

Acta Veterinaria Hungarica 59 (3), pp. 349–362 (2011)
DOI: 10.1556/AVet.2011.017

RELATIONSHIP BETWEEN MILK SOMATIC CELL COUNT AND POSTPARTUM OVARIAN CYCLICITY AND FERTILITY IN DAIRY COWS

Thin C. NGUYEN, Toshihiko NAKAO*, Gokarna GAUTAM, Long T. SU,
Ranasinghe M. S. B. K. RANASINGHE and Muhammad YUSUF

Laboratory of Theriogenology, Department of Veterinary Medicine, Faculty of Agriculture,
Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan

(Received 23 August 2010; accepted 9 February 2011)

The main objective of the study was to describe the relationship of high somatic cell count (SCC) with the incidence of abnormal postpartum resumption of ovarian cyclicity and reproductive performance in dairy cows. The factors influencing SCC were also investigated. Four hundred and forty-seven cows from six dairy herds in Japan were monitored for SCC and postpartum resumption of ovarian cyclicity. Cows with high SCC (200,000 to 500,000) had a higher incidence of prolonged luteal phase ($P < 0.01$) than cows with an SCC of 50,000 to 100,000. The high SCC cows ($> 500,000$) also showed a higher incidence of delayed first ovulation post partum than cows with an $SCC \leq 500,000$ ($P < 0.05$) during the first month post partum. Cows with an SCC of 200,000 to 500,000 showed lower conception and pregnancy rates, and more days from calving to conception than cows with an SCC of less than 200,000 ($P < 0.05$). Cows in parity 5 or more had a higher incidence of high SCC than cows in the first and second parities ($P < 0.05$). It is concluded that cows with a high SCC have a higher incidence of abnormal postpartum resumption of ovarian cyclicity, leading to reduced reproductive performance.

Key words: SCC, postpartum resumption of ovarian cyclicity, reproductive performance, dairy cow

Somatic cells in milk originate from leukocytes consisting of neutrophils, macrophages and lymphocytes derived from the blood circulation, as well as mammary epithelial cells (Concha, 1986). The peripheral blood leukocytes are stimulated to migrate into the milk through the milk-blood barrier by several chemokines such as IL-8 (Riollet et al., 2000). Since milk somatic cell count (SCC) is closely associated with inflammation and udder health (Yagi et al., 2004), increase of the SCC has been known as a reliable indicator of subclinical mastitis in dairy cows (Smith, 1996; Van Den Borne et al., 2010). Meanwhile, a high SCC in milk has been reported to be associated with decreased fertility in dairy cows (Moore et al., 1991; Barker et al., 1998; Risco et al., 1999; Santos et

*Corresponding author; E-mail: rakunonakao@kyp.biglobe.ne.jp; Fax: 0081 (83) 933-5935

al., 2004; Pinedo et al., 2009). This is in agreement with the previous studies reporting that clinical and subclinical mastitis caused poor reproductive performance (Moore et al., 1991; Barker et al., 1998; Risco et al., 1999; Santos et al., 2004; Schrick et al., 2001). The mechanism how high SCC is associated with poor fertility is yet to be determined. Tumour necrosis factor- α (TNF- α) (Pampfer et al., 1997; Pampfer, 2001), and nitric oxide (NO) (Lim and Hansel, 1998; Soto et al., 2003) released as the consequence of mastitis have been reported to cause embryonic mortality. PGF_{2 α} may also cause decreased fertility through its adverse effects on oocyte maturation and premature luteolysis (Soto et al., 2003; Hansen et al., 2004). In addition, it is presumed that high SCC or subclinical mastitis suppresses the release of gonadotropic hormones, leading to delayed resumption of ovarian cyclicity post partum. Only limited information, if any, has so far been available on the relationship between high SCC and the incidence of abnormal postpartum resumption of ovarian cyclicity. The description of this relationship may contribute to a better understanding of the mechanism how high SCC causes decreased reproductive performance in dairy cows.

The main objective of this study was, therefore, to describe the relationship between high SCC and reproductive performance, with especial regard to the incidence of different types of abnormal resumption of ovarian cyclicity. The factors influencing milk SCC were also investigated.

