Association of rumen fill score and energy status during the close-up dry period with conception at first artificial insemination in dairy cows

著者（英）	Kawashima Chiho, Karaki Chihiro, Munakata Megumi, Matsui Motozumi, Shimizu Takashi, Miyamoto Akio, Kida Katsuya
journal or publication title	Animal Science Journal
volume	87
number	10
page range	1218-1224
year	2016-10
URL	http://id.nii.ac.jp/1588/00001219/

doi: info:doi/10.1111/asj.12555

Title: Association of rumen fill score and energy status during the close-up dry period with conception at first artificial insemination in dairy cows

Authors: Chiho KAWASHIMA[1], Chihiro KARAKI[1], Megumi MUNAKATA[1], Motozumi MATSUI[2], Takashi SHIMIZU[3], Akio MIYAMOTO[3], and Katsuya KIDA[1]

Institute, address, country: [1]*Field Center of Animal Science and Agriculture,* [2]*Department of Applied Veterinary Medicine, and* [3]*Graduate School of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan*

Running Head: PREPARTUM RFS AND CONCEPTION AT 1st AI

Correspondence: Chiho Kawashima, Field Center of Animal Science and Agriculture,, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, 080-8555 Japan.

Phone: +81-155-49-5653; Facsimile: +81-155-49-5653; Email: kawasima@obihiro.ac.jp

ABSTRACT

Recent studies have shown significant associations between prepartum energy status and postpartum fertility in dairy cows; therefore, the assessment of energy status by blood metabolites and metabolic hormones and suitable improvement of management during prepartum period may enhance reproductive performance. Rumen fill score (RFS) is associated with feed intake; however, it is unknown whether RFS is also related to blood parameters. Therefore, this study investigated the relationship between RFS and energy status during prepartum period, and their associations with conception at first artificial insemination (AI) after parturition. In 42 multiparous Holstein cows, RFS assessment and blood sampling were carried out twice a week during 3 weeks of peripartum period. Ovarian cycles until AI were evaluated by measuring milk progesterone levels. Before calving, positive correlations were observed between RFS and total cholesterol, and RFS did not change in pregnant cows at first AI after parturition, whereas in non-pregnant cows, RFS decreased gradually as the calving day approached. After calving, non-pregnant cows showed lower energy status compared with pregnant cows, and some non-pregnant cows showed anovulation and cessation of estrous cycle. In conclusion, RFS during close-up dry period is related to real-time energy status, and is associated with postpartum energy status and conception at first AI in dairy cows.

Key words: *dairy cow, dry period, first artificial insemination, rumen fill score.*

INTRODUCTION

Milk production per cow has steadily increased in recent years owing to improved management practices and genetic selection; however, reproductive efficiency (e.g., calving interval, services per conception) has declined with this increase in milk production. One of the major causes of declined reproductive performance in high-yielding dairy cows is a severe negative energy balance (NEB). Modern high-producing dairy cows undergo a period of severe NEB during the early postpartum period, because the energy required for milk production and maintenance of tissue function exceeds the energy uptake during this period (Beam & Butler 1999; Lucy 2001). Butler and Smith (1989) observed that the levels of NEB were directly related to the postpartum interval to first ovulation. The resumption of ovarian activity plays a crucial role in subsequent fertility, in which earlier resumption of ovarian function is related to higher fertility (Staples *et al.* 1990; Senatore *et al.* 1996; Darwash *et al.* 1997; Kawashima *et al.* 2006).

Thus, to improve reproductive performance, most previous studies investigated the relationship between energy and metabolic status during the early postpartum period and the resumption of ovarian activity and fertility after parturition. In addition, recent studies have shown associations between energy and metabolic status during the prepartum period and fertility after calving. de Feu *et al.* (2009) showed evidence that eliminating the dry period advanced the first ovulation postpartum, but feeding a higher-energy diet over 12 weeks (wk) postpartum had no effect on the onset of cyclicity in the cows. Furthermore, Cavestany *et al.* (2009a, b) indicated that feeding a high-supplemented prepartum diet to cows also advanced first ovulation postpartum. In our recent study, we found that energy status during the prepartum period critically influenced ovarian function after parturition (Castro *et al.* 2012). These studies suggest that the energy status during the prepartum period critically affects nutrient partitioning, metabolism, and the reproductive axis after calving. Therefore, the assessment of energy status during the prepartum period and suitable improvement of management may enhance postpartum reproductive performance in high-producing dairy cows.

