

Postpartum Reproductive Management Based on the Routine Farm Records of a Dairy Herd: Relationship between the Metabolic Parameters and Postpartum Ovarian Activity

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ABSTRACT : The aim of this study was 1) to confirm the practical efficiency of a routine milk P4 monitoring system for postpartum reproductive management of a dairy herd, and 2) to evaluate the relationship between the blood metabolic profiles, milk quality and body weight of individual cows in the farm records, which may reflect the postpartum nutritional condition, and the time of postpartum resumption of ovarian activity of dairy cows. A total of 116 Holstein cows was used in the present study. First, during the period of Experiment 1, postpartum reproductive management based on weekly measured milk P4 concentration from individual cows was conducted. Compared with the reproductive records of the past two years without P4 monitoring, although the day from calving to first AI did not change, both the number of AI until pregnant (with P4; 1.9 times vs. without P4; 2.9 times) and the days open (with P4; 95.1 days vs. without P4; 135.8 days and 133.8 days) were significantly decreased. In Experiment 2, the measurement of blood constituents such as albumin, blood urea nitrogen, packed cell volume, ammonia, glucose, total cholesterol, non-esterified, AST and γ -GTP was performed on the blood samples taken once approximately 14 days postpartum, to monitor both health and nutritional conditions. The milk constituent parameters, such as milk protein (MP), milk fat (MF), SNF and lactose, collected from the monthly progeny test of individual cows, were used to monitor the postpartum nutritional status. Furthermore, the data obtained from the routine measurements of body weight were used to calculate the rate of peripartum body weight loss. The resumption day of the postpartum estrous cycle was assumed from the milk P4 profiles of individual cows. There was no clear relationship between each parameter from blood examination and those from resumption time. However, the cows had low values of MP, and SNF, which significantly affected the resumption of the postpartum estrous cycle. Similarly, a higher rate of body weight loss indicated a significant delay (more than 1 month) in the resumption of the postpartum estrous cycle, compared with the groups that had a medium or lower rate of body weight loss. The results of the present study demonstrated that the implementation of routine milk P4 monitoring-based postpartum reproductive management, together with milk quality parameters and routine BW data available in field conditions may be utilized as a practical approach for increasing the postpartum reproductive efficiency of a high yielding dairy herd. (*Asian-Aust. J. Anim. Sci.* 2005. Vol 18, No. 6 : 787-794)

Key Words : Dairy Herd, Reproductive Management, Metabolic Parameters, Ovarian Activity

INTRODUCTION

It is well accepted that a 365-day calving interval is an ideal goal for the reproductive management of a dairy herd. Therefore, early resumption of postpartum ovarian cyclicity is important for re-breeding cows, and attaining the pregnancy as early as possible. Practical approaches, such as improvement of estrus detection (Van Vliet and Van

Eerdenburg, 1996; Lyimo et al., 2000) or estrus/ovulation synchronization (Pursley et al., 1997; Keister et al., 1999; Pursley et al., 1999; Tallam et al., 2001), have been developed to improve the postpartum reproductive efficiency. Despite these efforts, reproductive efficiency has declined during the past two decades, in parallel with marked increases in milk production (Beam and Butler, 1999; Roche et al., 2000; Lucy, 2001). The recent consensus on this reduction is related to the increased incidence of negative energy balance (NEB) in the peri- and early postpartum periods (Beam and Butler, 1999; Rukkwamsuk et al., 1999; Butler, 2000; Roche et al., 2000), and this NEB may adversely impact postpartum health and fertility (Royal et al., 2000; Jorritsma et al., 2003).

One possible strategy for improving the reproductive efficacy of a dairy herd is the early detection of ovarian abnormalities. P4 profile analysis, especially in milk samples, has been suggested as an important method for evaluating ovarian function objectively (Lamming and

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Darwash, 1998; Opsomer et al., 1998; Royal et al., 2000). Thus, serial milk P4 profile analysis for early detection of ovarian abnormalities may be useful for practicing appropriate remedies to achieve early resumption of postpartum ovarian activity. However, the use of continuous serum or milk P4 profiles in postpartum reproductive management is still not well practiced.