Materials and methods

Animals and management

This study was conducted in 447 lactations of 362 high-producing Holstein-Friesian cows which calved during the period between January 2006 and December 2009 in six commercial dairy herds in Japan. Cows in the three herds were kept in free-stall barns with sawdust for bedding (herds A, B and C), and those in the other three herds were in 24-hour tie-stall barns with rubber mattresses on the floor (herds D, E and F). Herd size ranged between 30 and 200 cows. The average 305-day milk yield per cow per lactation of each herd was approximately 8,000 to 10,300 kg. The parities of cows ranged from one to ten. The cows were fed a total mixed ration consisting of grass silage, corn, soybean, hay, concentrate and mineral supplements. In herds A and B, cows were observed twice a day for oestrous signs around milking time at 06:00 and 17:00. In herds C, D, E and F oestrus was detected based on the secondary oestrous signs (swelling, relaxation and congestion of the vulva, mucous discharge, bellowing and restlessness, decreased milk yield). The cows were inseminated artificially by AI technicians 8–14 h after the detection of oestrus in herds A, B and C, while in the other three herds AI was done by the herd owners. Pregnancy was diagnosed by ultrasonography in cows 35 days after AI or later in four herds (C, D, E

and F) which were regularly visited by a team from Yamaguchi University. In the other herds, cows 35 days or more after breeding were checked for pregnancy by local veterinarians using rectal palpation.

Classification of cows based on SCC within 3 months post partum

Individual milk SCC from all udder quarters of cows was measured every month, starting within one month post partum, at Hokkaido Dairy Milk Recording and Testing Association and Okayama Animal Husbandry Association Dairy Milk Test Section. Milk samples were collected by the producers at the afternoon milking and were kept at 4 °C overnight. On the next morning, the samples were put in a cold box and sent to the laboratories. Within 3 h after receipt, the samples were tested for SCC using FossomaticTM Minor (Denmark).

All cows examined were classified into five groups based on SCC one to three months post partum: Group I: $SCC \leq 50,000/ml$, Group II: $50,000 < SCC \leq 100,000/ml$, Group III: $100,000 < SCC \leq 200,000/ml$, Group IV: $200,000 < SCC \leq 500,000/ml$, Group V: $SCC > 500,000/ml$. Cows with clinical mastitis were not included in this study.

Monitoring postpartum ovarian cycles based on milk progesterone profiles

Milk samples were collected twice a week (on Monday and Thursday) starting at approximately 10–14 days post partum until approximately 80 days after calving. About 10 ml of foremilk was collected at the afternoon milking and it was stored at 4 °C until assayed. Progesterone concentrations were determined using direct ELISA in whole milk (Isobe et al., 2004) in herds B, C, D and E or in skim milk (Shrestha et al., 2004b) in herds A and F.

Cows were monitored for resumption of ovarian cyclicity based on milk progesterone profiles. Ovulation was considered to have taken place 3–5 days before the first rise of progesterone concentrations ≥ 5.0 ng/ml in whole milk or ≥ 1.0 ng/ml in skim milk.

Cows with progesterone concentrations ≥ 5.0 ng/ml in whole milk or ≥ 1.0 ng/ml in skim milk in at least two consecutive milk samples were considered to have luteal activity (Isobe et al., 2004; Ling et al., 2007). Patterns of postpartum resumption of ovarian cyclicity based on progesterone profiles were classified as follow: (1) Normal resumption of ovarian cyclicity: first ovulation within 45 days after calving followed by two or more regular ovarian cycles; (2) Delayed first ovulation type I (DFO-I): first ovulation did not occur until 45 to 60 days after calving; (3) Delayed first ovulation type II (DFO-II): first ovulation did not occur until 61 days post partum or later; (4) Prolonged luteal phase (PLP): first ovulation occurred within 45 days after calving, but one or more ovarian cycles had luteal activity of more than 20 days (Ranasinghe et al., 2011); (5) Cessation of cyclicity (CC): first ovulation occurred within 45 days after

calving, but there was an absence of luteal activity for at least 14 days between the first and second luteal phases; (6) Short luteal phase (SLP): first ovulation occurred within 45 days after calving, but one or more ovarian cycles had luteal activity for less than 10 days, except for the first cycle.

Data collection and investigation of reproductive performance

Herds C, D, E and F were visited once a month for reproductive check: diagnosis and treatment of reproductive disorders, pregnancy diagnosis and body condition scoring (BCS: scale 1–5 with 0.25 increment points; Ferguson et al., 1994). Data in herds A and B were obtained through local veterinarians visiting the farms at weekly intervals.

All information about the herds (herd unique code identification, test-day, number of cows in milk and average milk production at the day of sampling) and about individual cows such as milk composition (SCC, milk urea nitrogen, fat, protein), calving date, breeding date, pregnancy, abortion, date of drying off and culling date were obtained from Dairy Herd Improvement (DHI) record sheets.