Measurement of feed intake is one of the most suitable variables to reflect energy status; however, monitoring of feed intake in individual cows is impractical for most commercial dairy farms. Evaluation of energy and metabolic status based on metabolic hormones and metabolites in the blood is a suitable and useful alternative, but blood sampling and laboratory measurements are costly, technically laborious, and time-consuming procedures. On the other hand, assessment of feed intake based on the rumen fill score (RFS) is a relatively simple method that is feasible for commercial dairy farms. Burfeind *et al.* (2010) showed that the RFS is associated with feed intake. However, it is unknown whether energy status based on RFS is related to energy and metabolic status assessed by blood metabolic hormone and metabolite concentrations, and there is little information on the relationship between feeding status based on RFS during the dry period and reproductive performance after parturition. Therefore, the present study investigated the relationship between RFS and energy status during the prepartum period, and the differences of RFS, blood energy and metabolic parameters and ovarian cycles between pregnant and non-pregnant dairy cows at the first artificial insemination (AI) after parturition.

MATERIALS AND METHODS

Animals, feeding, and management

The experimental procedures complied with the Guide for the Care and Use of Agricultural Animals of Obihiro University. The experiment was carried out in the Field Center of Animal Science and Agriculture, Obihiro University of Agriculture and Veterinary Medicine. Forty-two multiparous Holstein cows, which calved between September 2011 and August 2012, were used in this study. Cows were classified into 2 groups as pregnant cow and non-pregnant cow at first AI after parturition. Parity, dry period, and body condition score (BCS) of the experimental cows at initiation of the study were 2.4 ± 0.2, 67.5 ± 2.7 d, and 3.40 ± 0.04, respectively. The study was carried out from 3 wk before the expected parturition to the first AI. Cows were moved to a paddock for the close-up dry period about 1 month before the expected calving date, and were fed a limited total mixed ration (dry matter (DM) basis: 127 g of crude protein (CP)/kg and 6.6 MJ of net energy for lactation (NEL)/kg) consisting of grass silage, maize silage, concentrate for dry cows, and grass hay *ad libitum* until parturition. After parturition, cows were housed in a free-stall barn and received a lactation diet, which was a mixed ration (DM basis: 155 g of CP/kg and 6.2 MJ of NEL/kg) consisting of grass, maize silage, and concentrate for dairy cows *ad libitum*. In addition, the diets were supplemented with minerals, and the concentrate for dairy cows was provided according to each cow's specific requirements for milk production. Grass hay and water were always accessible. Cows were milked twice daily between 05.00 and 06.30 and between 17.00 and 18.30 hours.

Sampling

RFS and BCS were assessed twice a week from 3 wk before the expected parturition to 3 wk after calving. RFS was assessed within 2 h before feeding by the same operator using a 1 to 5 scale, where 1 = insufficient intake and 5 = sufficient intake (Burfeind *et al*. 2010). BCS was assessed by the same operator using a 1 to 5 scale with 0.25 intervals according to Ferguson et al. (1994). Blood samples were obtained by caudal venipuncture twice a week from 3 wk before the expected parturition to 3 wk after calving. Nonheparinized and silicone-coated 9-mL tubes (Venoject, Autosep, Gel + Clot. Act., VP-AS109K; Terumo Corporation, Tokyo, Japan) were used for biochemical analysis, and sterile 10-mL tubes containing 200 µL stabilizer solution (0.3 M EDTA, 1% acetyl salicylic acid, pH 7.4) were used for hormonal analysis. To obtain serum, blood samples were coagulated for 15 min at 38°C in an incubator. All tubes were centrifuged at 2,000 ×*g* for 20 min at 4°C, and plasma samples were kept at −30°C until analysis. In addition, milk samples were collected twice a week after milking from 1 month after calving to 3 wk after the first AI or 4 months after calving. After centrifugation at 1,500

×*g* for 15 min at 4°C, the skim milk samples were stored at −30°C until analysis for progesterone concentration.