On the other hand, both the metabolic profile test (MPT) as well as the body condition scoring (BCS) system have been reported to be useful methods for evaluating body energy reserves, and were used for evaluating the nutritional status in dairy cows (Wildman et al., 1982; Hady et al., 1994; Lopez-Gatius et al., 2003). Generally, the high-energy requirement at the onset of lactation results in an NEB condition that usually reaches its lowest level about 2 weeks postpartum (Butler, 2000). Indeed, in dairy cows, significant relationships have been reported between postpartum reproductive performance and changes in the metabolic parameters (Huszenicza et al., 1988; Ruegg et al., 1992; Butler, 1998; Kim and Suh, 2003; Pushpakumara et al., 2003).

Thus, the objective of this study was 1) to evaluate the efficiency of routine milk P4 monitoring-based reproductive management in acquiring early resumption of postpartum ovarian activity and thus, improving the reproductive efficacy of the postpartum dairy herd, and 2) to evaluate the relationship between the time of postpartum resumption of ovarian activity, and the nutritional status of the cow. The data available from farm records on blood metabolic profiles, milk quality parameters and body weight of individual cows were used for evaluating the nutritional status.

MATERIALS AND METHODS

Animals and herd

The study was conducted in the Field Center of Animal Science and Agriculture of Obihiro University of Agriculture and Veterinary Medicine in Hokkaido Prefecture of Japan. The ambient temperature there ranges from -20°C to 35°C from winter to summer. The cows were housed in free-stall facilities throughout the year and fed a mixture of grass silage and concentrate twice a day, with mineral supplements according to their requirements for maintenance and production. The cows were machine-milked twice daily, and the average 305-day fat-corrected milk yield in the preceding lactation was more than 8,500 kg. In total, 116 Holstein Friesian dairy cows, which calved from November 2000 to December 2002, were used in the present study.

Postpartum clinical examination

Within 24 h after the parturition, a general clinical

examination was conducted for each cow. Postpartum metabolic diseases, such as milk fever and ketosis, were diagnosed based on the definitions used in previous reports, and were treated immediately according to the routine treatment (Correa et al., 1993; Domecq et al., 1997; Loeffler et al., 1999). Additionally, the cows diagnosed with mastitis during the sampling periods based on both the California Mastitis Test (CMT) and bacterial examinations were treated with intra-mammary infusions of sensitive antibiotics. No cows were diagnosed with abomasal displacement, downer cow syndrome, fatty liver or retained placenta.

Experiment 1

Milk sampling and P4 measurements : Milk samples for the P4 measurement of the cows were collected twice weekly, from 2 weeks after calving until the time for the establishment of the next pregnancy. About 10 ml after-milk was collected in plastic tubes at morning milking (before morning feeding) from healthy udder quarters. The skim milk from milk samples was separated by centrifugation at 1,500×g for 30 min at 4°C, and stored at -30°C until P4 assay. To extract P4, 200 µl skim milk sample was added to 800 µl diethyl ether (1:5/v:v), and the mixture was vortexed for 30 min. The samples were then allowed to stand for 15 min and placed in a -30°C freezer overnight. The upper diethyl ether fraction was decanted and evaporated. The residue was dissolved in 200 µl assay buffer (40 mM PBS, 0.1% BSA, pH 7.2). The average recovery rate obtained was 85%.

