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Delayed Ovarian Cyclicity Does not Reflect Blood Metabolites Irregularity in Postpartum Holstein Cows

Research Article

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ABSTRACT

An experiment was conducted to investigate the effect of different types of ovarian cycles on reproductive indices and blood metabolites in 61 Holstein cows. Blood samples were taken from each cow between day 12 and day 50 postpartum (PP). Serum progesterone concentrations were measured to determine the onset of luteal function and different types of luteal dysfunctions in cows. Serum glucose, cholesterol and blood urea nitrogen concentrations were measured in the cows with normal and abnormal ovarian cyclicity. In this study, 29 (47.5%) cows showed normal ovarian cyclicity with first luteal activity detected before day 50 days PP, whereas 32 (52.5%) cows showed abnormal cyclicity. Of the 32 cows with abnormal patterns, 13 (21.3%) had prolonged luteal phase (PLP), 7 (11.5%) had short luteal phase (SLP) and 12 (19.7%) had delayed first ovulation (delayed ovulation). Progesterone concentration at different times was considerably varied from day to another day ($P \leq 0.01$), while the main effect of the group on the progesterone concentration was significant ($P \leq 0.01$). Cows that had normal cyclicity showed earlier luteal activity ($P \leq 0.01$) and shorter interval to first ovulation ($P \leq 0.01$) compared to cows with abnormal cyclicity. There were no significant differences in different blood metabolites between cows with normal or abnormal cyclicity. In conclusion, a high percentage (52.5%) of the postpartum cows had abnormal ovarian cycles. The abnormal ovarian cycles reduced the reproductive performance of dairy cows, including the onset of luteal activity and interval to first ovulation. Blood metabolites were not associated with postpartum abnormal cycles in cows.

KEY WORDS blood metabolites, postpartum cows, progesterone, reproductive indices.

INTRODUCTION

One calf each year is generally accepted to derive maximum economic benefit in dairy herds. To achieve this goal, early resumption of ovarian cyclicity in postpartum (PP) period is required. However, the increased incidence of abnormal ovarian cycles such as prolonged luteal phase and delayed first ovulation during pre-service period in high-producing dairy cows are believed to be responsible for the decline in reproductive performance (Lamming and Darwash, 1998; Opsomer *et al.* 1998; Shrestha *et al.* 2004).

A report about British Friesian cows between 1975 and 1982, showed that cows with abnormal ovarian cycles during the postpartum period had more days open, higher number of services per conception, lower first service conception rate, and reduced total conception rate than normal cows (Lamming and Darwash, 1998). After calving, the severe negative energy balance and the related metabolic changes have been associated with worldwide reported reduced fertility in high producing dairy cattle (Lucy, 2008). The low productivity of cattle under Egyptian condition needs improvement and this demands an evaluation of their nutritional status. The assessment of the nutritional and health status in cattle can be made by determining certain blood metabolite concentrations (Ndlovu *et al.* 2007). Metabolite concentrations indicate the extent of the metabolism of energy, proteins and other nutrients in the animals (Pambu-Gollah *et al.* 2000; Agenas *et al.* 2006). Changes in the circulating metabolites are important indicators of the metabolic status of an animal and luteal function (Lindsay *et al.* 1993; Wettemann *et al.* 2003). The metabolites include glucose and cholesterol which reflect energy status whilst total protein and urea indicate protein status. Factors including the physiological status of an animal, breed, nutrition, season and age may affect the concentration of these metabolites in the blood (Ndlovu *et al.* 2007).

Glucose is the principal source of energy for life processes in mammalian cells (Saleh *et al.* 2011). It provides information for the control of gonadotropin-releasing hormone (GnRH) secretion, as its availability influences tonic and surge modes of luteinizing hormone (LH) secretion, presumably through its effects on GnRH (Diskin *et al.* 2003).

Inadequate availability of utilizable glucose thus reduces the hypothalamic release of GnRH, leads to a decrease in LH release and eventually delays or prevents ovulation (Hess *et al.* 2005). A decrease in plasma cholesterol concentration has been reported that result in a reduction of plasma concentrations of insulin-like growth factor 1 and progesterone with a delayed or inhibited ovulation (Maciel *et al.* 2001).