AI submission rate within 100 days was defined as the proportion of cows that were subjected to AI within 100 days post partum in cows enrolled in the trial. First AI conception rate (FAICR) was the proportion of cows that conceived after first insemination in all inseminated cows. Pregnancy rates (PR) were calculated as the number of cows that became pregnant within 100, 150 and 210 days, divided by the total number of cows to be bred in the herd.

Statistical analysis

Statistical analysis was carried out using the statistical package SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Differences in AI submission rate within 100 days, FAICR, PR within 100, 150 and 210 days, and final pregnancy rate among the different groups of cows were analysed using Chi-square test. Likewise, the association between parity and calving season and the different levels of SCC was also analysed. When groups differed ($P \leq 0.05$), *post-hoc* test by Chi-square test (2×2 contingency table) was used to make comparisons between individual groups. Fisher's exact test was used when one or more cells had an expected value less than 5. The differences in calving to conception interval among the groups with different degrees of SCC or with different types of ovarian cycles were analysed using one-way ANOVA.

Results

Of the 447 cows monitored for SCC and resumption of ovarian cyclicity in the current study, 30 cows were culled without being inseminated and they were excluded from further analysis. Thus, 417 cows were available for the analysis.

Distribution of cows by SCC during a period of three months post partum

During a period of three months post partum 64.0 to 71.1% of the cows showed an SCC of less than 50,000 (Fig. 1). The percentages of cows having 200,000 to 500,000 SCC and those with more than 500,000 SCC during the same period were 4.3 to 6.2% and 3.8 to 5.5%, respectively.

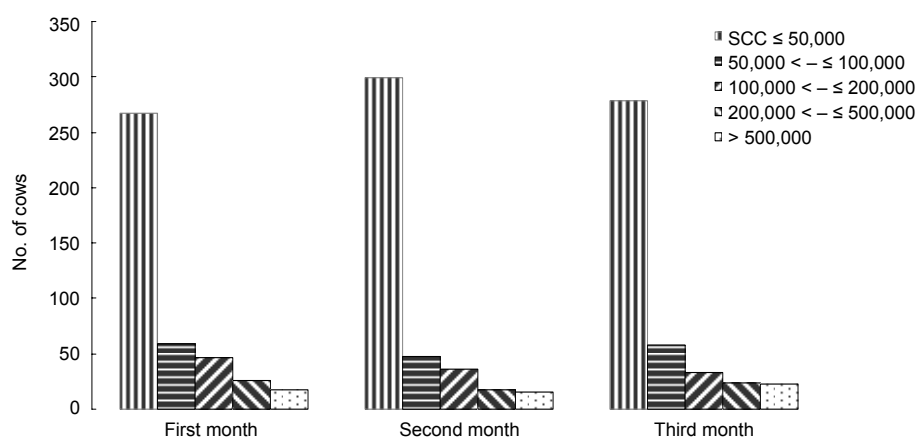


Fig. 1. Distribution of cows based on different levels of SCC in milk during 3 months post partum

Postpartum resumption of ovarian cyclicity and its relationship to SCC

Of the 417 cows examined, 233 (55.9%) cows had normal ovarian cycles post partum, while 184 (44.1%) cows had abnormal ovarian cycles, including 50 (12.2%) cows with prolonged luteal phase, 86 (20.5%) cows with delayed first ovulation, 29 (6.9%) cows with cessation of cyclicity, and 19 (4.5%) cows with short luteal phase (Table 1). There was a significant difference in the incidence of prolonged luteal phase among groups of cows with different levels of SCC in the first month ($P < 0.01$) and in the second month ($P < 0.05$) post partum. The group of cows with 200,000 to 500,000 SCC had a higher percentage of cows with prolonged luteal phase than that having 50,000 to 100,000 SCC ($P = 0.008$) in the first month post partum. In the second month post partum, 20.8% of cows with 50,000 to 100,000 SCC had prolonged luteal phase, while only 9% of cows with less than 50,000 SCC had prolonged luteal phase ($P = 0.022$). In addition, cows with 200,000 to 500,000 SCC and $> 500,000$ SCC tended to have a higher incidence of prolonged luteal phase than did cows with $< 50,000$ SCC ($P = 0.086$ and $P = 0.06$, respectively). The group of cows with $> 500,000$ SCC showed a higher incidence of delayed first ovulation type II than the other groups of cows with an SCC less than 500,000 in the first month post partum ($P < 0.05$).

Effect of SCC on conception and pregnancy rates and calving to conception interval

The levels of SCC in the first month post partum had no effect on reproductive performance (Table 2). In the second month post partum, cows with 200,000 to 500,000 SCC tended to show lower conception and pregnancy rates and required more days to conceive than cows with less than 200,000 SCC. In the third month post partum, cows with less than 50,000 SCC tended to have a higher conception rate than cows with 50,000 or more SCC.