For the quantitative determination of P4 concentrations, double antibody enzyme immunoassays (EIAs) were performed using 96-well ELISA plates (Nalge Nunc International, Denmark) coated with 50 µl anti-rabbit IgG (Seikagaku Co., Tokyo, Japan). The method for the EIA of the milk samples was based on the blood P4 EIA described elsewhere (Miyamoto et al., 1992) with minor modifications. Basically, 30 µl standard or sample was incubated with 100 µl polyclonal antibody solution (1:100,000) and 100 µl P4-horseradish peroxidase (1:20,000; P4 HRP) for 24 h at 4°C. The range of standards was 0.05-50 ng/ml and the effective dose 50 (ED 50) was 3.2 ng/ml. The intra- and interassay coefficients of variation of the assay were 6.2 and 9.3%, respectively. To assess our EIA assay system established for measuring the P4 concentration in milk samples, tail vein blood samples from 9 cows were also collected simultaneously with milk samples, and P4 concentration was measured as described previously (Miyamoto et al., 1992). The P4 concentrations from milk samples were highly correlated ($Y = 0.9499X + 0.751$, $R = 0.91$) with the tail vein plasma samples obtained at the same sampling time.

In individual cows, milk samples collected were stored

during the VWP (until 40 days postpartum) and P4 concentrations were measured at the same time in a single assay (first P4 assay). The P4 concentrations of consecutive bi-weekly milk samples were measured within the same week and added to the individual P4 profiles during the same week.

Reproductive management of cows : During the period of Experiment 1, postpartum reproductive management was started from day 30 after calving. Basically, during the first postpartum examination (fresh check), both the uterine involution and ovarian activity were monitored by vaginoscopy and per-rectal palpation of the genitalia. Uterine involution was considered to be completed when the sizes of both horns were similar. Immediately after this fresh check, tail paint was smeared on the top-line over the rump of each cow, to assist in the heat detection.

Based on the P4 profiles from the first P4 assay, the cows were grouped into 2 categories according to previous reports (Nakao et al., 1992; Opsomer et al., 1998): 1) Cows that had P4 concentrations of more than 2 ng/ml for at least three consecutive milk samples; such cows were considered to have luteal activity, and thus, the postpartum resumption of ovarian activity. Their next estrus was predicted based on changes in the P4 profiles. 2) Cows that did not have P4 concentrations of more than 2 ng/ml; such cows were considered to have not had any luteal activity during the VWP and were diagnosed with postpartum anestrus. These cows were treated with GnRH analogue (fertirelin acetate, 100 µg; IM) or CIDR (Beal, 1996) to induce the next ovulation. All cows that exhibited estrous behavior with or without treatments were artificially inseminated, and pregnancy diagnosis was performed 40 days after artificial insemination (AI) using an ultrasound scanner.

Experiment 2

Blood collection and analysis : Data on the blood metabolic profiles of individual cows were also obtained from the farm records. Blood examinations were routinely conducted once at the peak stage of lactation (approximately 14 days after the parturition) as a part of the postpartum clinical examination. Indicators used to assess the status of protein metabolism were packed cell volume (PCV), albumin (Alb), ammonia (NH₃) and blood urea nitrogen (BUN), and for energy metabolism were glucose (Glu), total cholesterol (T-cho) and non-esterified fatty acids (NEFA). As indicators for liver function, serum aspartate aminotransferase (AST) and γ -glutamyl transpeptidase (γ -GTP) were used.

Jugular vein blood samples (10 ml) were collected from each cow into vacutainer tubes, approximately 2 h after morning feeding. The vacutainers for Glu analysis contained NaF and Heparin; for NH₃ they contained

deproteinization medium; and for PCV, EDTA-2K. Plain vacutainer tubes were used to collect blood for other measured substances. All collected blood samples were immediately put on ice. Both plain tubes and tubes for measuring NH₃ were centrifuged for 15 min at 1500 × g, within 30 min of collection. The metabolic profiles were measured at Tokachi Clinical Diagnosis Laboratory, Ltd. (Obihiro, Japan), using automatic biochemical analyzers (No. 7450; Hitachi Med., Japan, Bio Magesty 2250; Nihon Kohden, Japan and SE 9000; SYSMEX, Japan).

