles within parity (primiparous and multiparous). For analyses of conception rates at 30 and 58 days after AI, and pregnancy loss between 30 and 58 days of gestation, the models included the same explanatory variables described for onset of postpartum ovulation, in addition to estrual or anestrual status, and insemination protocol (detection of estrus or timed AI). Adjusted odds ratio (AOR) and 95% confidence interval (CI) were generated during the logistic regression. Results are presented as proportions and AOR with respective 95% CI.

Regression analyses were performed to determine the best-fitted line plot between BCS at AI and the frequency of anovulation at 65 days postpartum using the regression procedure of MINITAB (MINITAB version 15.1, Minitab Inc., State College, PA). Sensitivity and specificity of detecting anestrual cows according to BCS at AI was calculated using the diagnostic test evaluation of Win Episcope 2.0.

Differences with $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ were designated as a tendency toward a difference for the explanatory variables evaluated.

3. Results

Farm influenced all reproductive outcomes evaluated, particularly because cows in farm 2 had the greatest prevalence of anovulation, the least conception rates, and the greatest pregnancy loss (Tables 2–5).

Overall, 75.9% of all cows had resumed estrous cyclicity by 65 days postpartum. Multiparous cows were more likely ($P < 0.0001$) to have initiated onset of estrous cycles than primiparous cows (Table 2). Both, a lesser BCS at calving and at AI were associated with decreased ($P < 0.001$) onset of estrous cycles by 65 days postpartum, although the effect seemed to be more pronounced in cows classified as thin at AI. A relationship between BCS at AI and prevalence of anestrual cows was observed (Fig. 1). As BCS at AI decreased, so did the proportion of estrual cows, although only 20.5% of all cows had BCS < 2.75, in which the prevalence of anovulation was greater than 30%. At AI, cows with BCS ≥ 3.75 were 2.4 times more likely ($P < 0.0001$) to have initiated onset of estrous cycles than cows with a BCS < 3.00. Similarly, cows that had a greater decrease in BCS from calving to AI were less likely ($P < 0.0001$) to have initiated onset of estrous cycles by 65 days postpartum than cows that had a lesser decrease in BCS. Cows calving in the summer and fall were more likely ($P < 0.0001$) to have initiated estrous cycles than cows in the winter and spring months. Finally, milk production in the first 90 days postpartum was associated with whether cows had initiated onset of estrous cycles, and those cows in the bottom quartile of

Table 2
Risk factors for resumption of estrous cycles by 65 days postpartum in lactating dairy cows

Variable	Estrual cyclic, % (number of cows)	Adjusted OR (95% CI)[a]	*P*-Value
Farm			
1	75.7 (669/884)	Referent	<0.0001
2	58.8 (599/1019)	0.55 (0.43, 0.69)	
3	78.4 (1998/2549)	1.04 (0.83, 1.23)	
4	81.4 (1591/1944)	1.28 (1.03, 1.57)	
Parity			
Multiparous	80.5 (2864/3558)	Referent	<0.0001
Primiparous	70.2 (1993/2838)	0.48 (0.42, 0.55)	
BCS at calving			
<3.00	74.2 (859/1158)	Referent	0.001
3.00–3.50	76.7 (3102/4042)	1.44 (1.19, 1.75)	
≥3.75	75.1 (896/1196)	1.49 (1.11, 2.00)	
BCS at AI			
<3.00	70.5 (1946/2761)	Referent	<0.0001
3.00–3.50	79.1 (2460/3109)	1.39 (1.17, 1.65)	
≥3.75	85.8 (451/526)	2.36 (1.52, 2.52)	
BCS change[b]			
Lost 1 unit or more	58.7 (279/475)	Referent	<0.0001
Lost < 1 unit	74.6 (2507/3361)	1.96 (1.52 2.52)	
No change	80.9 (2071/2560)	2.39 (1.74, 3.28)	
Season of calving			
Summer	84.1 (817/972)	Referent	<0.0001
Fall	82.9 (795/959)	0.83 (0.65, 1.07)	
Winter	68.8 (1595/2319)	0.54 (0.44, 0.67)	
Spring	76.9 (1650/2146)	0.65 (0.53, 0.80)	
Milk yield[c]			
Q1, 32.1 kg/d	72.7 (1011/1390)	Referent	0.002
Q2, 39.1 kg/d	77.6 (1204/1552)	1.34 (1.13, 1.60)	
Q3, 43.6 kg/d	77.6 (1350/1739)	1.36 (1.15, 1.62)	
Q4, 50.0 kg/d	75.3 (1292/1715)	1.21 (1.02, 1.43)	