Measurement of hormones and metabolites

Plasma and skim milk progesterone concentrations were determined by enzyme immunoassay after extraction using diethyl ether, as described previously (Miyamoto *et al.* 1992); the extraction efficiency was 90%. The 50% effective dose of the assay was 0.66 ng/mL. The mean intra-assay and inter-assay coefficients of variation were 6.0% and 9.2%, respectively. Insulin concentrations were determined using an ELISA kit (Bovine Insulin ELISA 10–1201-01; Mercodia, Uppsala, Sweden). The serum concentrations of glucose, NEFA, BHBA, total protein (TP), total cholesterol (T-CHO), aspartate aminotransferase (AST), and gamma-glutamyl transpeptidase (GGT) were measured using a clinical chemistry automated analyzer (TBA120FR; Toshiba Medical Systems Co., Ltd.; Tochigi, Japan).

Identification of the onset of luteal activity and normal or abnormal ovarian cycle

When the progesterone concentration in plasma or skim milk had increased to more than 1 ng/mL, the cow was considered as showing luteal activity (Stevenson & Britt 1979). In addition, the ovarian cycle was identified based on the plasma and skim milk progesterone profiles according to Shrestha *et al.* (2004):

1. Normal cycle: ovarian cycle with luteal activity of more than 10 d and less than 20 d (more than 4 and less than 7 samples).
2. Anovulation: luteal activity not identified until the first AI.
3. Prolonged luteal phase: ovarian cycle with luteal activity of more than 20 d.
4. Short luteal phase: ovarian cycle with luteal activity of less than 10 d.
5. Cessation of cycle: absence of luteal activity for at least 14 d between luteal phases.

Reproductive Management

Ovaries were scanned by ultrasonography or rectal palpation beginning at 40 d postpartum. All cows were inseminated at the observed estrus after 60 d postpartum and did not receive hormonal treatment until the first AI. In addition to internal signs of estrus as detected by rectal palpation, cows with clear vaginal mucous discharge were considered to be in estrus. Conception was confirmed by ultrasonography or rectal palpation at 60 d after AI.

Statistical Analysis

Before data analysis, RFS, BCS, plasma insulin concentration, and serum metabolite concentrations were averaged weekly. The period of 0–6 d after calving was regarded as the parturient week (0 wk postpartum). The relationship between RFS and blood parameters was analyzed by Spearman rank correlation using the CORR procedure after statistical testing of normality using the Kolmogorov-Smirnov test (SAS Enterprise Guide version 4.3, SAS Institute Inc.; Cary, NC, USA). StatView (StatView 5.0 software, Abacus Concepts Inc.; Berkeley, CA, USA) was used for data analysis, using the repeated-measures ANOVA procedure, including time (week), group (pregnancy at first AI or non-pregnancy at first AI), and their interaction in the model as fixed effects. In addition, these data were analyzed separately for the close-up dry period and the postpartum period. Cow was used as the repeated subject. Moreover, the significant effect of time (week) for RFS within a group (pregnancy or non-pregnancy at first AI) was analyzed using the Tukey-Kramer test. Other data between the pregnancy and non-pregnancy groups at the first AI were analyzed by the Student's t-test. Results are reported as mean ± standard error of the mean; differences with $P < 0.05$ were considered significant.

RESULTS

The relationship between RFS and blood parameters during the close-up dry period

Table 1 shows the relationship between RFS and blood metabolic parameters in each week during the close-up dry period. RFS was positively correlated with only serum T-CHO concentration during the close-up dry period (Spearman correlation coefficient = 0.35, $P < 0.05$ at 3 wk prepartum; 0.52, $P < 0.001$ at 2 wk prepartum; 0.43, $P < 0.01$ at 1 wk prepartum). For other parameter, there was no correlation with RFS.

[Table 1]

Situation of calving, milk yield, RFS and blood parameters in pregnant and non-pregnant cows at the first AI

Thirteen of 42 experimental cows (31%) were pregnant at the first AI after parturition, and the remaining experimental cows (n = 29, 69%) failed to conceive at the first AI after parturition. The interval between calving and the first AI did not differ between pregnant (83.1 ± 7.3 d) and non-pregnant (75.6 ± 3.4 d) cows at the first AI.

Table 2 shows the parity, calving difficulty, body weight of calf, difference between expected calving day and actual calving day, peripartum disease, and milk yield in pregnant and non-pregnant cows at the first AI. Peripartum diseases were diagnosed as mastitis (n = 4) and retained placenta (n = 1) in pregnant cows, and as milk fever (n = 2), ketosis (n = 2), and mastitis (n = 4) in non-pregnant cows. There was no significant difference in any factor between pregnant and non-pregnant cows at the first AI.