Milk examination : The data on milk fat (MF), solid non-fat (SNF), milk protein (MP) and lactose (LAC) content were obtained from farm records, and were routinely collected for the dairy herd in the progeny test at the beginning of each month. The measurements were done at the Laboratory Section of Tokachi Federation of Agriculture Cooperation (Obihiro, Japan), using an automatic milk component analyzer (FOSS SYSTEM 4000; Foss Electric, Inc.; Denmark). To avoid the individual variation of days from calving to examination, data obtained around 30 days (first or second postpartum milk examination) were used to compare MF, SNF, MP and LAC content.

Measurement of body weight : Data on the body weight of individual cows were also obtained from the farm records, and were routinely collected for the dairy herd at the time of the milk examinations. The body weight loss (BWL) before and after parturition was calculated according to the following formula.

$$\text{BWL (\%)} = 100 - \frac{\text{BW after parturition}}{\text{BW before parturition}} \times 100$$

The first or second BW measurement postpartum was selected for BW after parturition, to maintain at least 25 to 40 days between two BW measurements.

Data handling and statistical analysis : Reproductive records such as those of the day of the first AI after parturition, the number of AI until pregnancy, and the days open period of the cows (n = 20) after implementation of the P4 monitoring system were compared with those of the cows calved at the same period during the previous 2 years (before implementation of the P4 monitoring system), using ANOVA and post-hoc tests.

In the data analysis of blood metabolic status and BWL, the mean for each parameter of the herd was calculated. A reference range was calculated for each parameter; the upper value was obtained by adding SD to the mean, and the lower value was obtained by deducting the SD from the mean (mean ± 1 SD). The cows were divided into 3 groups: 1) within the reference range, 2) above the reference range,

Table 1. Efficiency of new reproductive management system over the past 2 years (January to April of each year)

Year	Number of Animals	Day from parturition to first AI	Number of AI	Days open
1999	11	74.5±6.5	2.6±0.4	135.8±17.1 ^a
2000	19	66.1±3.3	2.9±0.4 ^a	133.8±15.2 ^a
2001	20	66.3±3.9	1.9±0.2 ^b	95.1±7.6 ^b

^{a, b} Values within the column are significantly different ($p < 0.05$).

AI: Artificial insemination.

and 3) below the reference range. In the data analysis for MF, SNF, MP and Lac, the cows were divided into 2 groups depending on whether the values fell above or below the general standard. The standard values used in the present study were 3.5% for MF, 8.5% for SNF, 3.0% for MP, and 4.5% for LAC, according to previous experimental results (Yu et al., 1998; Robinson et al., 1999). The days spent before the resumption of postpartum ovarian activity between each group were compared using the unpaired t-test with STATVIEW computer software (Abacus Concepts, Inc., Berkeley, CA). Probabilities less than 0.05 ($p < 0.05$) were considered significant.

RESULTS

Experiment 1

Postpartum resumption of ovarian activity : The prediction of the postpartum resumption of ovarian activity was possible using the milk P4 profiles. Among the 50 cows examined, 37 cows (74.0%) showed raised P4 profiles at the first milk P4 assay, and thus indicating the postpartum resumption of ovarian activity. The first postpartum P4 rise was observed at day 35.3±1.5 (mean±SEM). The length of the first postpartum estrous cycle was 18.9±0.6 days (mean±SEM) and tended to be shorter than that afterward (21.6±0.3 days mean±SEM). The remaining 13 cows (26.0%) were considered to have not had any luteal activity during the VWP. Eleven of these 13 cows were treated with CIDR. After the treatment, 8 of the cows (72.7%) resumed their normal estrous cycle, and the first postpartum P4 rise was observed at day 69.5±3.0 (mean±SEM). The 3 cows that did not respond to CIDR were re-treated, and they responded to the second treatment. Their first postpartum P4 rise was observed at day 127.0±23.4 (mean±SEM).

According to the milk P4 profiles, 5 (10% of the total; 5/50) of the 37 cows considered to have had postpartum resumption of ovarian activity during the VWP were diagnosed with prolonged CL. These cows were immediately treated with PGF2 α . The first P4 rise of the cows diagnosed with prolonged CL was observed at day 27.6±1.9 (mean±SEM), which was rather earlier than that of the cows with normal cyclicity. After the PGF2 α treatment, 2 of the cows resumed the normal estrous cycle, but the remaining 3 cows were again diagnosed with prolonged CL based on the subsequent milk P4 profiles.

