

Relationship between the somatic cell count in milk and reproductive function in peripartum dairy cows

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Abstract. The aim of the present study was to examine the effect of the somatic cell count (SCC) in milk on reproductive performance, such as pregnancy status in the prepartum period and ovarian function in the postpartum period, in dairy cows. Blood samples were collected every week from one month prepartum to parturition in order to measure the concentrations of 13,14-dihydro-15-keto-PGF_{2α} (PGFM), estrone sulfate (E1S) and progesterone. Milk samples were collected three times per week in both the prepartum (for one month before the dry period) and postpartum periods (for 3 months immediately after parturition) to measure the SCC. Progesterone was also determined in the whole milk of postpartum cows to define the day of the first ovulation. In the prepartum period, the maximum SCC negatively correlated with the pregnancy period ($r = -0.77$), but not the calf birth weight. Positive and negative correlations were observed between the average SCC and PGFM or progesterone concentrations in plasma, respectively ($r = 0.84$ or -0.92 , respectively), at 39 weeks of pregnancy. In the postpartum period, a correlation was observed between the day of the first ovulation and both the average and maximum SCC ($r = -0.74$ and -0.75 , respectively), whereas days open was not related to the SCC. These results suggest that a high SCC in the prepartum period may advance parturition by increasing PGF_{2α} and decreasing progesterone and that the first ovulation in the postpartum period was affected by a high SCC.

Key words: Dairy cow, Mastitis, Progesterone, Prostaglandin, Somatic cell count

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Mastitis is an important disease in dairy cows and causes huge economic losses in the dairy industry. Various species of Gram-positive and Gram-negative bacteria can infect the mammary glands and cause mammary inflammation. These bacteria are recognized by the toll-like receptor (TLR) on the surface of epithelial cells and leukocytes. Different TLRs recognize different types of bacteria, e.g., TLR2 and 4 for Gram-positive and Gram-negative bacteria, respectively [1, 2]. The recognition of bacteria progresses the intracellular cascade and activates nuclear factor- κ B, which induces RNA transcription, followed by the translation and secretion of some cytokines and antimicrobial peptides [3–7]. Cytokines have various functions in inflammation. Tumor necrosis factor α (TNF- α), which is a cytokine, is produced in mastitic mammary glands and secreted into the blood stream, and it then reaches the oviduct [3], in which the production of prostaglandin F_{2α} is induced [8, 9]. Prostaglandin F_{2α} causes the contraction of smooth muscle in the oviduct, which may lead to abortions in pregnant animals [10]. Prostaglandin F_{2α} also causes the corpus luteum to regress and decreases the concentration of progesterone in the blood [11], which limits pregnancy [10]. Accordingly, mastitis in the prepartum period may have a detrimental effect on the maintenance of pregnancy.

Estrone sulfate (E1S), a conjugated estrogen, is the main estrogen

in the maternal circulation of cattle during pregnancy [12]. E1S concentrations in the plasma have been associated with calf birth weights and placental weights in cows [13]. Thus, maternal plasma E1S concentrations provide useful information for monitoring the fetoplacental function and predicting the maturity and viability of newborn calves [14]. As described above, mastitis-induced cytokines affect the function of the uterus; however, it remains unknown whether the development and condition of the fetus and placenta are also related to mastitis. E1S may be a useful indicator for monitoring the function of the fetus and placenta.

On the other hand, cytokines in the postpartum period have been shown to negatively impact hypothalamus-pituitary function, resulting in abnormal production of GnRH and gonadotropins [15]. Therefore, follicular development and ovulation do not occur, which results in reproductive disorders such as anovulation at estrus, fertilization failure, and embryonic mortality [16]. Administration of endotoxin to heifers near estrus was shown to inhibit the LH surge, cause anovulation and the formation of follicular cysts, or delay ovulation [17, 18]. Schrick *et al.* [19] reported that services per conception were significantly higher in cows diagnosed with clinical and subclinical mastitis than in uninfected cows. Accordingly, mastitis in the postpartum period may also be detrimental to reproductive function.

However, the relationship between mastitis and reproductive function, especially hormone dynamics, has not yet been investigated in detail. Therefore, the present study was undertaken to examine the effect of somatic cell count (SCC) in milk on reproductive performance, such as pregnancy status in the prepartum period and ovarian function in the postpartum period, in association with hormone dynamics.

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Materials and Methods

Animals

Ten multiparous Holstein-Friesian dairy cows (parity = 2 to 4) were fed at the Hiroshima University farm under the Guidelines for Animal Experimentation, Hiroshima University, Japan. The amount of feed supplied (energy, protein and minerals) met the requirements for dairy cows [20]. All cows were inseminated with Holstein-Friesian semen.