[a] OR = odds ratio; CI = confidence interval.
[b] BCS change from calving to AI at approximately 70 days postpartum.
[c] Milk yield in the first 90 days postpartum classified as quartiles within parity; mean value for each quartile is indicated.

milk production, with a mean daily milk yield of 32.1 kg/d, were less likely ($P = 0.002$) to have resumed estrous cycles than cows in quartiles 2, 3, and 4, with mean daily milk yields of 39.1, 43.6 and 50.0 kg/d, respectively.

Using BCS at AI thresholds to detect anestrual cows resulted in lesser sensitivity and specificity. For the overall true prevalence of anovulation in the study cows of 24.1%, using BCS ≤ 2.75 at 70 days postpartum as the threshold resulted in sensitivity of 53.0% (95% CI = 50.4, 55.5), and specificity of 60.0% (95% CI = 58.5, 61.3). When the threshold was changed to BCS ≤ 2.50, it resulted in sensitivity of 82.0% (95% CI = 81.0, 83.1), and specificity of 28.3% (95% CI = 26.1, 30.6).

Conception rate on day 30 after first postpartum AI was 38.3%. Primiparous cows had greater ($P = 0.01$) conception rate than multiparous cows (Table 3). Cows that had onset of estrous cycles

Table 3
Risk factors for conception rate on day 30 after AI in lactating dairy cows

Variable	Pregnancy, % (number of cows)	Adjusted OR (95% CI)[a]	*P*-Value
Farm			
1	36.2 (298/824)	Referent	0.05
2	32.5 (331/1019)	0.80 (0.65, 1.00)	
3	40.2 (1005/2500)	1.01 (0.85, 1.20)	
4	39.9 (710/1781)	0.96 (0.80, 1.15)	
Parity			
Multiparous	36.1 (1244/3446)	Referent	0.01
Primiparous	41.1 (1100/2678)	1.15 (1.03, 1.29)	
Cyclic by 65 DIM[b]			
Anestrual	29.0 (419/1445)	Referent	<0.001
Estrual cyclic	41.1 (1925/4679)	1.67 (1.46, 1.91)	
Method of AI			
Timed AI	37.9 (1653/4362)	Referent	0.43
Estrus	39.2 (691/1762)	1.05 (0.93, 1.18)	
BCS at calving			
<3.00	34.9 (388/1112)	Referent	0.004
3.00–3.50	38.7 (1487/3841)	1.23 (1.06, 1.43)	
≥3.75	40.1 (469/1171)	1.38 (1.14, 1.68)	
BCS at AI			
<3.00	33.6 (897/2667)	Referent	0.005
3.00–3.50	41.1 (1210/2945)	1.20 (1.06, 1.35)	
≥3.75	46.3 (237/512)	1.32 (1.07, 1.63)	
BCS change[c]			
Lost 1 unit or more	28.0 (132/472)	Referent	<0.0001
Lost < 1 unit	37.3 (1204/3230)	1.42 (1.13, 1.79)	
No change	41.6 (1008/2422)	1.69 (1.32, 2.17)	
Season of calving			
Summer	34.7 (319/920)	Referent	0.002
Fall	35.2 (332/943)	1.00 (0.83, 1.22)	
Winter	38.7 (856/2212)	1.27 (1.07, 1.52)	
Spring	40.9 (837/2049)	1.28 (1.08, 1.51)	
Milk yield[d]			
Q1, 32.1 kg/d	37.2 (496/1334)	Referent	0.74
Q2, 39.1 kg/d	38.9 (576/1481)	1.06 (0.91, 1.24)	
Q3, 43.6 kg/d	39.3 (652/1661)	1.09 (0.93, 1.26)	
Q4, 50.0 kg/d	37.6 (620/1648)	1.03 (0.88, 1.21)	

