

A Retrospective Study on the Risk Factors and the Effect of Higher Somatic Cell Count in Milk on Reproductive Performance in Dairy Cows

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Abstract: This retrospective study evaluated the effect of somatic cell count (SCC) in milk during early lactation on reproductive performance in dairy cows. Data were collected on 774 cows from six dairy farms, including cow parity, dates of previous calving, artificial insemination, pregnancy diagnosis, incidence of postpartum endometritis, reproductive performance (the intervals from calving to first insemination and conception), milk production and SCC. Data on 774 lactations were grouped based on the average first 3 months postpartum linear somatic cell score (SCS) as T1 (<3.0, n = 521), T2 (3.0 ≤ and <4.0, n = 113), and T3 (≥4.0, n = 140) groups. The odds ratio (OR) for the probability of endometritis increased 1.6 ($p < 0.05$) and 3.2 times ($p < 0.0001$) in the T2 and T3 groups, respectively, compared with that in the T1 group. The hazard of first insemination by 150 days in milk (DIM) was lower in the T3 group (hazard ratio [HR]: 0.76, $p < 0.01$) than in the T1 group. First insemination conception rate did not differ among the 3 groups (28.7-34.2%, $p > 0.05$). The hazard of pregnancy by 365 DIM in the T3 group was lower (HR: 0.75, $p < 0.05$ respectively) than in the T1 and T2 groups. The SCS during 4 to 7 months postpartum differed ($p < 0.0001$) among the 3 groups. Farm and cow parity were important risk factors for higher SCS (≥4.0). Multiparous cows were more likely to have a higher SCS (OR: 2.26, $p = 0.0005$) compared with primiparous cows. In conclusion, higher SCS (≥4.0) during early lactation was associated with decreased reproductive performance of dairy cows.

Key words: dairy cows, somatic cell score, endometritis, reproductive performance.

Introduction

Udder health is very important in dairy production, because occurrence of mastitis significantly decreases milk production, alters milk composition, and requires treatment, resulting in severe economic losses (4,14,19). Moreover, mastitis has been implicated in decreasing reproduction (25). Mastitis caused by gram-negative pathogens in the late puerperal phase, delayed the first postpartum ovulation and estrus (11). Another study demonstrated that a negative correlation between mastitis and reproduction was due to altered interestrus intervals and decreased length of the luteal phase caused by gram-negative pathogens (15). Furthermore, clinical mastitis resulted in prolonged intervals from calving to first service and conception, increased services per conception, and a higher risk of abortion (2,15,23).

Somatic cells in milk consist of several cell types, including neutrophils, macrophages, lymphocytes, and a smaller percentage of epithelial cells (29). Thus somatic cell count (SCC) has been used as an index of milk quality, as well as an indirect measure of mammary infection status (7,16,27). SCC of healthy quarters is usually below 100,000 cells/ml, whereas SCC 200,000 cells/ml is considered as subclinical mastitis (10,17,26). High linear SCC during early lactation had a significant effect on reproductive performance in Chilean dairy cattle (18). Another study reported that increased SCC

extended the intervals from calving to first service and conceptions by 1.3 to 2.0 days, respectively for each unit increase in somatic cell score (SCS) (20). On the other hand, Klass *et al.* (13) reported that subclinical and clinical mastitis did not increase days open, while subclinical mastitis during early lactation prolonged the interval to first insemination. Moreover, the impact of SCC on reproductive performance has not been determined in Korean dairy herds.

It is assumed that increased SCC in early lactation might reflect hygiene control at the farm- and cow-level and SCC during the period (e.g., the first postpartum 90 days) could be used as a predictor of reproductive performance in dairy herds. Therefore, the present study evaluated the effect of average SCS during the first 3 months postpartum on reproductive performance in dairy cows.