Blood and milk collection

Blood samples were collected from prepartum cows every week from one month prepartum to parturition in order to measure the concentrations of estrone sulfate (E1S), progesterone, and 13,14-dihydro-15-keto-PGF_{2α} (PGFM). Since prostaglandin is easily metabolized after its secretion into blood, its metabolite, PGFM, was measured. Milk samples were collected three times per week in both the prepartum (for one month before the dry period) and postpartum periods (for 3 months immediately after parturition) to measure the somatic cell count (SCC). Progesterone was also determined in the milk of postpartum cows only.

Examination of blood samples

Progesterone and E1S were measured using the enzyme immunoassay described previously by Sato *et al.* [21] and Isobe and Nakao [14], respectively.

The concentrations of PGFM in the plasma were determined using the enzyme immunoassay as previously described [8] with horseradish peroxidase-labeled PGFM and anti-PGFM serum (donated by Dr WJ Silvia, University of Kentucky, Lexington, KY, USA). The PGFM standard curve ranged from 30 to 3000 pg/ml, and the sensitivity of the assay was 10 pg/ml.

Examination of milk samples

Progesterone in whole milk was determined using the method described by Isobe *et al.* [22]. Five days before the progesterone concentration increased to 5 ng/ml or higher for the first time was considered to be the day of the first ovulation. Milk samples were spread on a glass slide for SCC measurements using the Breed method [23].

Statistical analysis

The average and maximum values of the SCC were determined in each animal in both the prepartum and postpartum periods. Correlation analysis was carried out using Spearman's correlation analysis between SCC data (average and maximum) and reproductive parameters (E1S, progesterone, PGFM concentrations, the pregnancy period, calf birth weight, day of the first ovulation, days open). Concentrations were compared among the weeks of pregnancy using the parametric Tukey multiple range test for PGFM and E1S and the nonparametric Steel-Dwass test for progesterone. A probability value < 0.05 was considered to be significant.

Results

The pregnancy period ranged from 265 to 291 days, whereas

one cow had a pregnancy period of 235 days. The maximum SCC in this cow was the highest among all cows. A negative correlation was observed between the pregnancy period and maximum SCC ($r = -0.77$, Fig. 1a) but not between the pregnancy period and the average SCC (Table 1).

Calf birth weights ranged from 33 to 55 kg. However, the cow with a pregnancy period of 235 days had a low calf weight (20 kg). A significant correlation was not observed between calf birth weight and SCC (both average and maximum), whereas a negative correlation was noted between calf birth weight and maximum SCC ($r = -0.65$, Fig. 1b), which was similar to the correlation noted between the pregnancy period and maximum SCC (Table 1).

Changes in hormone concentrations in prepartum cows are shown in Fig. 2. No significant differences were observed in the concentration of PGFM between 36 to 39 weeks of pregnancy. However, the E1S concentration was significantly higher at 39 weeks of pregnancy than at 36 and 37 weeks. In contrast, the progesterone concentration was lower at 39 weeks than at 37 weeks of pregnancy.

The concentrations of PGFM, E1S and progesterone were not related to the SCCs at 36, 37 and 38 weeks of pregnancy. However, at 39 weeks of pregnancy, a positive correlation was observed between the PGFM concentration in the plasma and the average SCC ($r = 0.84$, Fig. 1c). The progesterone concentration was negatively correlated with the average and maximum SCCs at 39 weeks of pregnancy ($r = -0.92$ and -0.74 , respectively, Fig. 1d).

In the postpartum period, the days of the first ovulation ranged between 9 and 60 days. A correlation was observed between the day of the first ovulation and both the average and maximum SCCs (Table 2, $r = -0.74$ and -0.75 , respectively, Fig. 1e). However, no significant relationship was found between days open and the SCC (days open: 93 to 260 days, Fig. 1f) or the day of the first ovulation and days open.

Discussion

In the present study, we investigated the association of mastitis with reproductive performance in dairy cows. We measured the SCC in milk and compared it with various reproductive parameters. A negative correlation was observed between the pregnancy period and maximum SCC in prepartum cows. These results suggest that a high SCC shortens the pregnancy period. This has been attributed to the production of prostaglandin because mammary infections are known to induce the production of cytokines from leukocytes recruited in the mammary gland. TNF- α , which is a cytokine, has been shown to stimulate the production of prostaglandin in the oviduct [8, 9]. The plasma concentration of the prostaglandin metabolite, PGFM, was measured and compared with the SCC to confirm this finding. The PGFM concentration at 39 weeks of pregnancy was positively correlated with the average SCC. This result indicated that a high SCC increased the prostaglandin F_{2α} concentration in the plasma, thereby supporting the above-described findings in which cytokines stimulated the production of prostaglandin in the oviduct [8, 9]. Prostaglandin may induce discharge of the fetus, resulting in a short pregnancy period.