Thus the main objective of the present study was to investigate the effects of abnormal ovarian cycles during the pre-service postpartum period on subsequent reproductive performance of Holstein cows. In addition, determination of blood concentrations of glucose, cholesterol and blood urea nitrogen and their relationship with the ovarian cyclicity in cows were also investigated.

MATERIALS AND METHODS

Animals

The study was conducted at dairy cattle farm at Elsalhya Agriculture Company near Elkasacin city, Ismailia province, Egypt, in 61 multiparous Holstein cows in their second to sixth parity. The cows were housed in open shelters equipped with automatically controlled water sprinklers and fans along the feeding line, had free access to tap water and were fed in groups according to milk production. The cows were fed a total mixed ration (TMR) consisted of Berseem (Egyptian clover) *Trifolium alexandrinum*, Berseem hay, silage and concentrates with mineral mixture. The dry matter intakes ranged from 19-21 and 14-16 kg/head/d in winter and summer, respectively.

The farm has a hot climate during summer season, with average temperatures between 27 ˚C and 33 ˚C. The climate during winter season is mild, with average temperatures between 13 ˚C and 21 ˚C. Cows were machine-milked three times daily. Milk production ranges from 7500 to 8500 kg.

Serum progesterone analysis

Blood samples were collected twice weekly from each cow by jugular puncture between day 12 and day 50 PP and the plain tubes were placed on ice immediately after collection. Serum was separated by centrifugation at 1800 × g for 15 min. Serum was stored at -20 ˚C, until assayed for progesterone and different blood metabolites concentrations. Progesterone concentration was measured using enzyme-linked immunesorbent assay (ELISA) kit (Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany). The intra-assay and interassay coefficients of variation (CVs) ranged from 3.5-4.6% and 3.5-5.3%, respectively. The cows were classified as having resumed ovarian cyclicity when progesterone concentration of ≥ 1 ng/mL was recorded for two consecutive samples in the same week (Tamadon *et al.* 2011). Based on this parameter, cows were classified as normal ovarian cyclicity (resumed cyclicity≤50 days PP followed with regular cycles). The cows with abnormal ovarian cyclicity were then classified according to Hommeida *et al.* (2005) into cows with prolonged luteal phase, cows with short luteal phase and cows with delayed ovulation (Table 1).

Blood metabolite analysis

Blood metabolites were analyzed weekly between day 12 and day 50 PP in some cows with normal (n=6) and abnormal (n=6) ovarian cyclicity using a spectrophotometer (Robert Riele Gmblt Co., Germany) and Biodiagnostic kits (Biodiagnostic, Egypt). Glucose was analyzed according to the method of Trinder (1969).

Total cholesterol (T-cho) concentration was analyzed according to the method of Allain *et al.* (1974). Blood urea nitrogen (BUN) was analyzed according to the method of Tabacco *et al.* (1979).

Reproductive indices

Reproductive parameters of the cows with normal and abnormal ovarian cyclicity were measured. Cows were artificially inseminated after the detection of estrus by the expert technicians confirming the absence of abnormal discharge or any uterine or cervical inflammations. Pregnancy diagnosis was performed by per rectal palpation 45 days after insemination. The calving to first estrus interval, first service conception rate, number of artificial insemination (AI) per conception, days open and calving interval were compared in the cows.

Table 1 Incidence of different types of postpartum luteal activity based on serum progesterone level in cows

Ovarian cyclicity	Definition	Number of cows	%
Normal	First luteal activity was detected ≤ 50 days PP followed with regular cycles	29	47.5
Abnormal	-	-	-
PLP	One or more ovarian cycles with luteal activity ≥ 20 days was diagnosed	13	21.3
	One or more ovarian cycles with luteal activity of < 10 days was recorded	7	11.5
Delayed ovulation	First luteal activity was not detected until > 50 days PP	12	19.7
Subtotal	-	32	52.5
Total	-	61	100

PLP: prolonged luteal phase and SLP: short luteal phase.

