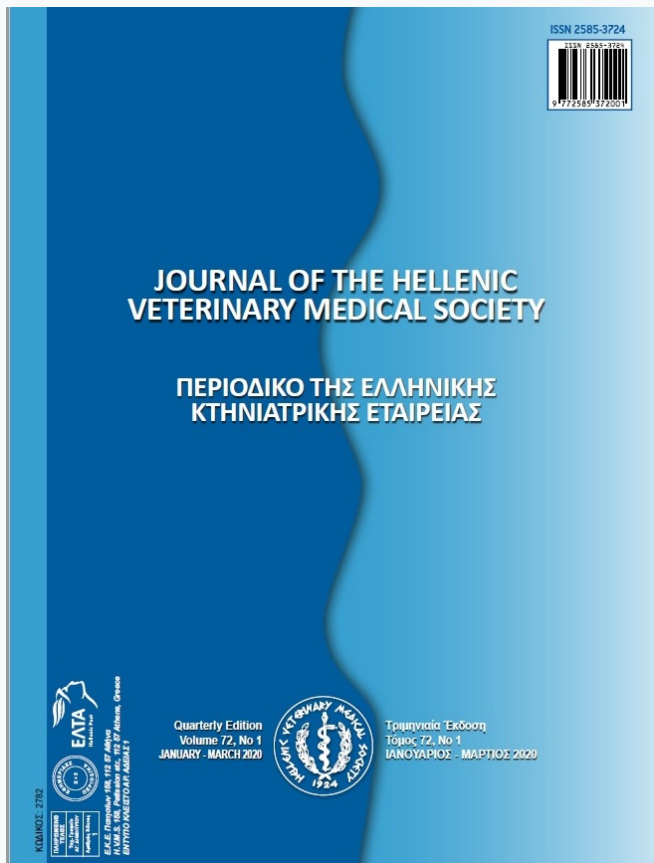


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Investigation of omentin-1 and metabolic parameters in periparturient cows

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ABSTRACT: We aimed to determine the level of omentin-1 hormone and other in periparturient period of dairy cows. It was also aimed to determine whether there is a correlation between omentin-1, glucose, Non-esterified fatty acids (NEFA), triglycerides (TG) and β -hydroxybutyrate (BHB). Blood samples were taken a month before parturition (PreP30), two weeks before parturition (PreP14), at parturition (P), two weeks after parturition (PostP14) and a month after parturition (PostP30). Concentrations of serum omentin-1 at P, serum glucose at P, PostP14, and PostP30, serum NEFA at P, serum TG PreP30 and PreP14, and serum BHB at P were statistically significantly higher than at other times. A positive correlation was observed between omentin-1 and glucose, NEFA and BHB, glucose and NEFA and BHB, and NEFA and BHB. A negative correlation was found between TG and omentin-1, glucose, NEFA and BHB. In conclusion, findings of the present study shows that omentin-1 may play an important role in the periparturient period. A positive correlation of omentin-1 with energy fuels NEFA, glucose, and BHB suggests that omentin-1 plays a role in energy metabolism like other adipokines. The fact that omentin-1 levels increase during delivery, when the fetus needs energy most, supports this hypothesis.

Keywords: Periparturient period, Omentin-1, NEFA, BHB

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INTRODUCTION

The “periparturient period” or “transition period” in dairy cows is defined in three phases: before, at, and post parturition (Ocal et al., 2015) including 2-4 weeks before parturition and the 3-4 weeks after parturition. The period before parturition is called the dry period and the period after parturition is called the lactation period (Moore et al., 2015). The periparturient period in cows is a critical time in which many metabolic and infectious diseases occur, the immune system is influenced, and physiological disturbances appear (Mendonça et al., 2014). There is an important increase in the need for nutrients in cows in the last phase of pregnancy and at the start of lactation after parturition. In the periparturient period, a negative energy balance occurs due to insufficient feed consumption as a result of the increasing nutritional needs of the offspring before parturition and the onset of lactation (Raboisson et al., 2014; Jaakson et al., 2018; Suzuki et al., 2018). Adipokines released by the adipose tissue include many substances such as omentin, leptin, adiponectin, resistin, tumor necrosis factor- α , interleukin-6, visfatin, and retinol-binding protein (Tan et al., 2015). Omentin-1 was first defined in 2001 as intelectin-1 and then named “omentin” in 2004 since it is expressed at a high level in the omentum (Sittichoroon et al., 2014). It was also defined as intestinal lactoferrin receptor and intelectin-1 in intestinal paneth cells in 2005, and named endothelial lectin as a result of being located in endothelial cells as well (Tan et al., 2015). Protein sequence analyses demonstrated that omentin-1 mRNA codes a protein of 313 amino acids with a peptide amino terminal that is highly hydrophobic. The portion that has 296 amino acid secretion functions is obtained following this edge as a result of the division between the 17th and 18th amino acids (Boron et al., 2015; Tan et al., 2015; Elsaid et al., 2018). The omentin-1 hormone, which was first found expressed in heart, lungs, ovary, small bowel, as well as placenta, muscle and kidneys at a lower rate, was later determined to be expressed in fat tissue too (Yang et al., 2006; Ohashi et al., 2014; Elsaid et al., 2018). Omentin has two homolog isoforms, omentin-1 and omentin-2. Omentin-1 is the most encountered isoform in the circulatory system (Pan et al., 2010; Kafalidis et al., 2013; Antonio de Luis et al., 2018). The amino acid sequences of omentin-1 and omentin-2 are 83% similar (Tan et al., 2015). Omentin-1 is an adipokine at a density of 33-40 kDa with 8 exon and 7 intron regions (Tohidi et al., 2012; Shen et al., 2016; Antonio de Luis et al., 2018). Omentin-1,