Another function of prostaglandin is degeneration of the corpus luteum. Therefore, the progesterone concentration was measured,

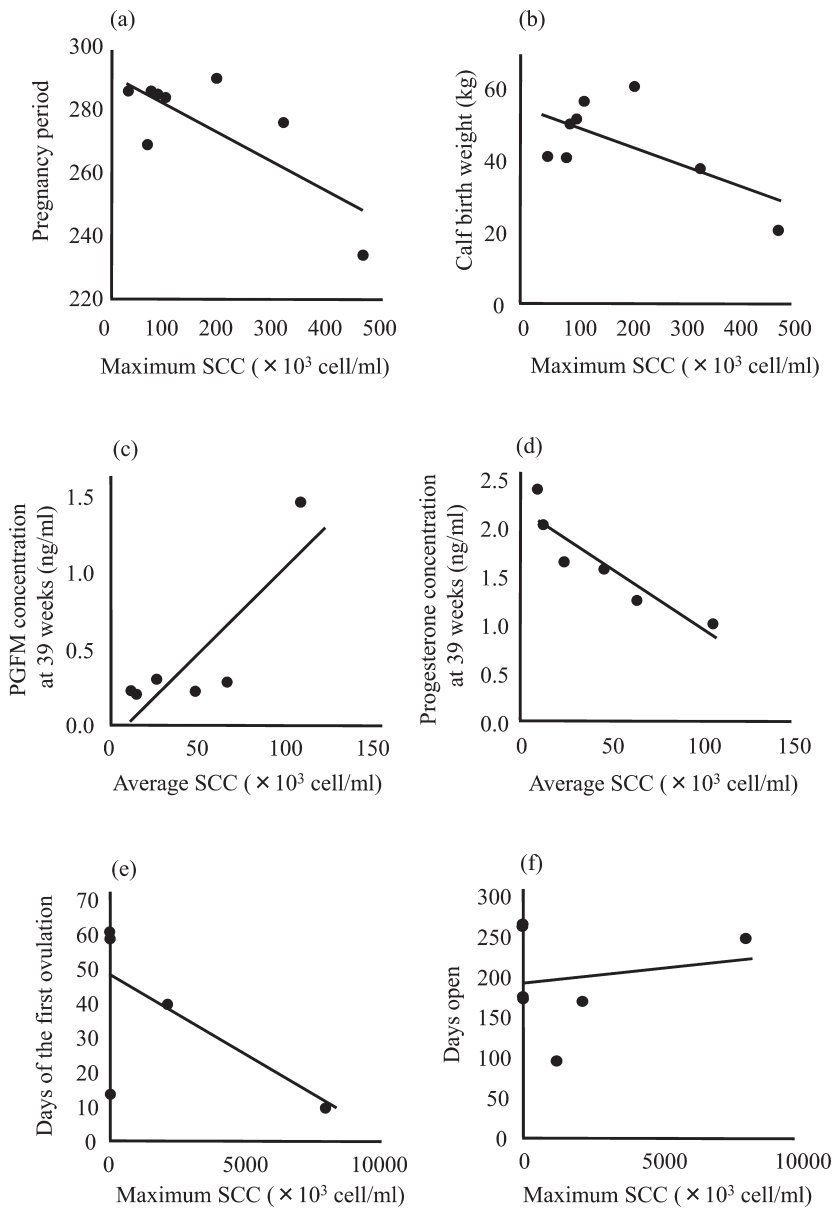


Fig. 1. Correlation of the SCC with reproductive function and hormone status. (a), (b), (e) and (f) represent correlations of the maximum somatic cell count with the pregnancy period, calf birth weight, day of the first ovulation and days open, respectively. (c) and (d) represent correlations of the average somatic cell count with the PGFM and progesterone concentrations at 39 week, respectively.