Efficiency of reproductive management with the milk P4 monitoring system : The reproductive parameters, such as days to first postpartum AI, number of AI and the days open before and after implementation of reproductive management based on milk P4 profiles, are shown in Table 1. The number of AI and the days open were significantly reduced after the implementation of milk P4 profile-based reproductive management. However, the interval from calving to first AI was not changed.

Experiment 2

In the present study, there were no significant differences in the postpartum resumption day between the cows diagnosed with mastitis within 30 days after calving (43.2±23.8 days; $n = 48$) and the normal cows (43.9±24.4 days; $n = 68$).

Blood examinations were conducted for 81 of the 116 cows in the present study. The data on each blood parameter examined are shown in Figure 1. There were no significant differences in postpartum resumption day in any blood parameter examined among the 3 groups.

The relationship between each parameter of milk examination of the 116 cows and the resumption of their ovarian activity is shown in Figure 2. The resumption of ovarian activity of the cow group with MP levels higher than 3.0% (38.8 days) was significantly earlier than that of the group with lower levels (50.5 days). Additionally, in the group with SNF levels higher than 8.5% (39.8 days), the ovarian resumption occurred significantly earlier, than that of the group with lower levels (56.2 days). However, no significant difference was observed for MF or LAC. Moreover, a higher number of cows had MP (67.6%), MF (65.7%) and SNF (62.9%) levels above the reference range and resumed postpartum ovarian activity within 40 days postpartum, compared to those with levels of MP (37.5%), MF (39.1%) and SNF (29.6%) below the reference range.

The relationship between the data of BWL from the 116 cows and the resumption of their ovarian activity is shown in Figure 3. The postpartum ovarian resumption day of the upper group was significantly later (59.1±7.4) than those of the other 2 groups (lower: 35.9±3.5, and range: 41.5±2.4). Additionally, the percentages of cows that resumed ovarian activity within 40 days after parturition of both the lower (70.8%) and range (57.1%) groups were significantly higher than that of the upper group (31.8%).