[Table 2]

Figure 1 shows RFS, BCS, circulating serum metabolite concentrations and enzyme levels, and plasma insulin concentrations during the experimental period. During the close-up dry period, RFS did not differ between pregnant and non-pregnant cows at the first AI; however, RFS did not change during the close-up dry period in pregnant cows at the first AI, but decreased in non-pregnant cows (-3 wk vs.

-2 wk, P = 0.06; -3 wk vs. -1 wk, P < 0.01). On the other hand, during the postpartum period, there were no differences in RFS between pregnant and non-pregnant cows at the first AI, or in the change in RFS within each group. In addition, plasma insulin concentrations tended to be higher in pregnant cows than in non-pregnant cows at the first AI during the close-up dry period (P = 0.06). During the postpartum period, pregnant cows had significantly higher serum glucose concentrations (P < 0.05), higher plasma insulin concentrations (P = 0.06), significantly lower serum BHBA concentrations (P < 0.05) and AST activity (P < 0.05), and lower serum NEFA concentrations (P = 0.07) than non-pregnant cows at the first AI. There were no significant differences in other parameters and BCS between pregnant and non-pregnant cows at the first AI in each period.

[Figure 1]

Ovarian cycle in pregnant and non-pregnant cows at the first AI

Ovarian activity before the first AI is shown in Table 3. The interval between calving and the onset of luteal activity was 37.3 ± 7.3 d in pregnant cows and was 36.1 ± 3.5 d in non-pregnant cows at the first AI. The number of cows with only normal ovarian cycles, normal and abnormal ovarian cycles, and only abnormal ovarian cycles did not differ between pregnant and non-pregnant cows at the first AI (n = 5, n = 4, and n = 4 in pregnant cows; n = 10, n = 10, and n = 9 in non-pregnant cows, respectively). In addition, there were 6 and 16 cows with a normal ovarian cycle before the first AI in the pregnant and non-pregnant groups, respectively. Moreover, 2 and 4 non-pregnant cows at the first AI showed anovulation and cessation of cycle, respectively.

[Table 3]

DISCUSSION

It is generally accepted that conception is affected by parity (Santos *et al.* 2009), calving difficulty (Gaafar *et al.* 2011; Ribeiro *et al.* 2013), presence of peripartum diseases (Santos *et al.* 2010; Walsh *et al.* 2011), and milk yield (Lucy 2001; Walsh *et al*. 2011). In the present study, there were no significant differences in any of these factors between pregnant and non-pregnant cows at the first AI; therefore, these factors did not affect conception at the first AI in the experimental cows evaluated herein.

Rumen fill is influenced by dry matter intake, feed contents, and the chemical composition of feed, especially the contents of dry matter and fiber (Hartnell & Satter 1979; Llamas-Lamas & Combs 1991; Boudon *et al*. 2009). Burfeind *et al.* (2010) showed that RFS is associated with feed intake, and suggested that it should be measured consistently at the same time of day to determine changes of dry matter intake. RFS was positively correlated with only serum T-CHO concentration during the close-up dry period in the present study. Spicer *et al.* (1993) showed that blood concentrations of T-CHO were positively correlated with dry matter intake and energy balance; therefore, it is considered that these concentrations in serum reflect energy intake from feed. Although the data of the present study did not incorporate real-time feed intake measures, the results suggest that RFS might reflect real-time feed intake based on its correlation with serum T-CHO levels. Moreover, RFS did not change in pregnant cows, whereas in non-pregnant cows, RFS decreased gradually as the calving day approached. In the present study, all cows were fed a limited total mixed ration and *ad libitum* grass hay until parturition. In addition, measurements of RFS were carried out within 2 h before feeding. Thus, it is considered that the differences observed in the change of RFS during the close-up dry period between the two groups were influenced mainly by differences in the intake of grass hay. Namely, RFS during the close-up dry period might depend on the appetite of an individual cow. In contrast, neither RFS nor the changes in RFS differed between pregnant and non-pregnant cows at the first AI during the postpartum period. One reason for this result might be that the cows were fed a total mixed ration *ad libitum* after calving. Hence, in the case of limited feeding, RFS may be used as an index of appetite as well as feed intake. Nevertheless, further studies are needed to investigate the relationship between RFS and metabolites as well as metabolic and appetite hormones.