Materials and Methods

Animals and management

This study was conducted on 6 Holstein dairy farms (A-F) located in Chungcheong Province. Cows were fed a total mixed ration and milked twice daily. They received regular reproductive health checks every 2 to 4 weeks, by veterinarians at the College of Veterinary Medicine, Chungbuk University. These included examination of ovarian structures and the uterus *via* transrectal palpation and ultrasonography. In addition to estrous detection, a herd reproductive management program was employed; estrus synchronization using PGF_{2α} and Ovsynch, which combined GnRH on day 0-PGF_{2α} on

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day 7-GnRH on day 9, with or without an internal drug-release device containing 1.9 g of progesterone insertion between days 0 and 7. Cows that exhibited estrus naturally or after estrus synchronization using PGF_{2α}, were inseminated according to the am-pm rule, whereas cows treated with Ovsynch received timed artificial insemination (AI). Pregnancy was diagnosed rectally 40-50 days after AI using both ultrasonography and manual palpation.

Data collection and study design

Data were collected on 774 cows (247 primiparous and 527 multiparous; mean [\pm SEM] parity = 2.6 ± 0.1) from 6 dairy farms. The data included detailed information on milk production and SCC, postpartum endometritis, and reproduction. Milk yield and SCC were collected monthly from the Korean Animal Improvement Association. In addition, cow parity, and dates of previous calving, insemination and conception were also included in the data set. The SCC values of individual cows were not normally distributed; therefore, values underwent logarithmic transformation for data analysis using a simple formula (e.g., $SCS = \log_2 [SCC/100,000] + 3$) (24). Lactation data were grouped based on the average linear SCS from the first 3 months postpartum as T1 (< 3.0 , $n = 521$), T2 ($3.0 \leq$ and < 4.0 , $n = 113$), and T3 (≥ 4.0 , $n = 140$) groups. The categories of the SCS < 3.0 , $3.0 \leq$ and < 4.0 , and ≥ 4.0 corresponded with the ranges of SCC values of $< 100,000$, $100,000 \leq$ and $< 200,000$, and $\geq 200,000$ cells/ml, respectively. The present study evaluated the effect of SCS on postpartum endometritis and reproductive performance (e.g., hazard of first insemination by 150 DIM, first insemination conception rate, and hazard of pregnancy by 365 DIM). Furthermore, the risk factors for higher SCS in milk (≥ 4.0) during the first 3 months postpartum were also determined.

Statistical analysis

Milk yield was categorized into $\geq 10,000$ vs. $< 10,000$ kg based on the 305-day milk yield. The calving seasons were grouped into spring (March to May), summer (June to August), autumn (September to November), and winter (December to February), while cow parity was grouped as primiparous or multiparous.

Occurrence rate of postpartum endometritis, first insemination conception rate, and risk factors for higher SCS in milk (≥ 4.0), during the first 3 months postpartum, were analyzed by logistic regression using the LOGISTIC procedure in SAS (version 9.2, SAS Institute Inc., Cary, NC, USA). The logistic regression model for the occurrence of endometritis and first insemination conception rate included farm, milk yield, calving season, cow parity, and group. Risk factors for higher SCS were identified by a model that included farm, milk yield, calving season, and cow parity. Backward stepwise regression was used in all models, and elimination was performed based on the Wald statistic criterion when $p > 0.11$. The odds ratio (OR) and 95% confidence interval (CI) were produced by logistic regression. Results were presented as proportions and OR with their respective 95% CIs.

Cox's proportional hazard model with the PHREG procedure in SAS was used for estimating hazard of first insemination by 150 DIM and the hazard of pregnancy by 365 DIM,

among groups. The hazard estimate of a cow being inseminated or pregnant at a given time, was thus obtained. The time variables used in the model were the interval in days between calving and first insemination, and the interval in days between calving and pregnancy. Cows that were sold, died or remained un-inseminated by 150 DIM or non-pregnant at 365 DIM were censored. The Cox models included farm, group, calving season, and cow parity. The proportional hazard rate was evaluated based on the interactions between explanatory variables and time, and by Kaplan-Meier curves. The median and mean days to first insemination or pregnancy were determined by survival analysis from the Kaplan-Meier model, using the LIFETEST procedure in SAS. A survival plot was generated using the survival module in MedCalc (version 13.2.2 for Windows, MedCalc Software, Mariakerke, Belgium).