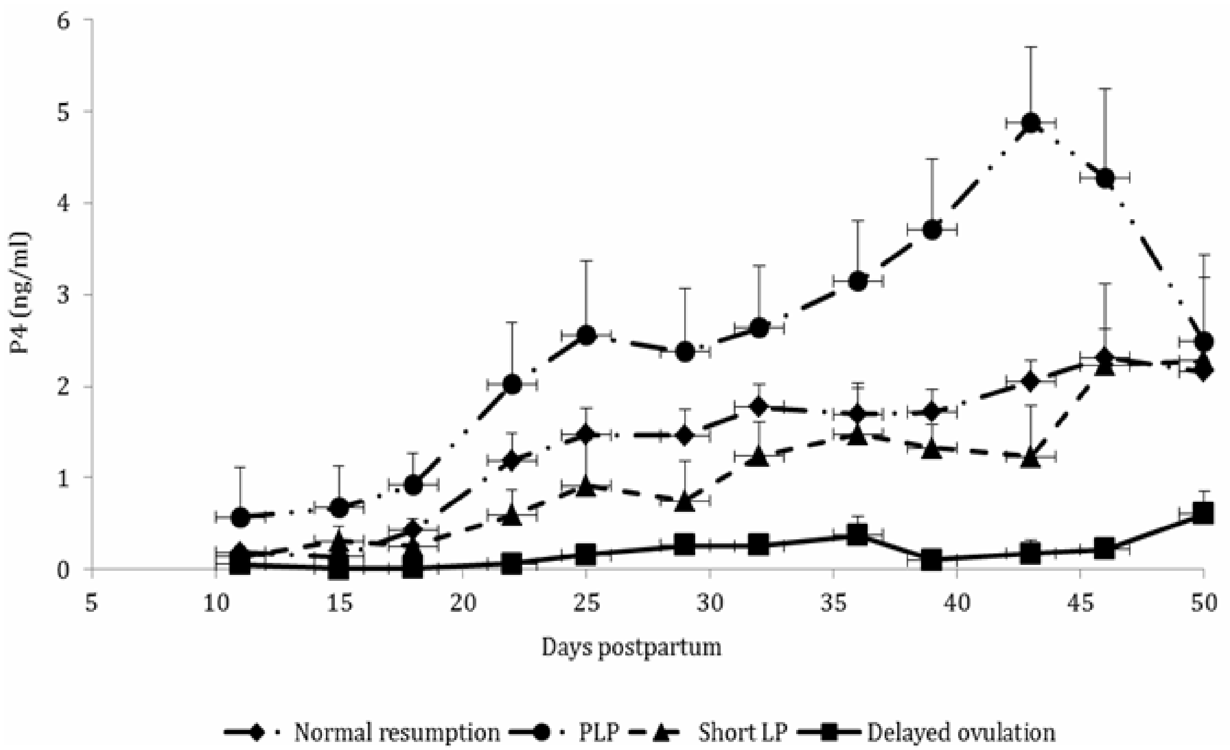

Figure 1 Different types of postpartum luteal activities based on serum progesterone (ng/mL) level (Mean±SEM) in cows

Statistical analysis

Data were analyzed using mixed model with repeat measure. The model included fixed effects: group, time and group × time interactions. The dependent continuous variable was the progesterone concentrations in all analyses. The first independent factor was the time (days postpartum) which had different repeated measure levels (i.e. day 12 - day 50 PP), while the second independent factor was the condition or treatment type with four levels (cows with normal ovarian cyclicity, cows with prolonged luteal function, cows with short luteal function and cows with delayed ovulation) (SPSS, 2011). Reproductive parameters and the concentrations of glucose, cholesterol and urea were compared in cows with normal and abnormal ovarian cyclicity using student t-test.

RESULTS AND DISCUSSION

Different types of luteal activity during postpartum period

In this study, 29 (47.5%) cows showed normal ovarian cyclicity with first luteal activity detected before 50 days PP followed by regular cycles whereas 32 (52.5%) cows showed abnormal cyclicity. Of the 32 cows with abnormal patterns, 13 (21.3%) had prolonged luteal phase (PLP), 7 (11.5%) had short luteal phase (SLP) and 12 (19.7%) with delayed first ovulation (delayed ovulation) (Table 1).