During the close-up dry period, plasma insulin concentrations tended to be higher in pregnant cows than in non-pregnant cows at the first AI in the present study. Insulin plays a central role in the homeostatic control of energy metabolism, and its concentration is positively correlated with energy intake (Chilliard *et al.* 1998). Hence, the differences in the changes of metabolites and insulin concentrations during the prepartum period between pregnant and non-pregnant cows at the first AI might be consistent with the results of feeding status based on RFS measurement at the same time. On the other hand, more clear differences in energy and metabolic status between the two groups were observed during the postpartum period than during the close-up dry period, supporting the results of previous studies (Kawashima *et al.* 2007; Kessel *et al.* 2008; Castro *et al.* 2012). Lower glucose and insulin contents indicate a lower energy status (Chilliard *et al.* 1998), higher NEFA and BHBA reflect greater mobilization of adipose tissue and failure of lipid metabolism in the liver (Grummer 1993, 1995), and higher AST activity suggests hepatic dysfunction (Swarup *et al.* 2007). Thus, based on our results, non-pregnant cows at the first AI had higher lipid mobilization, which is caused by a lower energy status, and showed hepatic dysfunction after parturition. Understandably, cows with severe NEB and/or metabolic disorders show lower fertility (Roche 2006; Walsh *et al.* 2011); therefore, a lower energy and metabolic status continuing from the prepartum period may have led to failed conception at the first AI in the present study.

Abnormal ovarian cycles before service have been shown to have negative effects on reproductive performance, including days to first AI and conception rate (Shrestha *et al.* 2004). In the present study, the onset of luteal activity and the interval between calving and the first AI did not differ between the two groups. However, Ranasinghe *et al.* (2011) showed that cows with cycle cessation or anovulation before service showed a lower conception rate at the first AI compared to cows with a normal ovarian cycle. Moreover, cows with anovulation until the first AI have been shown to have a lower energy status after calving (Shrestha *et al.* 2005). Hence, it is considered that the failure of conception in the non-pregnant cows at the first AI in the present study was due to their lower energy and metabolic status from the close-up dry period.

In conclusion, RFS during the close-up dry period may be a predictor of the energy and metabolic status after calving, and is associated with conception at first AI. These results should prove valuable for determining specific management strategies to improve reproductive performance in high-yielding dairy cows.

ACKNOWLEDGMENTS

The authors thank Dr. K. Okuda (Okayama University, Japan) for providing the P4 antiserum. This study was supported by JSPS KAKENHI Grant Number 23580363 and the Naito Foundation Subsidy for Female Researchers after Maternity Leave.

REFERENCES

Beam SW, Butler WR. 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. *Journal of Reproduction and Fertility Supplement* **54**, 411–424.

Boudon A, Peyraud JL, Faverdin P, Delagarde R, Delaby L, Chaves AV. 2009. Effect of rumen fill on intake of fresh perennial ryegrass in young and mature dairy cows grazing or zero-grazing fresh perennial ryegrass. *Animal* **3**, 1706–1720.

Burfeind O, Sepúlveda P, von Keyserlingk MA, Weary DM, Veira DM, Heuwieser W. 2010. Technical note: Evaluation of a scoring system for rumen fill in dairy cows. *Journal of Dairy Science* **93**, 3635–3640.

Butler WR, Smith RD. 1989: Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *Journal of Dairy Science* **72**, 767–783.

Castro N, Kawashima C, van Dorland HA, Morel I, Miyamoto A, Bruckmaier RM. 2012. Metabolic and energy status during the dry period is crucial for the resumption of ovarian activity postpartum in dairy cows. *Journal of Dairy Science* **95**, 5804–5812.

Cavestany D, Kulcsar M, Crespi D, Chilliard Y, La Manna A, Balogh O, Keresztes M, Delavaud C, Huszenicza G, Meikle A. 2009a. Effect of prepartum energetic supplementation on productive and reproductive characteristics, and metabolic and hormonal profiles in dairy cows under grazing conditions. *Reproduction in Domestic Animals* **44**, 663–671.

Cavestany D, Vinoles C, Crowe M A, La Manna A, Mendoza A. 2009b. Effect of prepartum diet on postpartum ovarian activity in Holstein cows in a pasture-based dairy system. *Animal Reproduction Science* **114**, 1–13.