Factors affecting SCC in the first three months post partum

The calving season had no effect on SCC ($P > 0.05$) while there was a significant association between parity and high SCC ($P = 0.04$, $P = 0.014$, $P = 0.003$ in the first, second and third month post partum, respectively) (Table 3). Cows in parity 5 or more had a higher incidence of high SCC than cows in the first and second parities in the first three months after calving ($P < 0.01$).

Effect of abnormal resumption of ovarian cyclicity on pregnancy rate and calving to conception interval

There were significant differences in FAICR ($P < 0.05$) and PR within 100 days post partum ($P < 0.05$) among the different types of postpartum ovarian cycles (Table 4). Cows with delayed first ovulation types I and II showed lower FAICR and PR within 100 days post partum than cows with normal postpartum ovarian cycles ($P < 0.05$). Likewise, cows with abnormal resumption of ovarian cyclicity had a longer calving to conception interval than cows with normal ovarian cycles ($P < 0.05$).

Discussion

In the present study, the proportions of cows with 200,000 to 500,000 SCC and those with more than 500,000 SCC in six commercial dairy herds during a period of three months post partum were 4.3 to 6.2% and 3.8 to 5.5%, respectively, which values are lower than those reported previously. Hagnestam-Nielsen et al. (2009) reported that the proportions of cows with 200,000 to 500,000 and $> 500,000$ SCC were 18.2% and 18.6%, 9.8% and 7.5%, 11.6% and 8.4% at 1–2 weeks, 3–8 weeks and 9–16 weeks post partum, respectively. Van Den Borne et al. (2010) also reported that 12.8% of primiparous and 27.1% of multiparous cows had more than 200,000 SCC. In this study only 8.1 to 11.3% of the cows showed more than 200,000 SCC in the first to third month post partum.

The incidence of abnormal resumption of ovarian cyclicity has been reported as 32% to 57% in earlier studies (Nakao et al., 1992; Lamming and Darwash, 1998; Opsomer et al., 1998; Shrestha et al., 2004a). In this study, 44.1% of

the cows had abnormal ovarian cycles, which is comparable with the findings of previous studies.

Huszenicza et al. (2005) reported that mastitis was associated with delayed first ovulation in acyclic cows or lengthened the follicular phase in cyclic cows. Similarly, Lavon et al. (2010) reported that 30% of cows with subclinical mastitis exhibited delayed ovulation. On the other hand, Morris et al. (2009) indicated that high SCC and lameness reduced the likelihood of ovulation, but the same was not true for animals with a high SCC only. Our data have described for the first time the relationship between SCC and different patterns of abnormal resumption of ovarian cyclicity post partum, including delayed first ovulation and prolonged luteal phases. Cows with more than 500,000 SCC had a higher incidence of delayed first ovulation type II ($P < 0.05$). Cows with high SCC in the first and second month post partum had a higher incidence of prolonged luteal phase than cows with low SCC ($P < 0.01$ and $P < 0.05$, respectively). The causes of high incidence of prolonged luteal phase in cows with high SCC are yet to be determined. The direct association between subclinical mastitis and endometritis has been suggested by Bacha and Regassa (2010). Endometritis could be one of the causes of prolonged luteal phase (Sheldon et al., 2009; Ranasinghe et al., 2011).

Contradictory results regarding the effect of high SCC on reproductive performance have been reported so far. Huszenicza et al. (2005) failed to show an influence of clinical mastitis on the pregnancy rate or the calving to conception interval. However, some other authors have demonstrated that high SCC resulted in longer intervals from calving to first breeding and to conception (Moore et al., 1991; Barker et al., 1998; Schrick et al., 2001; Pinedo et al., 2009), as well as in lower FAICR and total pregnancy rate (Santos et al., 2004). The data of the current study show that cows with 50,000 to 100,000 SCC had a significantly lower FAICR than cows with less than 50,000 SCC ($P = 0.012$). Moreover, cows with 200,000 to 500,000 SCC in the second month post partum had a significantly lower ($P = 0.006$) pregnancy rate within 150 days post partum than cows with less than 50,000 SCC. The cows with 200,000 to 500,000 SCC also had a longer calving to conception interval than the normal cows ($P = 0.012$). Our data support the findings of earlier studies reporting an association between mastitis and poor fertility (Pampfer et al., 1997; Pampfer, 2001; Lim and Hansel, 1998; Soto et al., 2003; Hansen et al., 2004).