Progesterone concentration in different types of luteal activity

Progesterone concentration at different times (days postpartum) were considerably varied from day to another day ($P \leq 0.01$). The main effect of the group (cows with normal ovarian cylicity, cows with prolonged luteal function, cows with short luteal function and cows with delayed first ovulation) on the progesterone concentration was significantly different ($P \leq 0.01$).

Table 2 Reproductive parameters (Mean±SE) of cows with normal and abnormal postpartum ovarian cyclicity

Parameters	Cows with normal ovarian cyclicity	Cows with abnormal cycles	P-value
Parity	2.8±0.3	3.1±0.4	0.25^{ns}
Interval to 1^{st} PP ovulation (days)	20.6±2.5	30.5±8.9	0.001
Commencement of luteal activity (days)	25.6±2.5	34.5±8.1	0.002
Calving to 1^{st} observed heat (days)	35.9±7.2	53.5±7.0	0.82^{ns}
Interval from calving to 1^{st} service (days)	59.0±3.4	65.0±3.9	0.51^{ns}
No. of services / conception	2.0±0.3	2.4±0.3	0.69^{ns}
Days open	93.3±8.4	113.0±11.2	0.37^{ns}

SE: standard error.
NS: non significant.

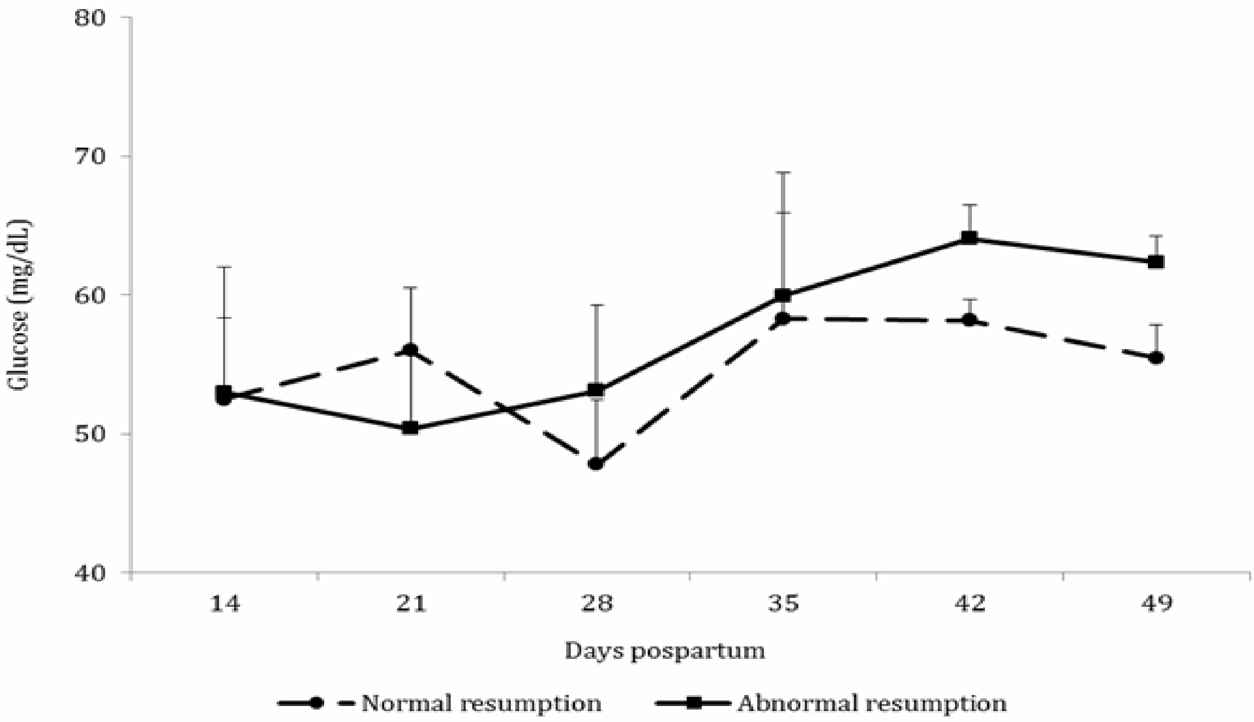

Figure 2 Glucose (Mean±SEM) levels (mg/dL) in cows with normal and delayed postpartum ovarian cyclicity