Chilliard Y, Bocquier F, Doreau M. 1998. Digestive and metabolic adaptations of ruminants to undernutrition, and consequences on reproduction. *Reproduction, Nutrition, Development* **38**, 131–152.

Darwash AO, Lamming GE, Woolliams JA. 1997. The phenotypic association between the interval to post-partum ovulation and traditional measures of fertility in dairy cattle. *Animal Science* **65**, 9–16.

de Feu MA., Evans AC, Lonergan P, Butler ST. 2009. The effect of dry period duration and dietary energy density on milk production, bioenergetic status, and postpartum ovarian function in Holstein-Friesian dairy cows. *Journal of Dairy Science* **92**, 6011–6022.

Ferguson JD, Galligan DT, Thomsen N. 1994. Principal descriptors of body condition score in Holstein cows. *Journal of Dairy Science* **77**, 2695–2703.

Gaafar HM, Shamiah Sh M, El-Hamd MA, Shitta AA, El-Din MA. 2011. Dystocia in Friesian cows and its effects on postpartum reproductive performance and milk production. *Tropical Animal Health and Production* **43**, 229–234.

Grummer RR. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. *Journal of Dairy Science* **76**, 3882–3896.

Grummer RR. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *Journal of Animal Science* **73**, 2820–2833.

Hartnell GF, Satter LD. 1979. Determination of rumen fill, retention time and ruminal turnover rates of ingesta at different stages of lactation in dairy cows. *Journal of Animal Science* **48**, 381–392.

Kawashima C, Kaneko E, Amaya Montoya C, Matsui M, Yamagishi N, Matsunaga N, Ishii M, Kida K, Miyake Y-I, Miyamoto A. 2006. Relationship between the first ovulation within three weeks postpartum and subsequent ovarian cycles and fertility in high producing dairy cows. *The Journal of Reproduction and Development* **52**, 479–486.

Kawashima C, Fukihara S, Maeda M, Kaneko E, Amaya Montoya C, Matsui M, Shimizu T, Matsunaga N, Kida K, Miyake Y-I, Schams D, Miyamoto A. 2007. Relationship between metabolic hormones and ovulation of dominant follicle during the first follicular wave postpartum in high-producing dairy cows. *Reproduction* **133**, 155–163.

Kessel S, Stroehl M, Meyer HH, Hiss S, Sauerwein H, Schwarz FJ, Bruckmaier RM. 2008. Individual variability in physiological adaptation to metabolic stress during early lactation in dairy cows kept under equal conditions. *Journal of Animal Science* **86**, 2903–2912.

Llamas-Lamas G, Combs DK. 1991. Effect of forage to concentrate ratio and intake level on utilization of early vegetative alfalfa silage by dairy cows. *Journal of Dairy Science* **74**, 526–536.

Lucy MC. 2001. Reproductive loss in high-producing dairy cattle: where will it end? *Journal of Dairy Science* **84**, 1277–1293.

Miyamoto A, Okuda K, Schweigert FJ, Schams D. 1992. Effects of basic fibroblast growth factor, transforming growth factor-beta and nerve growth factor on the secretory function of the bovine corpus luteum in vitro. *Journal of Endocrinology* **135**, 103–114.

Ranasinghe RM., Nakao T, Yamada K, Koike K, Hayashi A, Dematawewa CM. 2011. Characteristics of prolonged luteal phase identified by milk progesterone concentrations and its effects on reproductive performance in Holstein cows. *Journal of Dairy Science* **94**, 116–127.

Ribeiro ES, Lima FS, Greco LF, Bisinotto RS, Monteiro AP, Favoreto M, Ayres H, Marsola RS, Martinez N, Thatcher WW, Santos JE. 2013. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. *Journal of Dairy Science* **96**, 5682–5697.

Roche JF. 2006. The effect of nutritional management of the dairy cow on reproductive efficiency. *Animal Reproduction Science* **96**, 282–296.

Santos JE, Rutigliano HM, Sa Filho MF. 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. *Animal Reproduction Science* **110**, 207–221.

Santos JE, Bisinotto RS, Ribeiro ES, Lima FS, Greco LF, Staples CR, Thatcher WW. 2010. Applying nutrition and physiology to improve reproduction in dairy cattle. *Society of Reproduction and Fertility Supplement* **67**, 387–403.