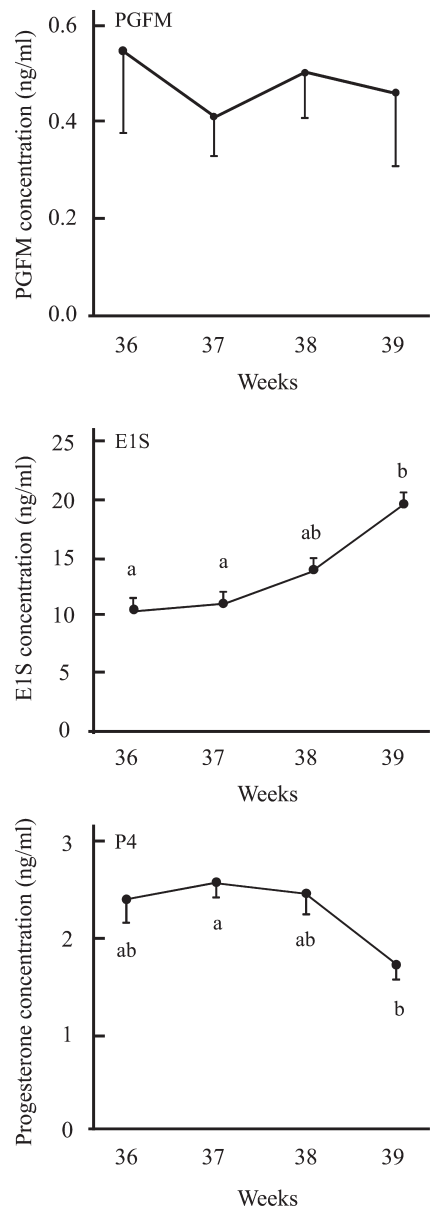


Fig. 2. Changes in the concentrations of PGFM, estrone sulfate (EIS) and progesterone at 36 to 39 weeks of pregnancy. a, b: Different letters indicate a significant difference between weeks.

and the results obtained showed a negative correlation between the concentration and both the average and maximum SCCs at 39 weeks of pregnancy. This result suggested that a high SCC may inhibit the function of the corpus luteum, which may, in turn, shorten the pregnancy period. Taken together, these findings indicated that the pregnancy period must have been shortened due to the uterine contractions and regression of the corpus luteum caused by prostaglandin, which was induced by $TNF-\alpha$ secreted from somatic cells.

A strong negative association was observed between the SCC and

calf birth weight. This may have been due to a high SCC inducing premature calving (short pregnancy period), resulting in low calf weights.

Mastitis in the postpartum period stimulates the production of cytokines, which inhibits hypothalamus-pituitary function, thereby preventing the onset of ovarian function. Therefore, the day of the first ovulation was evaluated based on the progesterone concentration in milk. The day of the first ovulation was negatively correlated with both the average and maximum SCCs, which indicated that a

Table 1. Effects of the somatic cell count in the prepartum period on the pregnancy period, calf birth weight and hormone concentration

	Weeks	Somatic cell count	
		Average	Maximum
Pregnancy period		0.12	-0.77*
Calf birth weight		0.24	-0.65
PGFM	36	0.06	0.58
	37	0.06	0.43
	38	0.29	-0.37
	39	0.84*	0.32
E1S	36	0.13	0.36
	37	0.14	0.53
	38	-0.19	0.44
	39	-0.52	-0.22
Progesterone	36	-0.59	-0.35
	37	-0.49	-0.62
	38	-0.39	-0.25
	39	-0.92*	-0.74*

PGFM, 13,14-dihydro-15-keto-PGF_{2α}; E1S, estrone sulfate. Values are correlation coefficients. * The correlation coefficient was significant ($P < 0.05$).

Table 2. Effects of the somatic cell count in the postpartum period on the day of the first ovulation and days open

	Somatic cell count	
	Average	Maximum
Day of the first ovulation	-0.74*	-0.75*
Days open	0.27	0.19

Values are correlation coefficients. * The correlation coefficient was significant ($P < 0.05$).

high SCC resulted in an earlier day of first ovulation. This result is opposite to our hypothesis, and the reason for this currently remains unclear. However, infection causes stress in the animal, which stimulates the production of cortisol from the adrenal gland [24]. Since cortisol is synthesized through progesterone, an increase in cortisol concentrations is sometimes accompanied by the production of progesterone [25]. Progesterone may stimulate hypothalamus-pituitary function, as Crane *et al.* [26] reported that administration of progesterone prior to a natural ovulation may correct the underlying hypothalamic defect and improve the pregnancy rate in cows with cystic follicles. However, days open was neither significantly related to the SCC nor the days of the first ovulation. The first ovulation in the early postpartum period was recently shown to not guarantee that subsequent fertilization, implantation, embryo development and growth of a fetus will result in a viable pregnancy [27].

E1S is produced in the fetus and placenta and is then transferred to the maternal circulation [12]. A relationship has been reported between the E1S concentration and calf viability and birth weight [13]; therefore, E1S can be used to predict these parameters. In the present study, E1S was measured to determine whether the SCC affected the fetus. No relationship was observed between the E1S

concentration and the SCC. Therefore, these results suggest that fetus development may not be affected by the SCC. It has been reported that estrogen increased permeability of umbilical vein endothelial cells in humans [28]. The leukocytes included in the milk SCC derived from the mammary blood circulation through the blood vessel wall. Since E1S was not correlated with the SCC, the permeability of the blood vessels in the mammary gland may be not changed by E1S.

In conclusion, the results of the present study suggest that a high SCC in the prepartum period may advance parturition by increasing PGF_{2α} and decreasing progesterone and that the first ovulation in the postpartum period may be affected by the SCC. The present study implies that mastitis affects reproduction in peripartum dairy cows.

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