The interaction of time and group was significantly different ($P \leq 0.05$) and indicated that the change in progesterone concentrations in different categories of cows was significantly different across the time factor (Figure 1).

Reproductive performance

Cows that had normal ovarian cyclicity showed earlier luteal activity ($P \leq 0.01$) and shorter interval to first ovulation ($P \leq 0.01$) compared to cows with abnormal patterns.

However, there were no significant differences between those cows in terms of interval from calving to first observed heat and first service, number of services required for conception and days open (Table 2).

Blood metabolites

Blood glucose (Figure 2), T-cho (Figure 3) and BUN (Figure 4) were not significantly different between cows that had shown normal and abnormal postpartum ovarian cyclicity.

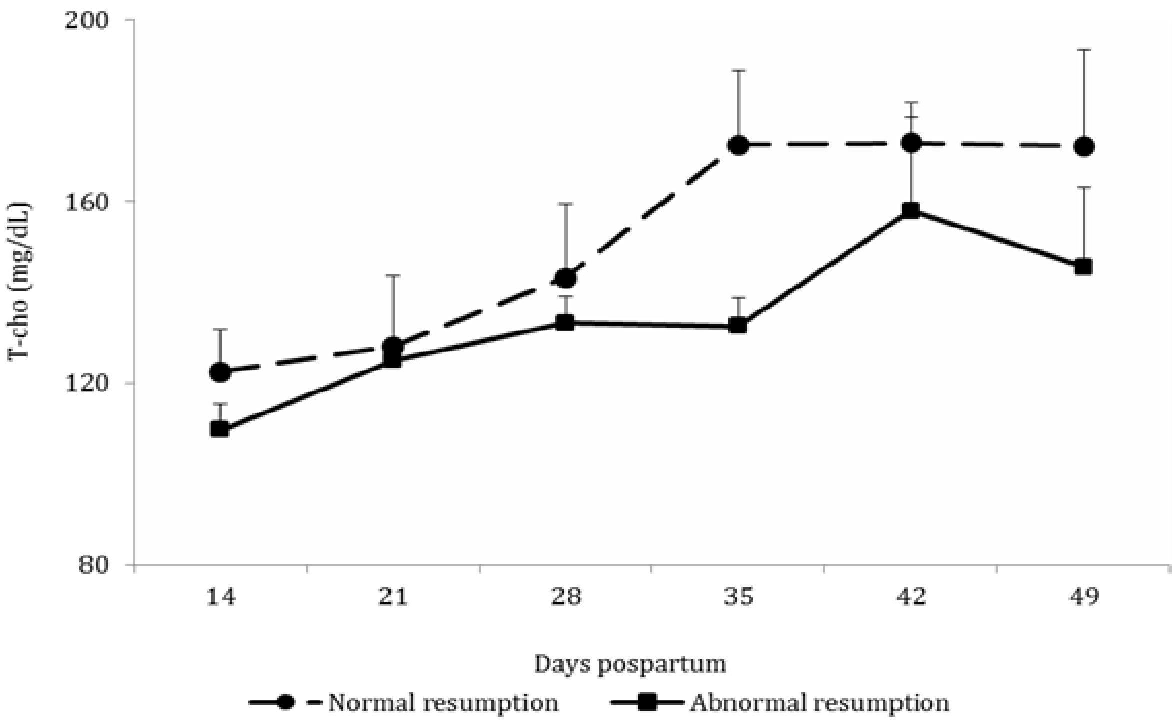

Figure 3 Cholesterol (Mean±SEM) levels (mg/dL) in cows with normal and delayed postpartum ovarian cyclicity

The incidence of abnormal cyclicity (52%) and the incidence of cows with prolonged luteal phase (21%) in the present study, was similar with the finding of Hommeida *et al.* (2005), while our findings of cows with delayed first ovulation (19%) was lower than that was reported earlier by Hommeida *et al.* (2005).