Changes of SCS in milk during 4-7 months postpartum were compared among the 3 groups using repeated measures of ANOVA. If there were interactions between variables, one-way ANOVA was performed to evaluate significant differences within groups. Differences with $p \leq 0.05$ were considered significant, and $0.05 < p \leq 0.1$ were designated as a tendency toward a significant difference.

Results

Mean (\pm SEM) SCC values were $51,670 \pm 1,570$, $194,650 \pm 15,300$, and $1,178,610 \pm 84,560$ cells/ml for the T1, T2, and T3 groups, respectively. The incidence rate of postpartum endometritis was 26.7% (61/236), 35.4% (134/339), and 51.7% (88/216) in T1, T2, and T3 groups, respectively. Logistic analysis revealed that farm, group, and milk yield affected the OR for the probability of endometritis (Table 1). The OR increased 1.6 ($p < 0.05$) and 3.2 times ($p < 0.0001$) in the T2 and T3 groups, respectively, compared with that in the T1 group. Moreover, the OR for the probability of endometritis increased 2.1 times ($p < 0.01$) in the T3 group compared with that in the T2 group. The OR (0.60) was lower for cows with

Table 1. Logistic regression model of factors contributing to postpartum endometritis

Variable (n)	OR	95% CI	p-value
Farm			
A (203)	Reference		
B (309)	2.68	1.064-6.734	< 0.05
C (115)	5.62	2.284-13.831	< 0.0005
D (54)	1.25	0.462-3.375	> 0.05
E (46)	1.24	0.397-3.892	> 0.05
F (47)	0.71	0.205-2.444	> 0.05
Group			
T1 (521)	Reference		
T2 (113)	1.64	1.046-2.580	< 0.05
T3 (140)	3.16	2.146-4.666	< 0.0001
Milk yield*			
$< 10,000$ (309)	Reference		
$\geq 10,000$ (465)	0.60	0.426-0.828	< 0.005

*305-day milk yield (kg).

Table 2. Factors affecting the hazard of first insemination by 150 DIM analyzed by PHREG procedure

Variable (n)	HR	95% CI	p-value
Farm			
A (203)	Reference		
B (309)	0.65	0.469-0.908	< 0.05
C (115)	1.11	0.807-1.525	> 0.05
D (54)	1.35	0.945-1.914	0.1
E (46)	1.22	0.820-1.827	> 0.05
F (47)	1.27	0.838-1.927	> 0.05
Group			
T1 (521)	Reference		
T2 (113)	1.00	0.814-1.238	> 0.05
T3 (140)	0.76	0.620-0.926	< 0.01
Calving season			
Spring (188)	Reference		
Summer (237)	1.19	0.968-1.457	< 0.1
Autumn (171)	1.61	1.295-2.007	< 0.0001
Winter (178)	1.09	0.879-1.355	> 0.05
Cow parity			
Primiparous (247)	Reference		
Multiparous (527)	0.81	0.689-0.947	< 0.01

Table 3. Factors affecting the hazard of pregnancy by 365 DIM analyzed by PHREG procedure

Variable (n)	HR	95% CI	p-value
Farm			
A (203)	Reference		
B (309)	0.80	0.571-1.133	> 0.05
C (115)	1.00	0.719-1.388	> 0.05
D (54)	1.41	0.984-2.023	< 0.1
E (46)	1.39	0.922-2.106	> 0.05
F (47)	2.11	1.376-3.223	< 0.001
Group			
T1 (521)	Reference		
T2 (113)	1.00	0.801-1.249	> 0.05
T3 (140)	0.77	0.623-0.959	< 0.05
Calving season			
Spring (188)	Reference		
Summer (237)	1.20	0.972-1.491	< 0.1
Autumn (171)	1.40	1.112-1.757	< 0.005
Winter (178)	1.20	0.958-1.511	> 0.05
Cow parity			
Primiparous (247)	Reference		
Multiparous (527)	0.83	0.702-0.977	< 0.05

milk yield $\geq 10,000$ kg compared with those with milk yield $< 10,000$ kg ($p < 0.005$).