In the third month post partum, cows with more than 100,000 to 200,000 SCC needed more days to the first postpartum AI than cows with less than 50,000 SCC ($P = 0.019$). This is partly due to a higher incidence of abnormal resumption of ovarian cyclicity post partum in cows with high SCC than in cows with low SCC, as shown in this study.

The significant effect of parity on SCC in the first three months after calving ($P < 0.05$), shown in this study, is in agreement with the findings of previous studies (Sheldrake et al., 1983; Laevens et al., 1997).

Table 1
Relationship between SCC in milk and resumption of ovarian cyclicity in postpartum cows

Ovarian cycles post partum	SCC in the first month post partum ($\times 1000/\text{ml}$)					Total	SCC in the second month post partum ($\times 1000/\text{ml}$)					Total
	≤ 50	50 <– ≤ 100	100 <– ≤ 200	200 <– ≤ 500	> 500		≤ 50	50 <– ≤ 100	100 <– ≤ 200	200 <– ≤ 500	> 500	
No. of cows examined	267	59	47	26	18	417	299	48	36	18	16	417
Percent	64.0	14.1	11.3	6.2	4.3	100	71.7	11.5	8.6	4.3	3.8	100
Normal	148	35	28	14	8	233	169	25	21	9	9	233
Percent	55.4	59.3	59.6	53.8	44.4	55.9	56.5	52.1	58.3	50.0	56.3	55.9
Abnormal	119	24	19	12	10	184	130	23	15	9	7	184
Percent	44.6	40.7	40.4	46.2	55.6	44.1	43.5	47.9	41.7	50.0	43.8	44.1
PLP	30	3	8	7	2	50	27	10	5	4	4	50
Percent	11.2 ^{xy}	5.1 ^x	17.0 ^{xy}	26.9 ^y	11.1 ^{xy}	12.0	9.0 ^a	20.8 ^b	13.9 ^{ab}	22.2 ^{ab}	25.0 ^{ab}	12.0
DFO-I	35	11	5	4	3	58	42	9	4	2	1	58
Percent	13.1	18.6	10.6	15.4	16.7	13.9	14.0	18.8	11.1	11.1	6.3	13.9
DFO-II	20	2	2	0	4	28	21	0	4	1	2	28
Percent	7.5 ^{ab}	3.4 ^a	4.3 ^a	0.0	22.2 ^b	6.7	7.0	0.0	11.1	5.6	12.5	6.7
CC	22	3	3	0	1	29	25	2	1	1	0	29
Percent	8.2	5.1	6.4	0.0	5.6	7.0	8.4	4.2	2.8	5.6	0.0	7.0
SLP	12	5	1	1	0	19	15	2	1	1	0	19
Percent	4.5	8.5	2.1	3.8	0.0	4.6	5.0	4.2	2.8	5.6	0.0	4.6

^{a, b}Numbers in the same rows with different superscripts differ ($P < 0.05$); ^{x, y}Numbers in the same rows with different superscripts differ ($P < 0.01$); PLP: prolonged luteal phase; DFO-I, DFO-II: delayed first ovulation type I, II; CC: cessation of cyclicity; SLP: short luteal phase

Table 2

Relationship between SCC in milk during early lactation and subsequent reproductive performance in dairy cows

	SCC in the first month post partum (× 1000/ml)					SCC in the second month post partum (× 1000/ml)					SCC in the third month post partum (× 1000/ml)				
	≤ 50	50 <– ≤ 100	100 <– ≤ 200	200 <– ≤ 500	> 500	≤ 50	50 <– ≤ 100	100 <– ≤ 200	200 <– ≤ 500	> 500	≤ 50	50 <– ≤ 100	100 <– ≤ 200	200 <– ≤ 500	> 500
No. of cows	267	59	47	26	18	299	48	36	18	16	279	58	33	24	23
AI submission rate within 100 days post partum (%)	78.8	86.4	76.6	69.2	66.7	80.3	75.0	75.0	66.7	81.3	82.1 ^a	72.4 ^{ab}	63.6 ^b	79.2 ^{ab}	69.6 ^{ab}
First AI conception rate (%)	47.6	44.1	40.4	23.1	33.3	47.5 ^a	27.1 ^b	41.7 ^a	27.8 ^{ab}	56.3 ^{ab}	47.7	39.7	33.3	41.7	30.4
Pregnancy rate within 100 days (%)	54.7	54.2	48.9	38.5	50.0	55.9	43.8	50.0	33.3	50.0	55.9	48.3	42.4	50.0	43.5
Pregnancy rate within 150 days (%)	80.3	87.7	76.1	76.0	62.5	80.2 ^a	67.4 ^{ab}	82.9 ^a	50.0 ^b	81.3 ^{ab}	82.0	78.2	71.0	73.9	75.0
Pregnancy rate within 210 days (%)	90.6	96.5	93.3	92.0	81.3	88.9	86.4	94.3	77.8	100.0	92.2	92.5	83.9	82.6	100.0
Calving to conception interval (Mean ± SE)	108.5 ± 34.0	100.3 ± 24.2	107.8 ± 27.6	110.4 ± 19.6	114.3 ± 36.5	104.3 ± 30.7 ^a	119.9 ± 35.1 ^{ab}	106.2 ± 24.7 ^a	144.6 ± 37.8 ^b	97.7 ± 31.3 ^a	104.5 ± 29.6 ^a	109.0 ± 28.2 ^{ab}	132.3 ± 38.5 ^b	116.0 ± 48.6 ^{ab}	107.6 ± 23.0 ^{ab}