[a] OR = odds ratio; CI = confidence interval.
[b] DIM = days in milk.
[c] BCS change from calving to AI at approximately 70 days postpartum.
[d] Milk yield in the first 90 days postpartum classified as quartiles within parity; mean value for each quartile is indicated.

by 65 days postpartum had greater ($P < 0.001$) conception rate on day 30 after first postpartum AI than anestrual cows. Of the 6396 cows evaluated, 4362 were inseminated following timed AI, and conception was similar ($P = 0.43$) for cows inseminated at a synchronized estrus or timed AI. BCS at calving influenced ($P = 0.004$) conception on day 30. Similarly, both BCS at AI and changes in BCS from calving to AI were associated with conception rates. As BCS at AI increased, risk

Table 4
Risk factors for conception rate on day 58 after AI in lactating dairy cows

Variable	Pregnancy, % (number of cows)	Adjusted OR (95% CI)[a]	*P*-Value
Farm			
1	32.0 (264/824)	Referent	0.002
2	25.8 (263/1019)	0.69 (0.55, 0.87)	
3	35.4 (882/2490)	0.97 (0.81, 1.15)	
4	34.6 (616/1781)	0.90 (0.75, 1.09)	
Parity			
Multiparous	29.7 (1021/3436)	Referent	<0.0001
Primiparous	37.5 (1004/2678)	1.31 (1.16, 1.47)	
Cyclic by 65 DIM[b]			
Anestrual	24.5 (353/1444)	Referent	<0.0001
Estrual cyclic	35.8 (1672/4670)	1.67 (1.45, 1.92)	
Method of AI			
Timed AI	32.4 (1411/4352)	Referent	0.13
Estrus	34.9 (614/1762)	1.10 (0.98, 1.24)	
BCS at calving			
<3.00	30.0 (334/1112)	Referent	0.001
3.00–3.50	33.1 (1267/3831)	1.20 (1.03, 1.40)	
≥3.75	36.2 (424/1171)	1.46 (1.20, 1.79)	
BCS at AI			
<3.00	28.0 (746/2661)	Referent	0.003
3.00–3.50	35.9 (1057/2941)	1.19 (1.05, 1.35)	
≥3.75	43.4 (222/512)	1.40 (1.13, 1.74)	
BCS change[c]			
Lost 1 unit or more	22.3 (105/472)	Referent	<0.0001
Lost < 1 unit	31.7 (1020/3220)	1.56 (1.21, 2.00)	
No change	37.2 (900/2422)	1.94 (1.49, 2.53)	
Season of calving			
Summer	30.3 (279/920)	Referent	0.008
Fall	29.8 (278/934)	0.97 (0.79, 1.19)	
Winter	33.7 (746/2212)	1.20 (1.01, 1.43)	
Spring	35.3 (722/2048)	1.26 (1.05, 1.51)	
Milk yield[d]			
Q1, 32.1 kg/d	32.4 (432/1333)	Referent	0.49
Q2, 39.1 kg/d	34.4 (509/1481)	1.09 (0.93, 1.28)	
Q3, 43.6 kg/d	34.1 (566/1658)	1.09 (0.93, 1.28)	
Q4, 50.0 kg/d	31.6 (518/1642)	1.00 (0.85, 1.18)	

[a] OR = odds ratio; CI = confidence interval.
[b] DIM = days in milk.
[c] BCS change from calving to AI at approximately 70 days postpartum.
[d] Milk yield in the first 90 days postpartum classified as quartiles within parity; mean value for each quartile is indicated.

of conception also increased ($P = 0.005$); however, as BCS loss in the first 70 days postpartum increased, risk of conception decreased ($P < 0.0001$), such that cows that had no change in BCS were 1.7 times more likely to conceive than cows that lost one or more units of body condition. Season of calving influenced ($P = 0.002$) risk of pregnancy, and cows inseminated during the summer and fall months were less likely to become pregnant than cows inseminated during the