Table 2 shows factors affecting the hazard of first insemination by 150 DIM analyzed by PHREG procedure. Farm, group, calving season, and cow parity affected the hazard. The hazard of first insemination by 150 DIM was lower in T3 group (hazard ratio [HR]: 0.76, $p < 0.01$) than in T1 group. Median and mean (\pm SEM) days to first insemination were 71 and 78.5 ± 1.3 in the T1 group, 74 and 79.1 ± 2.7 in the T2 group, and 81 and 85.7 ± 2.7 in the T3 group, respectively.

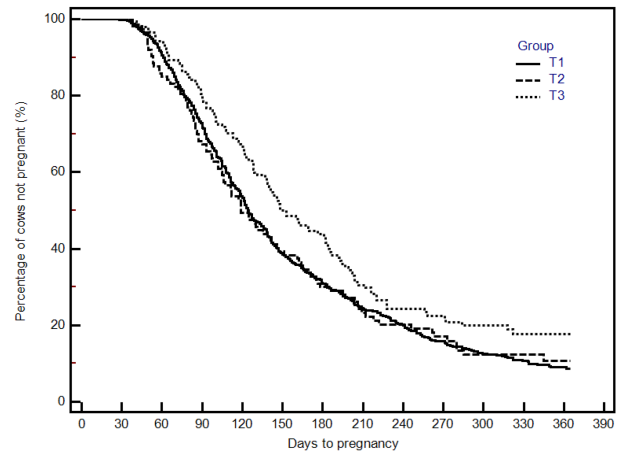


Fig 1. Survival curves for interval to pregnancy in T1 (n = 521), T2 (n = 113), and T3 (n = 140) groups based on the somatic cell score. The hazard of pregnancy by 365 DIM was lower in the T3 group than in the T1 group (HR: 0.75; CI = 0.618-0.910; $p < 0.01$) and in the T2 group (HR: 0.75; CI = 0.569-0.996; $p < 0.05$), whereas the hazard in the T1 and T2 groups did not differ.

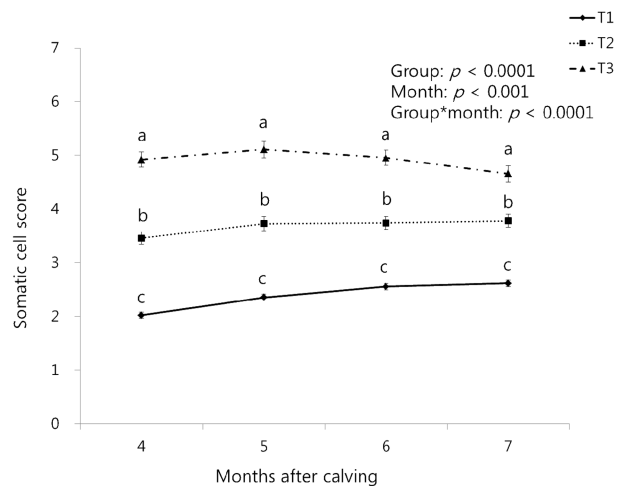


Fig 2. Changes of somatic cell score in milk during 4 - 7 months postpartum among the T1 (n = 521), T2 (n = 113), and T3 (n = 140) groups.

^{a,b,c} means with different superscripts differ ($p < 0.0001$) among groups.

Additionally, the hazard was higher for cows calved during autumn compared with those calved during spring (HR: 1.61, $p < 0.0001$); it was lower for multiparous cows compared with primiparous cows (HR: 0.81, $p < 0.01$). First insemination conception rate did not differ among the T1 (30.0%, 155/515), T2 (34.2%, 38/111), and T3 groups (28.7%, 39/136, $p > 0.05$).