Senatore EM, Butler WR, Oltenacu PA. 1996. Relationship between energy balance and postpartum ovarian activity and fertility in first lactation dairy cows. *Animal Science* **62**, 17–23.

Shrestha HK, Nakao T, Suzuki T, Higaki T, Akita M. 2004. Effects of abnormal ovarian cycles during pre-service period postpartum on subsequent reproductive performance of high-producing Holstein cows. *Theriogenology* **61**, 1559–1571.

Shrestha HK, Nakao T, Suzuki T, Akita M, Higaki T. 2005. Relationships between body condition score, body weight, and some nutritional parameters in plasma and resumption of ovarian cyclicity postpartum during pre-service period in high-producing dairy cows in a subtropical region in Japan. *Theriogenology* **64**, 855–866.

Spicer LJ, Vernon RK, Tucker WB, Wettemann RP, Hogue JF, Adams GD. 1993. Effects of inert fat on energy balance, plasma concentrations of hormones, and reproduction in dairy cows. *Journal of Dairy Science* **76,** 2664-2673.

Staples CR, Thatcher WW, Clark JH. 1990. Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. *Journal of Dairy Science* **73**, 938–947.

Stevenson JS, Britt JH. 1979. Relationships among luteinizing hormone, estradiol, progesterone, glucocorticoids, milk yield, body weight and postpartum ovarian activity in Holstein cows. *Journal of Animal Science* **48**, 570–577.

Swarup D, Naresh R, Varshney VP, Balagangatharathilagar M, Kumar P, Nandi D, Patra RC. 2007. Changes in plasma hormones profile and liver function in cows naturally exposed to lead and cadmium around different industrial areas. *Research in Veterinary Science* **82**, 16–21.

Walsh SW, Williams EJ, Evans AC. 2011. A review of the causes of poor fertility in high milk producing dairy cows. *Animal Reproduction Science* **123**, 127–138.

Figure legends

Figure 1 RFS, BCS, concentrations of serum metabolites, insulin levels in plasma, and serum enzyme activities during the experimental period (mean ± SEM; solid, pregnant (n =13); open, non-pregnant (n = 29)).

Table 1 Spearman rank correlation between RFS and blood metabolic parameters in each week during the close-up dry period

	RFS					
	3 wk prepartum		2 wk prepartum		1 wk prepartum	
	r	*p*	*r*	*p*	*r*	*p*
BCS	0.191	0.226	-0.074	0.640	-0.098	0.535
Glucose (mg/dL)	0.068	0.667	0.199	0.208	-0.036	0.820
Insulin (ng/mL)	-0.118	0.457	-0.093	0.557	-0.050	0.751
NEFA (μEq/L)	-0.217	0.168	-0.056	0.725	-0.159	0.314
BHBA (μEq/L)	0.253	0.101	0.127	0.423	-0.058	0.715
T-CHO (mg/dL)	0.354	0.022	0.516	0.001	0.431	0.004
TP (g/dL)	-0.265	0.090	-0.059	0.711	-0.101	0.524
AST (IU/L)	-0.055	0.728	0.245	0.118	0.099	0.533
GGT (IU/L)	-0.228	0.147	-0.301	0.053	-0.031	0.847

Table 2 Parity, calving difficulty, body weight of calf, difference between expected calving day and actual calving day, peripartum disease, and milk yield in pregnant and non-pregnant cows at the first AI[1]

	Pregnancy (n = 13)			Non-pregnancy (n = 29)			P-value
Parity at onset of the experiment	2.6	±	0.3	2.3	±	0.2	0.37
Calving difficulty[2]	1.1	±	0.1	1.2	±	0.1	0.55
Body weight of the calf at birth (kg)	46.5	±	1.9	47.3	±	0.8	0.08
Difference between expected calving day and actual calving day	1.5	±	1.3	1.7	±	0.8	0.90
Diagnosis of peripartum disease	4/13 (30.8%)			8/29 (27.6%)			0.83
Total milk yield from day 7 to 100 postpartum (kg)	3735.8	±	135.0	3929.7	±	86.6	0.23
Average of daily milk yield between day 7 and 100 postpartum (kg)	39.7	±	1.4	41.8	±	0.9	0.21
Peak milk yield (kg)	43.4	±	1.5	45.9	±	1.1	0.18
Peak milk week (wk)	5.2	±	0.4	5.8	±	0.4	0.34

[1]Values are the mean ± SEM.