In the present study, the interval to first PP ovulation and commencement of luteal cyclicity were significantly (P≤0.05) delayed in cows with abnormal cyclicity compared to cows with normal one. The other reproductive indices were non significantly improved in cows with normal ovarian cylicity compared to cows with abnormal one. Postpartum cows with abnormal cycles, both with prolonged luteal phase and delayed ovulation, in the pre-service period had poorer reproductive performance than those with normal ovarian cycles (Shrestha *et al.* 2004; Hommeida *et al.* 2005). Cows with prolonged luteal phase had longer intervals to first AI and conception than normal cows.

A decrease in proportion of cows served, a lower first service conception rate, an increase in the interval to conception and more services per conception were reported in cows having prolonged luteal phases (Lamming and Darwash, 1998).

Previous reports have shown that cows with anovulation required longer intervals from calving to first AI and to conception, and more number of inseminations per conception than cows with normal cycles (Shrestha *et al.* 2004). Hommeida *et al.* (2005) reported that cows with delayed cyclicity postpartum had increased interval to first insemination. The discrepancy in the results of reproductive indices between the present study and other studies might be due to the differences in the number of experimental cows, farm management and reproductive strategies in the farm after calving. The determination of blood metabolites could provide an understanding of the impact of nutritional status on reproduction and thus guide in the development of management strategies for early postpartum ovarian cyclicity. Blood glucose concentration is an important indicator of dietary energy intake. Low glucose concentrations due to dietary and tissue energy deficiencies towards milk synthesis results in releasing of non esterified fatty acids and ketones which may affect the reproductive performance of the cows. Our findings on the relationship between the concentrations of certain nutrient-sensitive blood metabolites and the ovarian cyclicity in postpartum cows were in agreement with previous reports (Ahmad *et al.* 2004; Jayachandran *et al.* 2013).