Table 5
Risk factors for pregnancy loss between 30 and 58 days of gestation in lactating dairy cows

Variable	Pregnancy loss, % (number of cows)	Adjusted OR (95% CI)[a]	*P*-Value
Farm			
1	11.4 (34/298)	Referent	0.005
2	20.5 (68/331)	2.06 (1.30, 3.26)	
3	11.3 (113/998)	1.23 (0.81, 1.88)	
4	13.2 (94/710)	1.53 (0.99, 2.37)	
Parity			
Multiparous	17.2 (213/1237)	Referent	<0.0001
Primiparous	8.7 (96/1100)	0.52 (0.40, 0.68)	
Cyclic by 65 DIM[b]			
Anestrual	15.6 (65/418)	Referent	0.25
Estrual cyclic	12.7 (244/1919)	0.83 (0.61, 1.14)	
Method of AI			
Timed AI	14.1 (232/1646)	Referent	0.12
Estrus	11.1 (77/691)	0.78 (0.59, 1.06)	
BCS at calving			
<3.00	13.9 (54/388)	Referent	0.03
3.00–3.50	14.2 (210/1480)	0.98 (0.70, 1.38)	
≥3.75	9.6 (45/469)	0.59 (0.37, 0.96)	
BCS at AI			
<3.00	16.2 (145/893)	Referent	0.04
3.00–3.50	12.3 (149/1207)	0.85 (0.66, 1.10)	
≥3.75	6.3 (15/237)	0.48 (0.27, 0.85)	
BCS change[c]			
Lost 1 unit or more	20.5 (27/132)	Referent	0.002
Lost < 1 unit	14.5 (174/1197)	0.52 (0.31, 0.86)	
No change	10.7 (108/1008)	0.38 (0.22, 0.66)	
Season of calving			
Summer	12.5 (40/319)	Referent	0.51
Fall	13.8 (45/326)	1.07 (0.67, 1.71)	
Winter	12.9 (110/856)	0.97 (0.63, 1.50)	
Spring	13.6 (114/836)	1.22 (0.81, 1.82)	
Milk yield[d]			
Q1, 32.1 kg/d	12.7 (63/495)	Referent	0.57
Q2, 39.1 kg/d	11.6 (67/576)	0.86 (0.59, 1.25)	
Q3, 43.6 kg/d	12.8 (83/650)	0.93 (0.65, 1.34)	
Q4, 50.0 kg/d	15.6 (96/616)	1.09 (0.76, 1.56)	

[a] OR = odds ratio; 95% CI = confidence interval.
[b] DIM = days in milk.
[c] BCS change from calving to AI at approximately 70 days postpartum.
[d] Milk yield in the first 90 days postpartum classified as quartiles within parity; mean value for each quartile is indicated.

winter and spring months. When milk yield in the first 90 days of lactation was evaluated for relationships with conception, cows in the greater quartiles for milk yield had similar risk of pregnancy as cows within the lesser quartiles for this variable.

Because of late embryonic and early fetal losses, overall conception rate decreased from 38.3% on day 30 to 33.1% on day 58. Conception rate on day 58 was not influenced by method of AI and