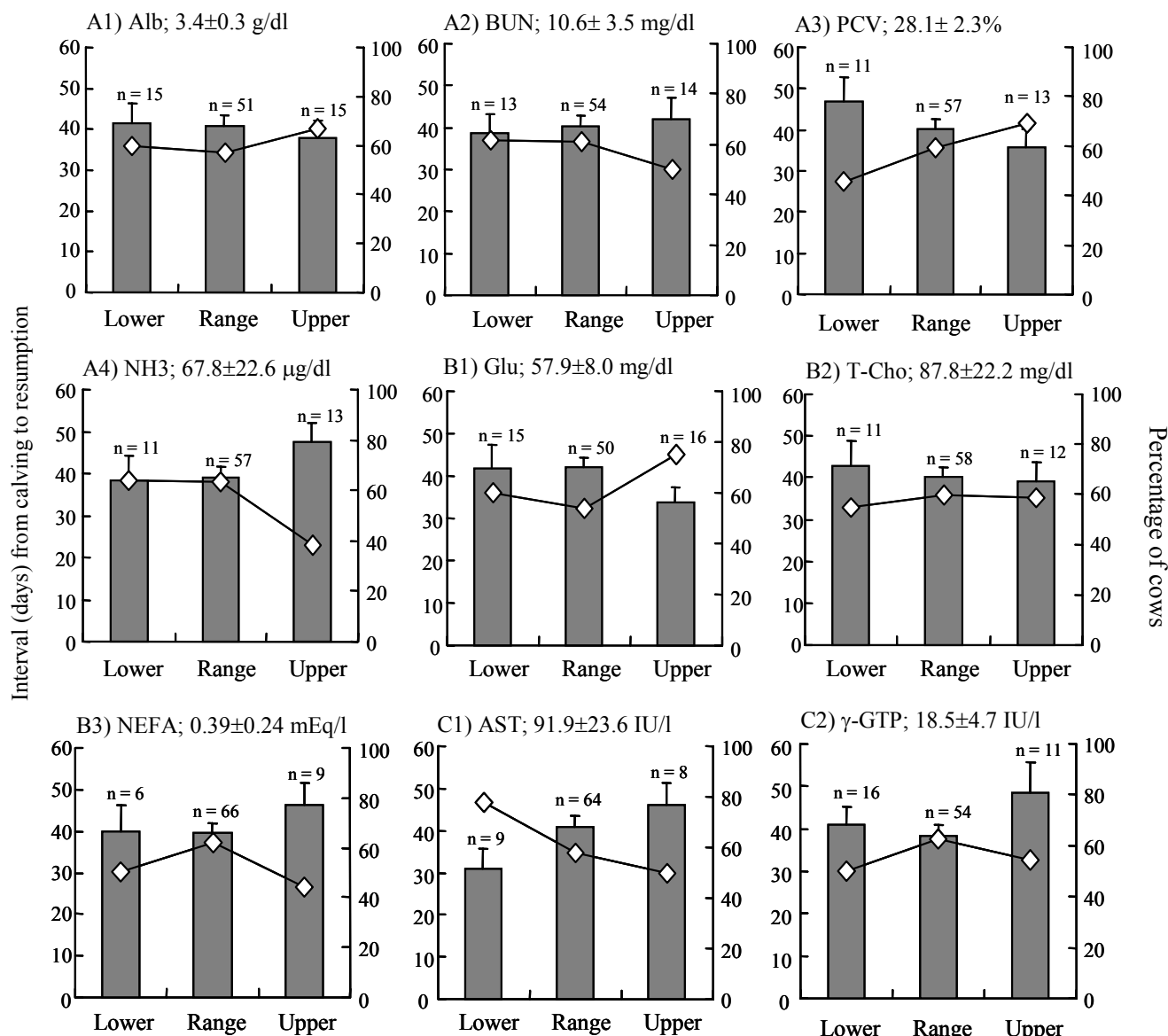


Figure 1. Relationship between blood metabolic parameters and the postpartum resumption of ovarian activity of the cows. ■ : interval (days) from calving to resumption of ovarian activity. ◇ : percentage of the cows that resumed ovarian activity within 40 days after calving. Range, within the reference range (mean±1 SD); Upper, above the reference range; and Lower, below the reference range. Parameters used to assess the status of protein metabolism were A1) albumin (Alb), A2) blood urea nitrogen (BUN), A3) packed cell volume (PCV) and A4) ammonia (NH3); those for energy metabolism were B1) glucose (Glu), B2) total cholesterol (T-Chol) and B3) non-esterified fatty acids (NEFA); and those for liver functions were C1) serum aspartate aminotransferase (AST) and C2) γ-glutamyl transpeptidase (γ-GTP). The mean (±SEM) value for each parameter is indicated above each figure.

DISCUSSION

The results of the present practical study clearly demonstrated that the implementation of routine milk P4 monitoring-based postpartum reproductive management shortened the days open and reduced the number of AI. The cows with high profiles of MP, MF and SNF, had early postpartum resumption of ovarian activity. Moreover, the cows with higher BWL had late postpartum resumption of ovarian activity.

Opsomer et al. (1998) reported that approximately 90%

of the ovarian functional abnormalities of postpartum dairy cows were due to anestrus and prolonged CL. The results of the present study also indicated that both anestrus and prolonged CL accounted for 94.7% of the ovarian functional abnormalities. Using the milk P4 profiles, all anestrus cows were detected during the VWP (with 40 days), although at least 2 more P4 profiles are needed to detect prolonged CL or irregular cyclicity (approximately 60 days postpartum). It is generally accepted that anovulation in dairy cows during the postpartum VWP period occurs due to the failure of dominant follicles to ovulate, rather than to