Table 3 shows factors affecting the hazard of pregnancy by 365 DIM analyzed by PHREG procedure. Farm, group, calving season, and cow parity affected the hazard. The hazard was lower in T3 group (HR: 0.77, $p < 0.05$) than in the T1 group. Survival curves generated using the survival option in MedCalc, showed that the hazard of pregnancy by 365 DIM in the T3 group was lower (HR: 0.75, $p < 0.05$ respectively) than in the T1 and T2 groups (Fig 1). Median and mean (\pm

Table 4. Logistic regression model of risk factors for higher SCS in milk (≥ 4.0) in dairy cows during the first 3 months postpartum

Variable (n)	OR	95% CI	<i>p</i> -value
Farm			
A (203)	Reference		
B (309)	3.55	0.813-15.492	< 0.1
C (115)	6.24	1.468-26.480	< 0.05
D (54)	4.60	1.029-20.566	< 0.05
E (46)	2.40	0.456-12.584	> 0.05
F (47)	2.54	0.464-13.900	> 0.05
Cow parity			
Primiparous (247)	Reference		
Multiparous (527)	2.26	1.423-3.578	0.0005

SEM) days to pregnancy were 124 and 157.6 ± 4.3 in the T1 group, 119 and 156.0 ± 9.5 in the T2 group, and 153 and 182.7 ± 9.0 in the T3 group, respectively. Furthermore, the hazard was higher for cows calved during autumn compared with those calved during spring (HR: 1.40, $p < 0.005$), and lower for multiparous cows compared with primiparous cows (HR: 0.83, $p < 0.05$).

Fig 2 represents the changes of SCS in milk during 4-7 months postpartum among the 3 groups. There were significant effects for group ($p < 0.0001$), sampling time ($p < 0.001$), and group-by-sampling time interaction ($p < 0.0001$) on the SCS during the period (Fig 2). The SCS at each month differed ($p < 0.0001$) among the 3 groups.

Table 4 shows the risk factors for higher SCS in milk (≥ 4.0) in dairy cows during the first 3 months postpartum. Farm and cow parity were the risk factors for higher SCS. Higher SCS was more likely at 2 farms (C and D) by 6.2 and 4.6-fold compared with farm A. Multiparous cows were more likely to have a higher SCS (OR: 2.26, $p = 0.0005$) compared with primiparous cows.

Discussion

Our retrospective analyses showed that SCC during the first 3 months postpartum was associated with an increased incidence of postpartum endometritis and decreased reproductive performance in dairy cows. It was determined that reinforcement of farm health management, including udder health, and disposal of cows with high SCC and parities might be needed to decrease SCC in dairy herds.

Incidence of postpartum endometritis increased with increasing SCC (T3 > T2 > T1), although the reason was not clarified in this study. Inferior herd hygiene and a compromised systemic immune function of lactating dairy cows during early lactations might have negatively affected udder health and might have also caused postpartum endometritis. Tsolov *et al.* (32) found significant interactions between fertility disorders and the incidence of mastitis, and correlations between pathogen isolated from the uterus and mammary gland, supportive of our observations.

Lower hazard of the first insemination by 150 DIM in the T3 group than in the T1 group resulted in a 10-day delay of the interval from calving to first insemination, which was

similar to a previous study in which the interval from calving to first service was 21.8 days longer in cows with higher SCS (≥ 4.5) before the first breeding, compared with controls (18). Another study reported that the interval from calving to first service increased 1.3 days for each unit increase in test-day SCS (20), which also agreed with our results. Increased SCC in early lactation negatively affected LH pulses which may delay ovulation (2). This might explain in part the prolonged interval from calving to first insemination in cows with higher SCC. Higher SCS did not affect the first insemination conception rate in the present study, contrary to results of a previous study in which cows with higher SCC showed a lower odds of conception (0.85 times) compared with healthy cows (18).

Lower hazard of pregnancy by 365 DIM in the T3 group than the T1 or T2 groups resulted in prolonged intervals from calving to conception of 29 and 34-days, respectively, compared with T1 and T2 groups. The negative impact of higher SCC on the reproductive performance in this study was similar to the results of Pinedo *et al.* (18) in which the risk of pregnancy decreased by 44% on a high SCS (≥ 4.5) before breeding. Similarly, increased test-day SCS extended the calving to conception interval by 2.0 days for each unit increase in SCS (20). Besides, our observations that higher hazards of the intervals from calving to first insemination and conception in cows calved during autumn compared with cows calved during spring, and a lower hazard of the intervals for multiparous cows compared with primiparous cows, as well as significant difference among farms were consistent with other reports (8,33). The exact mechanisms to explain the decrease in reproductive performance by increasing SCC were not clarified in the present study. It is speculated that, however, endocrine disturbance and postpartum immune suppression of postpartum cows (2,12), together with unfavorable environmental conditions and hygiene control within herds, and other factors (5,31) might in part attribute to uterine disease (endometritis) and subsequent fertility.