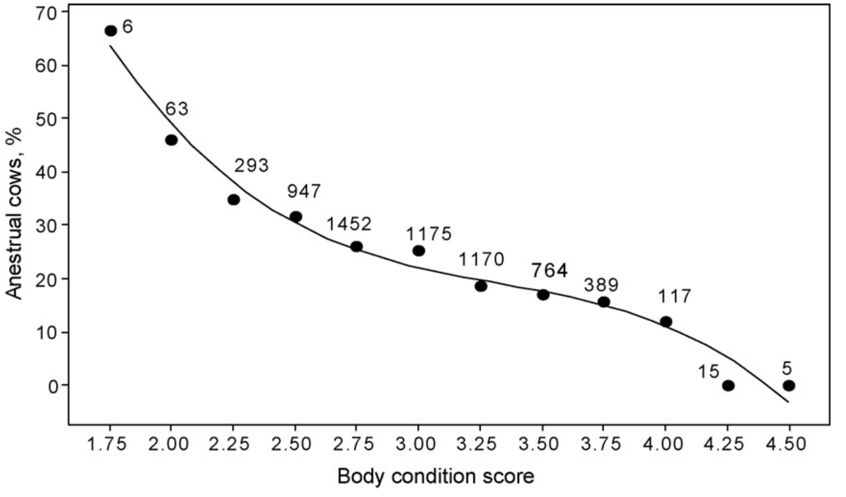

Fig. 1. Relationship between body condition score (BCS) at 70 days postpartum and proportion of anestrual cows at 65 days in milk. Values indicate the number of cows in each category of BCS. A cubic relationship was observed ($P < 0.001$): Anestrual cows; $\% = 342.3 - 274.3\,\text{BCS} + 79.79\,\text{BCS}^2 - 7.975\,\text{BCS}^3$, $r^2 = 0.97$.

milk yield (Table 4). Similar to conception on day 30 after AI, parity, estrual or anestrual status, BCS at calving and AI, and BCS change from calving to AI, season of calving were all associated with risk of pregnancy. Cows that had initiated estrous cycles had greater ($P < 0.0001$) conception rate than anestrual cows. As BCS at calving and AI increased, so did conception rate ($P < 0.0001$), as cows lost more BCS from calving to AI, the risk of conception decreased ($P = 0.001$), and cows inseminated during the summer and fall months were less likely ($P = 0.008$) to become pregnant than cows inseminated during winter and spring.

A total of 2337 cows were pregnant on day 30 after AI and, of those, 309 lost their pregnancy (13.2%). Primiparous cows were less likely ($P < 0.001$) to lose a pregnancy than multiparous cows (Table 5). Estrual status, method of AI, season of calving, and milk yield were not ($P > 0.10$) associated with risk of pregnancy loss; however, cows calving with greater BCS, those with greater BCS at AI, and those that had either no change or lost <1 unit of BCS from calving to AI all had a reduced ($P < 0.05$) risk of pregnancy loss.

4. Discussion

Reproductive performance of dairy cows is influenced by ability to resume estrous cycles early postpartum (Butler, 2003) and, after AI, to maintain pregnancy (Santos et al., 2004). Delayed postpartum ovulation is associated with more pronounced negative energy balance (Butler, 2003), typically induced by inadequate dry matter intake (Villa-Godoy et al., 1988). Although energy status partially determines when cows first ovulate postpartum, it is likely that other factors might also be involved. More primiparous than multiparous cows were anestrous in the first 60–70 days postpartum (Moreira et al., 2001; Gümen et al., 2003; Rhodes et al., 2003; Cerri et al., 2004), which was also observed in the current study. Primiparous cows were 52% less likely (adjusted OR = 0.48) to have initiated estrous cycles by 65 days postpartum than multiparous cows. Energy balance of cows during the last 2 weeks of gestation was lesser in primigravid than multiparous cows (Moore et al., 2000), and lactations 1 and 4 cows had more pronounced negative energy balance early postpartum than cows of lactation 2 and 3 (Heuer et al., 2000).

Primiparous cows have greater concentrations of blood non-esterified fatty acids than multiparous cows 1 week before parturition (Meikle et al., 2004; Wathes et al., 2007), and early postpartum (Wathes et al., 2007), and these differences have been associated with longer calving to conception interval (Meikle et al., 2004). Elevated concentrations of non-esterified fatty acids and ketones before parturition have been related with reduced periparturient immunological function and uterine disease (Hammon et al., 2006), which are known to influence postpartum ovulation (Sheldon et al., 2002). Additionally, primiparous cows typically have more early postpartum uterine problems (Goshen and Shpigel, 2006), which can delay resumption of ovulation. Cows diagnosed with uterine infection had dominant ovarian follicles that grew at a slower rate than those without uterine problems (Sheldon et al., 2002). Thus, it is possible that primiparous might be more sensitive to metabolic and endocrine signals during the periparturient period such as those influenced by the nutrient balance, and also by alterations in uterine health, thereby delaying the resumption of postpartum ovulation.