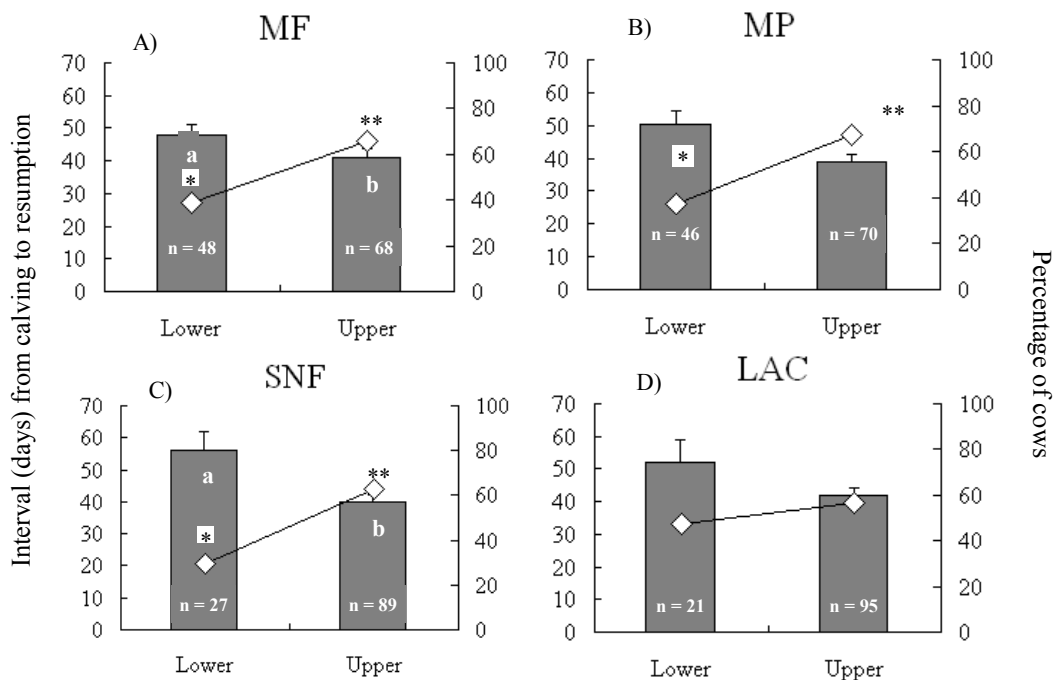


Figure 2. Relationship between milk parameters and the resumption of postpartum ovarian activity of the cows. ■ : interval (days) from calving to resumption of ovarian activity. ◇ : percentage of cows that resumed ovarian activity within 40 days after calving. Upper: values falls above the general standard; Lower, values falls below the general standard. Milk parameters observed were A) milk protein (MP), B) milk fat (MF), C) solid non-fat (SNF) and D) lactose (LAC). The general standard value for each parameter is indicated above each figure. a, b: Significantly different (day), * ** Significantly different (%).

their absence (Roche et al., 2000). From the results of the first P4 assay, the status of ovarian activity of each cow during the VWP was revealed.

The first AI within 85 days after parturition is fundamental for the 1-calf-per-year concept. In the present study, 75% of the treated cows resumed their estrous cycles, and underwent the first postpartum AI within 85 days after parturition. Moreover, the prediction of the next estrus and the close observation of heat signs became possible using the milk P4 profiles. As such, a significant improvement of postpartum reproductive efficiency, both in the reduction of the number of AI and the days open period, was obtained with the routine milk P4 monitoring system during the early postpartum period. Thus, the routine milk P4 monitoring system may be utilized as a practical approach for the reproductive management of a high yielding dairy herd.

It has been reported that MF tends to increase while MP tends to decrease during the period of postpartum NEB, and suggested that the data of milk examinations may be useful for monitoring feed intake and nutrient balance (Grieve et al., 1986). In the present study, the cows with high profiles of MP, MF and SNF had early postpartum resumption of ovarian activity. Thus, available data on MP, MF and SNF, which are routinely collected in dairy enterprises for milk quality assessment in progeny testing, can be efficiently utilized as useful parameters for identifying the nutritional status of a cow, and thus as indicators for avoiding late

postpartum resumption of ovarian activity due to nutritional imbalance conditions.