^{a, b}Numbers in the same rows with different superscripts differ (P < 0.05); SE: Standard error

Table 3
Effects of parity and season of calving on the incidence of high SCC in postpartum cows

Factor	Category	No. of lactations	Percentage of cows based on the levels of SCC in the first month post partum (%)				Percentage of cows based on the levels of SCC in the second month post partum (%)				Percentage of cows based on the levels of SCC in the third month post partum (%)			
			≤ 50	50 < – ≤ 100	100 < – ≤ 200	> 200	≤ 50	50 < – ≤ 100	100 < – ≤ 200	> 200	≤ 50	50 < – ≤ 100	100 < – ≤ 200	> 200
			Parity	1	114	59.6	20.2	11.4	8.8 ^b	74.6	14.0	7.0	4.4 ^c	76.3
	2	109	70.6	11.9	10.1	7.3 ^b	76.1	11.0	8.3	4.6 ^{bc}	63.3	18.3	8.3	10.1 ^{bc}
	3	68	72.1	8.8	11.8	7.3 ^b	73.5	7.4	11.8	7.4 ^{abc}	67.6	13.2	8.8	10.3 ^{abc}
	4	51	64.7	13.7	7.8	13.7 ^{ab}	68.6	7.8	9.8	13.7 ^{ab}	68.6	9.8	7.8	13.7 ^{ab}
	5 or more	74	54.1	13.5	14.8	18.9 ^a	62.2	13.5	8.1	16.2 ^a	56.8	10.8	9.5	23.0 ^a
Season of calving	Spring	98	64.3	17.3	9.2	9.2	69.4	18.4	6.1	7.1	62.2	12.2	10.2	16.3
	Summer	111	60.4	13.5	13.5	12.6	66.7	9.9	11.7	11.7	61.3	18.0	9.0	11.7
	Autumn	129	64.3	14	11.6	10.1	78.3	7.8	6.2	7.8	71.3	12.4	7.8	8.5
	Winter	78	67.9	11.5	10.3	10.2	71.8	11.5	11.5	5.1	74.4	12.8	3.8	9.0

^{a, b, c}Values in a column in the same factor with different superscripts differ (P < 0.05)

Table 4
Reproductive performance of dairy cows with different types of abnormal resumption of ovarian cyclicity post partum

	Normal	Abnormal resumption of ovarian cyclicity				
		PLP	DFO-I	DFO-II	CC	SLP
No. of cows examined	233	50	58	28	29	19
AI submission rate within 100 days post partum (%)	82.0	76.0	84.5	67.9	69.0	63.2
First AI conception rate (%)	49.8 ^a	38.0 ^{ab}	31.0 ^b	32.1 ^{ab}	58.6 ^a	26.3 ^b
Pregnancy rate within 100 days (%)	60.9 ^a	46.0 ^{ab}	44.8 ^b	32.1 ^b	51.7 ^{ab}	26.3 ^b
Pregnancy rate within 150 days (%)	84.5	73.5	71.9	76.9	82.1	64.7
Pregnancy rate within 210 days (%)	94.6	87.8	89.3	84.6	85.7	93.8
Final pregnancy rate (%)	94.0	94.0	94.8	89.3	93.1	84.2
Calving to conception interval (Mean ± SE)	94.9 ± 25.6 ^a	119.7 ± 31.8 ^b	125.3 ± 38.4 ^b	127.3 ± 35.0 ^b	124.6 ± 44.9 ^b	125.4 ± 23.0 ^{ab}

^{a, b}Numbers in rows with different superscripts differ ($P < 0.05$); PLP: prolonged luteal phase; DFO-I, DFO-II: delayed first ovulation type I, II; CC: cessation of cyclicity; SLP: short luteal phase; SE: Standard error

It has been well documented that abnormal resumption of ovarian cyclicity post partum reduced reproductive performance in postpartum dairy cows (Lamming and Darwash, 1998; Shrestha et al., 2004b). Our results confirm that cows with abnormal ovarian cycles post partum had poor reproductive performance.