Long-term selection for milk yield has been negatively associated with the ability of lactating dairy cows to conceive and maintain a pregnancy to term. Greater milk yields may delay the resumption of ovarian function in high yielding dairy cows in part because of increased catabolic state (Butler, 2003). Metabolic alterations such as a decline in plasma leptin to a nadir immediately after the parturition (Kadokawa et al., 2000; Liefers et al., 2003), associated with simultaneous decline in plasma insulin and IGF-I and an increase in growth hormone concentrations (Block et al., 2003) have been related with the severity of the negative nutrient balance and with a delay in the postpartum luteal activity in dairy cows (Kadokawa et al., 2000), possibly because these endocrine cues influence LH pulses (Butler, 2003).

The growth hormone/IGF system in the hepatic tissue of early lactation cows in negative energy balance is uncoupled, which results in lesser concentration of IGF-I. Increased insulin and IGF-I concentrations were associated with more estrogenic follicles (Butler et al., 2004), from which ovulations were more likely to occur early postpartum (Butler, 2003). Because insulin appears to be a metabolic signal re-coupling the growth hormone-IGF system (Butler et al., 2003), reductions in energy status early postpartum may impair ovulation by reducing blood insulin and IGF-I concentrations. Although energy balance was not measured in this study, as BCS at AI increased, so did the proportion of estrual cows. Furthermore, a greater loss in BCS from calving to AI markedly reduced the proportion of cows that had initiated estrous cycles. These data clearly demonstrate that changes in energy status as evidenced by body reserves are important factors regulating when cows resume ovulation after calving.

Of the 1539-anestrual cows, 815 (53%) had a BCS at AI $\leq$ 2.75, and the other 47% had a BCS between 3.00 and 4.50 (Fig. 1). Although BCS is not an absolute indicator of resumption of estrous cycles in dairy cows, these data indicate that risk of anovulation is greater in those cows with BCS $\leq$ 2.75 at 70 days postpartum, approximately 30%, and they represent more than half of all anestrual cows in these herds. In spite of that, these data also indicate that using BCS $\leq$ 2.75 at 70 days postpartum to detect anestrual cows, when the overall prevalence of anovulation in the herd is 24.1% would result in poor sensitivity and specificity, 53.0 and 60.0%, respectively.

Cows classified within the bottom quartile of milk production were less likely to have initiated estrous cycles than those belonging to quartiles with greater milk yields. Increased milk yield could delay onset of estrous cycles if associated with exacerbated negative nutrient balance. However, milk yield is closely associated with dry matter intake (Liefers et al., 2003), and energy intake accounted for most of the variation in energy balance in postpartum cows, whereas milk yield only accounted for 20–25% of this variation (Villa-Godoy et al., 1988). Therefore, earlier resumption of postpartum ovulation in cows with greater milk yields may be explained by greater production associated with greater dry matter intake, which results in a more desirable energy status in early lactation. It is also possible that cows with lesser production were those having postpartum health problems (Goshen and Shpigel, 2006), which not only could reduce lactation performance, but also delayed postpartum ovulation.

Although cattle are not seasonal breeders like other ruminants, season influenced resumption of estrous cyclicity in the current study. Walsh et al. (2007) evaluated cows from 18 herds and observed that those calving in the spring and winter had the greatest risk to be in anestrus. Opsomer et al. (2000) also found that cows calving in spring had delayed resumption of postpartum ovulation. In systems in which grazing is the basis for nutrition of cows, season has a marked effect on resumption of estrous cycles in dairy cows (Rhodes et al., 2003). It can be postulated that alterations in photoperiodic stimulation (Dahl et al., 2000) and nutritional changes (Rhodes et al., 2003) associated with specific times of the year are potential explanation for the effect of season or risk of delayed onset of estrous cycles in lactating dairy cows.

In spite of primiparous cows being less likely to initiate estrous cycles by 65 days postpartum, their conception rates at 30 and 58 days after AI were greater than those of multiparous cows. The improved conception rate 58 days after AI for primiparous compared with multiparous cows was in part because of lesser pregnancy loss. Others have also found greater conception rates for primiparous than multiparous cows, and these responses were observed regardless of method of estrous or ovulation synchronization (Tenhagen et al., 2001, 2004).