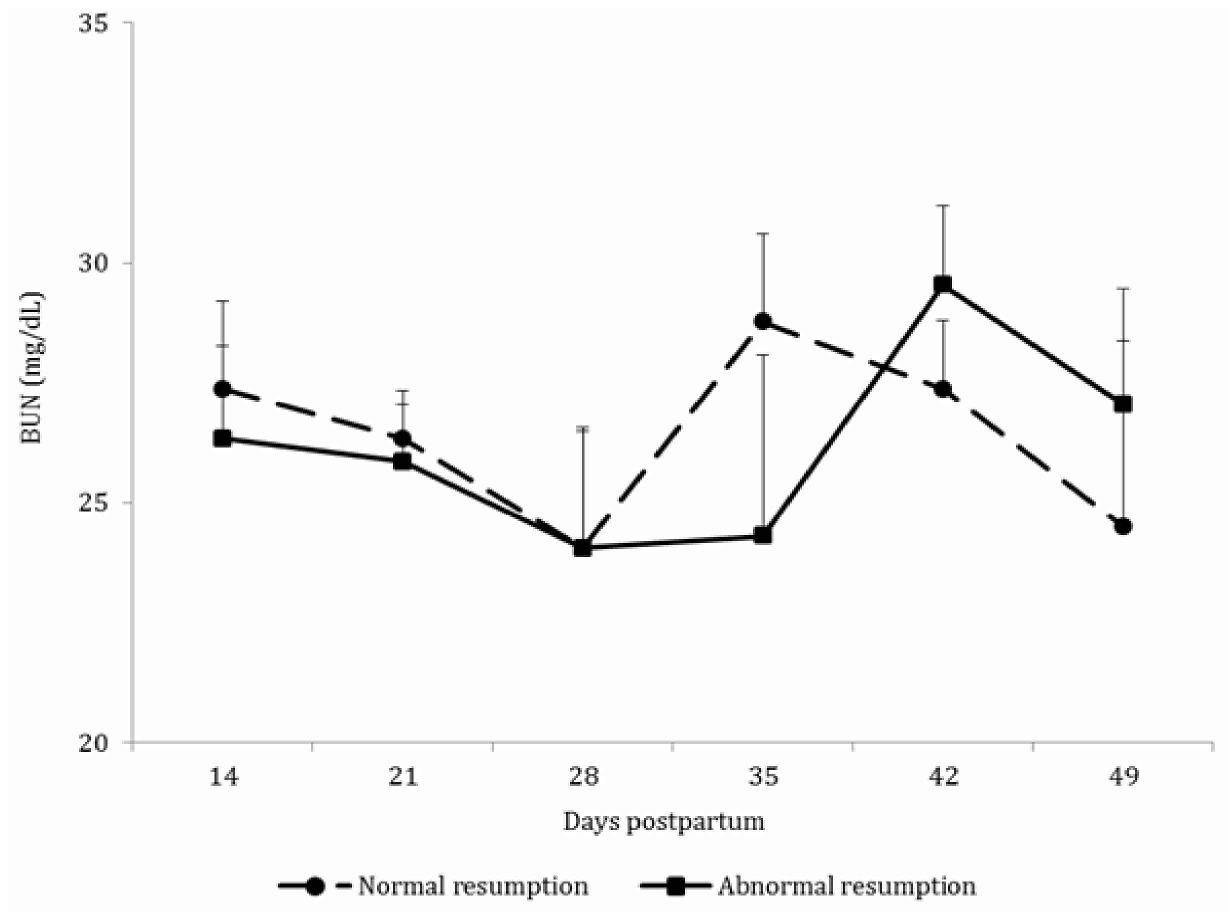

Figure 4 Blood urea nitrogen (Mean±SEM) levels (mg/dL) in cows with normal and delayed postpartum ovarian cyclicity

Nevertheless, in the present study, glucose concentration at days 42 and 50 PP was little bit increased in cows with abnormal than those with normal cyclicity. Based on previous reports, serum glucose level was higher in cows with uterine inflammation than in healthy and cyclic ones (Majeed *et al.* 1990; Ahmad *et al.* 2004).

It has been hypothesized that byproducts of the protein metabolism (such as ammonia or urea) may impair the reproductive efficiency of the cows Butler (1998). During early postpartum period, high producing dairy cows had elevated plasma urea concentrations due to protein catabolism (Leroy *et al.* 2008). High urea concentrations have been associated with a lower uterine pH which may result in fertilization failure through reducing the viability of the spermatozoa (Westwood *et al.* 1998). In the present study, the early cycling cows recorded lower non significant concentrations of urea than delayed cycling cows at day 42 and 50 PP.

The decrease in urea concentrations in the early cyclic cows may be ascribed to an improvement in the nutritional status of the cows. Previous reports revealed that no significant differences were found in BUN between early cyclic and late cyclic cows (Majeed *et al.* 1990; Shrestha *et al.* 2005).

Cholesterol is a precursor of steroid hormones and its presence in higher concentrations in blood of animals with advanced reproductive performance suggested a better ovarian activity (Yotov *et al.* 2013), which is supported by previous reports in dairy animals whereby animals with higher concentration of cholesterol in plasma exhibited estrus more frequently (Jorritsma *et al.* 2003). Negative energy balance after calving in high producing dairy cows was associated with low cholesterol level in blood and / or in the follicular fluid (Pedron *et al.* 1993; Leroy *et al.* 2004). In the present study, total cholesterol concentrations were not associated with normal postpartum ovarian cyclicity in cows. However, in other studies, lower serum concentration of cholesterol was found in anestrus than in regular cyclic ones (Burle *et al.* 1995; Jayachandran *et al.* 2013).

CONCLUSION

The present study has shown that large percentage of cows suffered from delayed postpartum ovarian cyclicity. Abnormal ovarian cycles delayed the onset of luteal activity and extended the interval to first ovulation. Blood metabolites were not associated with abnormal ovarian cycles in cows. The issues behind this required further study and clarification.

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