Significant differences in SCS during 4-7 months postpartum among all the 3 groups in the present study demonstrated that higher SCC during early lactation led to a chronic decrease in milk quality throughout lactation, presumably leading to economic loss. Similarly, Santos *et al.* (25) found that clinical mastitis had chronically higher linear SCC scores throughout lactation compared to cows without clinical mastitis, which might be due to persistent mammary infections caused by environmental pathogens (28). Mastitis was an important risk factor for culling in dairy herds (9,25). In a study on Irish dairy herds, SCC in the first month of lactation during parity 1 was positively associated with risk of disposal from the herd, although the size of this effect appeared relatively small (1). In particular, higher SCC throughout lactation plays an important role in determining milk price and may thus be detrimental to dairy producers in the Korean dairy industry.

Determination of the risk factors for higher SCC may be helpful in decreasing the incidence of subclinical mastitis. The results of the present study revealed that farm and higher parity were important risk factors for higher SCC. It is assumed that farm factors might be related to differences in each farm environment, and hygiene control in the herds

(5,21). Increased risk for higher SCC by higher cow parity in this study was consistent with previous studies (5,26). Increased SCC might be due to worsening hygiene as cows advanced in parity, followed by increased prevalence of intramammary infection (22,24). Milk yield and the calving season were not risk factors for higher SCC in this study. Contrary to our finding, lower milk yield was a reported risk factor for higher SCC (5). Seasonal variation for higher SCC was also reported; cows are more likely exposed to pathogens during warm and wet seasons, resulting in a higher SCC, compared to cooler periods (3,30). Additionally, heat stress reduces the phagocytic ability of neutrophils against invading pathogens (6).

In conclusion, higher SCS (≥ 4.0) during the first 3 months postpartum decreased reproductive performance in dairy cows, compared to SCS < 4.0 (comparable to the SCC values of $< 200,000$ cells/ml). Based on the risk factors for higher SCS (≥ 4.0), improvement of farm hygiene and careful consideration of disposal of cows with high SCC and parities from herds may in part contribute to decreased SCC levels in dairy herds.

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젖소에서 비유초기 체세포 증가 위험 요인 및 번식효율에 미치는 영향 분석 연구

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요 약 : 본 연구는 젖소에서 분만 후 비유초기 체세포 증가가 번식효율에 미치는 영향에 대하여 조사하였다. 774두의 젖소 자료를 분만 후 3개월 평균 체세포지수에 따라 T1(3.0 미만, n = 521), T2(3.0 이상 4.0 미만, n = 113) 및 T3(4.0 이상, n = 140) 군으로 구분하였다. 자궁내막염의 발생위험(odds ratio)은 T1군에 비해 T2군 및 T3군이 각각 1.6배($p < 0.05$) 및 3.2배($p < 0.0001$) 증가하였다. 분만 후 150일까지 첫 수정율은 T1군에 비해 T3군이 낮았으나 (hazard ratio [HR] = 0.76, $p < 0.01$), 분만 후 첫수정 수태율은 각 군간에 차이가 나타나지 않았다(28.7-34.2%, $p > 0.05$). 분만 후 365일까지의 임신율은 T3군이 T1군 및 T2군에 비해 낮았다(HR = 0.75, $p < 0.05$). 분만 후 4-7개월까지 체세포지수는 각 군간에 차이가 인정되었다($p < 0.0001$). 체세포지수 4이상 도달 위험요인은 목장($p < 0.05$) 및 산차($p = 0.0005$)와 관련되었으며, 초산차에 비해 2산이상의 젖소에서 2.3배의 높은 위험도를 나타내었다. 결론적으로, 젖소에서 비유초기 4.0 이상의 높은 체세포지수는 이후 번식효율의 감소를 초래하였다.

주요어 : 젖소, 체세포지수, 자궁내막염, 번식 능력