The results of this study suggest that cows with high SCC have a higher incidence of abnormal resumption of ovarian cyclicity post partum, which leads to reduced reproductive performance.

Acknowledgements

The first author is supported by the Japanese Government Scholarship for PhD study. The authors would like to thank the owners of herds involved in this study for their co-operation in milk sampling.

References

- Bacha, B. and Regassa, F. G. (2010): Subclinical endometritis in Zebu × Friesian crossbred dairy cows: its risk factors, association with subclinical mastitis and effect on reproductive performance. *Trop. Anim. Health. Prod.* **42**, 397–403.
- Barker, A. R., Schrick, F. N., Lewis, M. J., Dowlen, H. H. and Oliver, S. P. (1998): Influence of clinical mastitis during early lactation on reproductive performance of Jersey cows. *J. Dairy Sci.* **81**, 1285–1290.
- Concha, C. (1986): Cell types and their immunological functions in bovine mammary tissues and secretions – A review of the literature. *Nord. Vet. Med.* **38**, 257–272.
- Ferguson, J. D., Galligan, D. T. and Thomsen, N. (1994): Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci.* **77**, 2695–2703.
- Hagnestam-Nielsen, C., Emanuelson, U., Berglund, B. and Strandberg, E. (2009): Relationship between somatic cell count and milk yield in different stages of lactation. *J. Dairy Sci.* **92**, 3124–3133.
- Hansen, P. J., Soto, P. and Natzke, R. P. (2004): Mastitis and fertility in cattle – possible involvement of inflammation or immune activation in embryonic mortality. *Am. J. Reprod. Immunol.* **51**, 294–301.
- Huszenicza, G., Jánosi, Sz., Kulcsár, M., Kóródi, P., Reiczigel, J., Kátai, L., Peters, A. R. and De Rensis, F. (2005): Effects of clinical mastitis on ovarian function in postpartum dairy cows. *Reprod. Domest. Anim.* **40**, 199–204.
- Isobe, N., Yoshimura, T., Yoshida, C. and Nakao, T. (2004): Incidence of silent ovulation in dairy cows during post partum period. *Dtsch. Tierärztl. Wochenschr.* **111**, 35–38.
- Laevens, H., Deluyker, H., Schukken, Y. H., De Meulemeester, L., Vandermeersch, R., De Muele-naere, E. and De Kruif, A. (1997): Influence of parity and stage of lactation on the somatic cell count in bacteriologically negative dairy cows. *J. Dairy Sci.* **80**, 3219–3226.
- Lamming, G. E. and Darwash, A. O. (1998): The use of milk progesterone profiles to characterise components of subfertility in milked dairy cows. *Anim. Reprod. Sci.* **52**, 175–190.
- Lavon, Y., Leitner, G., Voet, H. and Wolfenson, D. (2010): Naturally occurring mastitis effects on timing of ovulation, steroid and gonadotropic hormone concentrations, and follicular and luteal growth in cows. *J. Dairy Sci.* **93**, 911–921.