Rhodes et al. (2003) indicated that between 11 and 38% of the cows in year-round calving production systems and 13–43% of the cows in pasture-based systems were anestrous before the beginning of insemination postpartum. Even when cows initiated onset of estrous cycles following a period of anovulation or anestrus, conception was compromised (Gümen et al., 2003; Cerri et al., 2004; Galvão et al., 2004; Rhodes et al., 2003), which also extended the interval to pregnancy (Walsh et al., 2007). Anestrual cows (24.1%) in the current study had lesser conception rates at 30 and 58 days after AI, but similar pregnancy loss to estrual cows. Generally, late embryonic losses were numerically greater for anestrual than estrual cows (Santos et al., 2004). Data from the current study indicated a numerical increase in pregnancy loss for anestrual compared with estrual cows. Galvão et al. (2004) observed that anestrual cows had increased risk of pregnancy loss. Anovulation is associated with reduced concentrations of estradiol (Butler, 2003), and both lack of adequate progesterone in the preceding estrous cycle to AI and lesser concentrations of estradiol during proestrus might increase incidence of estrous cycles of less than typical length as a result of premature luteal regression (Mann and Lamming, 2000; Shaham-Albalancy et al., 2001). Also, anestrual cows subjected to synchronization protocols had reduced behavioral estrous expression (Galvão et al., 2004), which compromised ovulation and conception rate. Therefore, re-establishment of postpartum ovulation prior to first AI is critical for optimum establishment and maintenance of pregnancy.

Generally, risks of conception and pregnancy loss are similar in cows inseminated following synchronization of estrus or ovulation or following detection of spontaneous estrus (Chebel et al., 2004; Santos et al., 2004). Others also observed similar (Tenhagen et al., 2001; Gümen et al., 2003), or even greater (Cerri et al., 2004) conception rates when cows were received timed AI compared with AI following a synchronized estrus. Therefore, properly implemented synchronization programs for fixed time AI results in similar fertility in high producing dairy cows when compared with insemination following detection of a synchronized estrus.

Milk yield has not been associated with conception or embryonic survival (Chebel et al., 2004; Tenhagen et al., 2001). In the current study, conception rates at 30 and 58 days after AI, and pregnancy loss in the first 58 days of gestation were all similar for cows producing an average of 32–50 kg/d in the first 90 days postpartum.

The BCS influences establishment and maintenance of pregnancy in lactating dairy cattle (Domecq et al., 1997; López-Gatius et al., 2002). Changes in BCS early postpartum were important indicators of the risk of conception at first AI in dairy cows (Domecq et al., 1997). Cows having excessive loss of BCS in early lactation had prolonged interval to first AI and were less likely to conceive (Domecq et al., 1997). López-Gatius et al. (2002) indicated that a 1 unit decline in BCS, using a 1–5 scale, from calving to 30 days postpartum increased 2.4-fold the risk of pregnancy loss. Data from the current study corroborate with these findings, and taken together they suggest that both the BCS and the change in BCS from calving to AI are important indicators of establishment and maintenance of pregnancy in high-producing dairy cows.

5. Conclusion

The current study characterized the frequency of onset of estrous cycles in primiparous and multiparous dairy cows in the first 65 days postpartum in four high-producing dairy herds, and attempted to identify factors associated with resumption of postpartum ovulation and maintenance of pregnancy in lactating dairy cows. Parity, BCS at calving and AI, BCS change from calving to AI, season of calving and milk production were all associated with time of onset of postpartum estrous cycles. Similarly, parity, estrual or anestrual status, BCS and BCS changes, and season were also associated with conception rates at 30 and 58 days after first postpartum AI. Amount of milk produced in the first 90 days postpartum and insemination of dairy cows following either timed AI or synchronized estrus, however, did not influence the risk of conception and pregnancy loss. Improvements in fertility of high producing lactating dairy cows should be achieved when nutrition and health programs improve energy status early postpartum, thereby minimizing the loss of BCS early in lactation, which is expected to result in faster resumption postpartum ovulation and increased conception rate and maintenance of pregnancy.

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