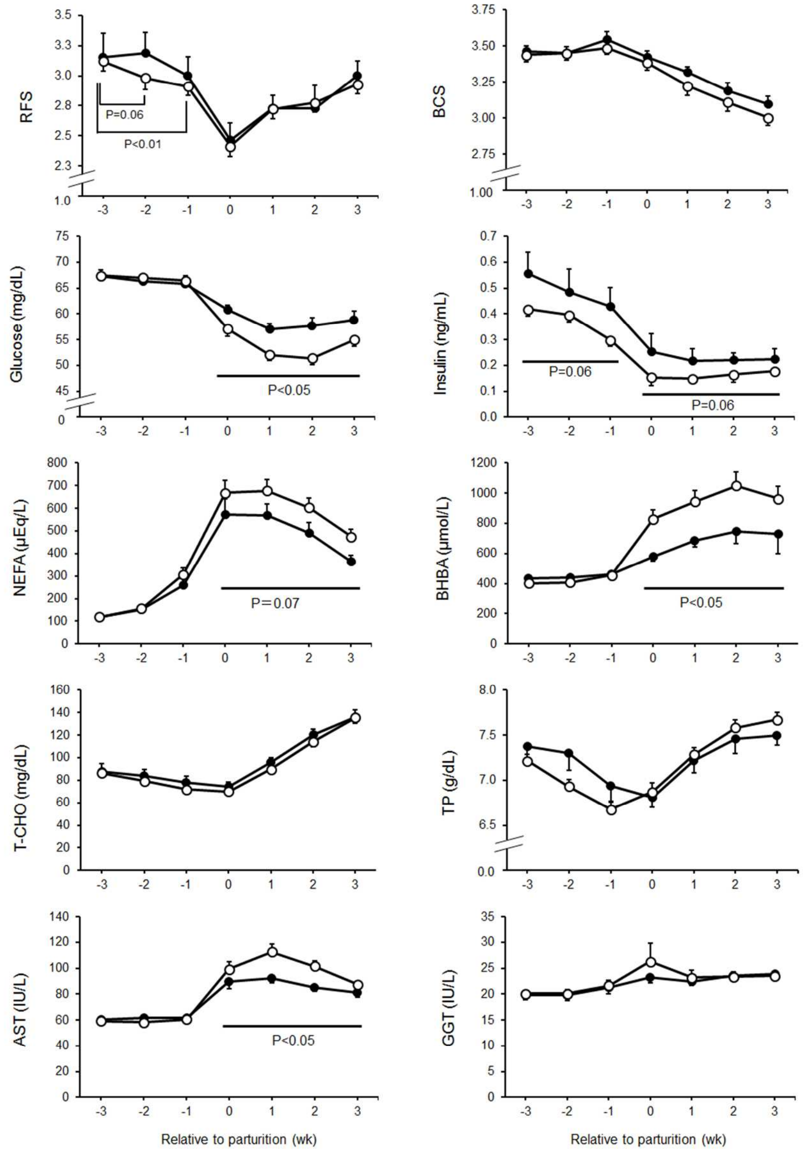
[2]1, unassisted birth (natural, without human assistance); 2, easy calving with human assistance; 3, difficult calving with a few humans; 4, dystocia (requiring much more force than normal); and 5, surgical treatment or death of cow

Table 3 Ovarian cyclicity before the first AI in pregnant and non-pregnant cows[1]

	Pregnant (n = 13)	Non-pregnant (n = 29)	P-value
Onset of luteal activity (day)	37.3 ± 7.3	36.1 ± 3.5	0.86
Characteristics of ovarian cyclicity until the first AI			
Normal ovarian cycles	5/13 (38.5%)	10/29 (34.5%)	0.96
Normal and abnormal ovarian cycles	4/13 (30.8%)	10/29 (34.5%)	
Abnormal ovarian cycles	4/13 (30.8%)	9/29 (31.0%)	
Number of cows with normal ovarian cycle before the first AI	6/13 (46.2%)	16/29 (55.2%)	0.59
Number of cows with cessation of cycle or anovulation			
Cessation of cycle	0/13 (0.0%)	4/29 (13.8%)	-
Anovulation before insemination	0/13 (0.0%)	2/29 (6.9%)	-

[1]Values are the mean ± SEM.

Fig.1



356x504mm (300 x 300 DPI)