On the other hand, the BCS system is widely used to evaluate the nutritional status in dairy cows, and it is suggested that BWL during the pre- and postpartum periods may affect reproductive performance and increase susceptibility to postpartum diseases (Butler, 2000; Pryce et al., 2001; Kim et al., 2003). Moreover, some previous studies have shown that a prolonged interval from calving to the first ovulation is associated with an excessive postpartum BWL (Heinonen et al., 1988; Pushpakumara et al., 2003; Taylor et al., 2003). The present results also clearly indicated that BWL during the postpartum period was positively correlated with late resumption of ovarian activity. The available records of BW at the farm, which had been measured as a routine management practice, were evaluated in the present study. Thus, similar to the milk quality parameters, routine BW data at the farm might be employed as a useful practical indicator for predicting postpartum ovarian activity.

In the present study, albumin, BUN, NH₃ and PCV were determined as indicators of protein metabolism, and revealed that no significant relationships were observed between any parameter. Indeed, it has been reported that the intake of high dietary CP resulted in elevated blood concentrations of NH₃ and urea, and suggested that high CP in the diet does not have a strong impact on the resumption

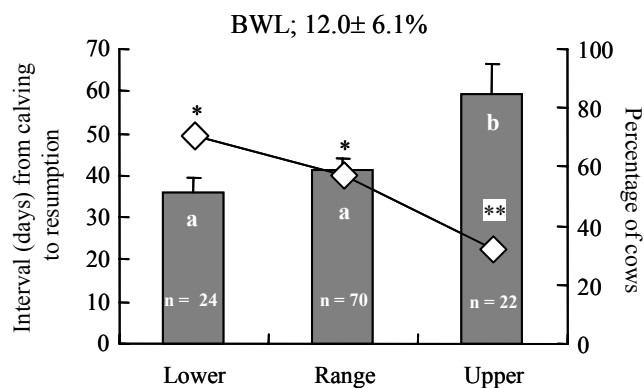


Figure 3. Relationship between the body weight loss (BWL) and the resumption of postpartum ovarian activity of the cows. ■ : interval (days) from calving to resumption of ovarian activity. ◇ : percentage of the cows that resumed ovarian activity within 40 days after calving. Range, within the reference range (mean±1 SD); Upper, above the reference range; and Lower, below the reference range. The mean (±SEM) value for each parameter is indicated above each figure. a, b: Significantly different (days). * ** Significantly different (%).

of postpartum ovarian activity, but does affect the conception rate (Butler, 1998). Similarly, in the present study, Glu, T-Chol and FA were determined as indicators of energy metabolism while AST and γ -GTP were determined as indicators of liver function, and no significant relationships were observed among them. Thus, the results of the present study demonstrated that metabolic parameters derived from one blood sampling at the nadir period of NEB might not be a practical indicator for monitoring the postpartum ovarian activity in a dairy herd. On the other hand, the levels of blood components reflect not only the nutritional status but also the health and physiological condition of each cow. Therefore, blood metabolic parameter examination at the nadir period of NEB, at least, might be a useful option for the detection of postpartum health conditions which may ultimately result in the delayed resumption of ovarian activity of the dairy herd.

The clinical status or occurrence of metabolic diseases or mastitis during the early lactation period did not affect the postpartum ovarian activity of the examined cows. Because the cows showing clinical symptoms at this stage received suitable treatment based on the clinical examinations, these results strongly suggest the effectiveness of the proper diagnosis and treatment of diseases, not only for recovery from the disease, but also for the postpartum resumption of ovarian activity.

The results of the present study demonstrated that the implementation of routine milk P4 monitoring-based postpartum reproductive management, together with milk quality parameters and routine BW data available at the farm, may be utilized as a practical approach to increasing

the postpartum reproductive efficiency of a high yielding dairy herd.

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