- Lim, J. M. and Hansel, W. (1998): Improved development of *in vitro*-derived bovine embryos by use of a nitric oxide scavenger in a cumulus-granulosa cell coculture system. *Mol. Reprod. Dev.* **50**, 45–53.
- Ling, K., Waldmann, A., Samarutel, J., Jaakson, H., Kaart, T. and Leesmae, A. (2007): Field trial on the relationship of blood metabolites and body condition score with the recurrence of luteal activity in Estonian Holstein cows. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* **54**, 337–341.
- Moore, D. A., Cullor, J. S., Bondurant, R. H. and Sischo, W. M. (1991): Preliminary field evidence for the association of clinical mastitis with altered interestrus intervals in dairy cattle. *Theriogenology* **36**, 257–265.
- Morris, M. J., Walker, S. L., Jones, D. N., Routly, J. E., Smith, R. F. and Dobson, H. (2009): Influence of somatic cell count, body condition and lameness on follicular growth and ovulation in dairy cows. *Theriogenology* **71**, 801–806.
- Nakao, T., Moriyoshi, M. and Kawata, K. (1992): The effect of post-partum ovarian dysfunction and endometritis on subsequent reproductive performance in high and medium producing dairy cows. *Theriogenology* **37**, 341–349.
- Opsomer, G., Coryn, M., Deluyker, H. and De Kruif, A. (1998): An analysis of ovarian dysfunction in high yielding dairy cows after calving based on progesterone profile. *Reprod. Domest. Anim.* **33**, 193–204.
- Pampfer, S. (2001): Dysregulation of the cytokine network in the uterus of the diabetic rat. *Am. J. Reprod. Immunol.* **45**, 375–381.
- Pampfer, S., Vanderheyden, I., McCracken, J. E., Vesela, J. and De Hertogh, R. (1997): Increased cell death in rat blastocyst exposed to maternal diabetes *in utero* and to high glucose or tumor necrosis factor- α *in vitro*. *Development* **124**, 4827–4836.
- Pinedo, P. J., Melendez, P., Villagomez-Cortes, J. A. and Risco, C. A. (2009): Effect of high somatic cell counts on reproductive performance of Chilean dairy cattle. *J. Dairy Sci.* **92**, 1575–1580.
- Ranasinghe, R. M. S. B. K., Nakao, T., Yamada, K., Koike, K., Hayashi, A. and Dematawewa, C. M. B. (2011): Characteristics of prolonged luteal phase identified by milk progesterone concentrations and its effects on reproductive performance in Holstein cows. *J. Dairy Sci.* **94**, 116–127.
- Riollet, C., Rainard, P. and Poutrel, B. (2000): Cells and cytokines in inflammatory secretions of bovine mammary gland. *Adv. Exp. Med. Biol.* **480**, 247–258.
- Risco, C. A., Donovan, G. A. and Hernandez, J. (1999): Clinical mastitis associated with abortion in dairy cows. *J. Dairy Sci.* **82**, 1684–1689.
- Santos, J. E. P., Cerri, R. L. A., Ballou, M. A., Higginbotham, G. E. and Kirk, J. H. (2004): Effect of timing of first clinical mastitis occurrence on lactational and reproductive performance of Holstein dairy cows. *Anim. Reprod. Sci.* **80**, 31–45.
- Schrick, F. N., Hockett, M. E., Saxton, A. M., Lewis, M. J., Dowlen, H. H. and Oliver, S. P. (2001): Influence of subclinical mastitis during early lactation on reproductive parameters. *J. Dairy Sci.* **84**, 1407–1412.
- Sheldon, I. M., Price, S. B., Cronin, J., Gilbert, R. O. and Gadsby, J. E. (2009): Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle. *Reprod. Domest. Anim.* **44**, 1–9.
- Sheldrake, R. F., Hoare, R. J. T. and McGregor, G. D. (1983): Lactation stage, parity, and infection affecting somatic cells, electrical conductivity, and serum albumin in milk. *J. Dairy Sci.* **66**, 542–547.
- Shrestha, H. K., Nakao, T., Higaki, T., Suzuki, T. and Akita M. (2004a): Resumption of ovarian cyclicity in high-producing Holstein cows. *Theriogenology* **61**, 637–649.
- Shrestha, H. K., Nakao, T., Suzuki, T., Higaki, T. and Akita, M. (2004b): Effect of abnormal ovarian cycles during pre-service period post-partum on subsequent reproductive performance of high-producing Holstein cows. *Theriogenology* **61**, 1559–1571.

- Smith, K. L. (1996): Standards for somatic cells in milk: physiological and regulatory. International Dairy Federation Mastitis Newsletter, September 1996, pp. 7–9.
- Soto, P., Natzke, R. P. and Hansen, P. J. (2003): Identification of possible mediators of embryonic mortality caused by mastitis: actions of lipopolysaccharide, prostaglandin F_{2α}, and the nitric oxide generator, sodium nitroprusside dihydrate, on oocyte maturation and embryonic development in cattle. *Am. J. Reprod. Immunol.* **50**, 263–272.
- Van Den Borne, B. H. P., Van Schaik, G., Lam, T. J. G. M. and Nielen, M. (2010): Variation in herd level mastitis indicators between primi- and multiparae in Dutch dairy herds. *Prev. Vet. Med.* **96**, 49–55.
- Yagi, Y., Shiono, H., Chikayama, Y., Ohnuma, A., Nakamura, I. and Yayou, K. I. (2004): Transport stress increases somatic cell counts in milk, and enhances the migration capacity of peripheral blood neutrophils of dairy cows. *J. Vet. Med. Sci.* **66**, 381–387.