with the UniProt code of Q8WWAQ and GenBank expression number of AY549722, has been studied more than omentin-2 (Tan et al., 2015). Omentin-1 is an anti-inflammatory adipokine. Since it increases the insulin transduction signal, omentin-1 plays an important role in increasing insulin sensitivity and glucose metabolism in local omental adipose tissue and modulating paracrine and endocrine factors (Boron et al., 2015; Elsaid et al., 2018). Glucose, a substantial energy source for animals, meets the energy needs of the organism and is used in the synthesis of milk compounds. Lactose synthesis is increased with parturition, thus more glucose is required by the body (Larsen and Kristensen, 2013). Non-esterified fatty acids (NEFA) are the main component of triglycerides (TG) and are used as an energy source by many tissues (LeBlanc et al., 2005). Because of the formation of a negative energy balance, fat reserves pass into the blood as NEFA in dairy cows during early lactation and contribute to the energy requirements. NEFA are used or stored as very low-density lipoproteins in the liver, or they are esterified to TG. Metabolic problems arise in dairy cows in cases where NEFA and β -hydroxybutyrate (BHB) increase to extreme levels. In the transition period, measurement of NEFA and BHB can be used to determine a negative energy balance or ketosis index (Barletta et al., 2017). In light of this knowledge we aimed to determine the level of omentin-1 hormone and other blood metabolites in periparturient period of dairy cows. It was also aimed to determine whether there is a correlation between omentin-1, glucose, NEFA, TG and BHB.

MATERIALS AND METHODS

Experimental animals and animal management

This study was conducted at Kafkas University, Faculty of Veterinary Medicine, Education and Research Farm. Ethical approval was obtained (KAÜ-HADYK/2017-078) before starting the study. A total of 15 multiparous Brown Swiss and Simmental dairy cows, average 2-4 years of age, were used in the study. These 15 animals were moved to a closed area of concrete floor space 20 days before starting the study. The animals were fed with pelleted dairy feed (feeding ingredient: raw protein 18%, raw cellulose 14%, raw oil 2,5%, raw ash 9%; feed raw materials: corn, barley, wheat, cottonseed meal, soy meal, sunflower seed meal, wheat bran, vetch, molasses, calcium carbonate, sodium chloride). Feed and water were provided *ad libitum* to animals regularly every morning starting at 7 am. To find the periparturient period of the 15 cows,

the study was conducted between September 2017 and February 2018. The animals started parturition between November and December. After parturition cows were milked and the calves fed this milk.

Blood sampling

Blood samples (a total of 75 samples at 5 timepoints) were collected before feeding between 6-7 am every morning from the tail vein into non-anticoagulant tubes a month before parturition (PreP30), two weeks before parturition (PreP14), at parturition (P), two weeks after parturition (PostP14), and a month after parturition (PostP30). Serum samples obtained by centrifuging the blood at 3000 rpm at 4°C for 15 minutes were stored at -20°C with aprotinin (SIGMA, A1153) added. When all measurements were complete, ELISA and other colorimetric analyses were performed in duplicate using the BIO-TEK EPOCH, GEN 5 software.

Biochemical analysis

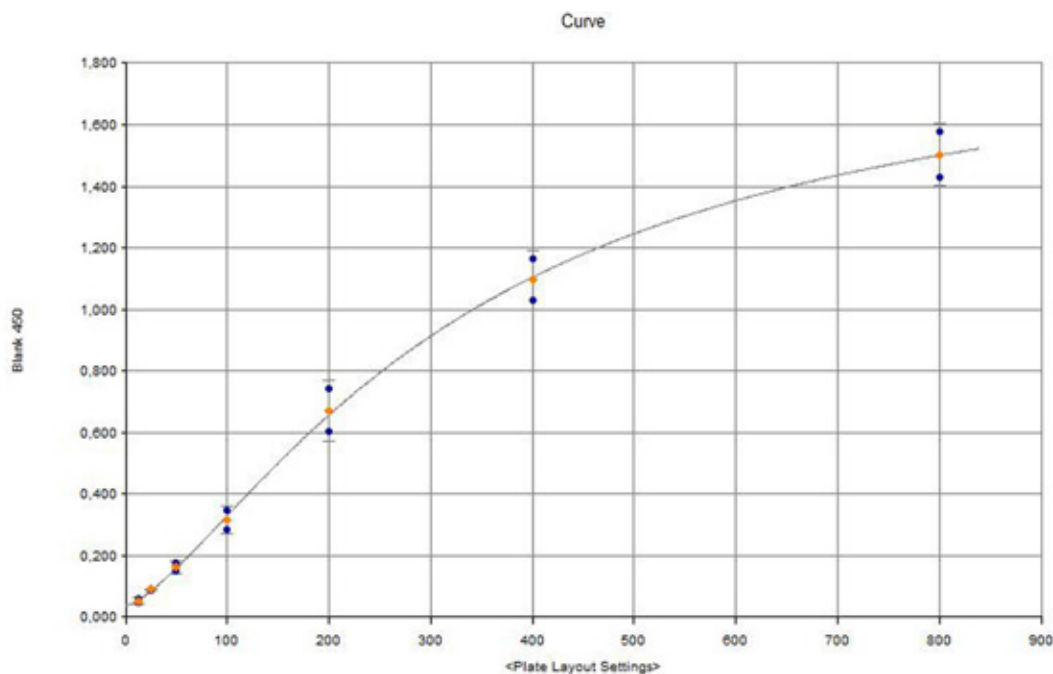
Serum omentin-1 levels were measured using the

commercial ELISA kits (SunRed, China, specific for Bovine, Catalogue No.: 201-04-0280). To determine the omentin-1 levels in the samples, double-antibody sandwich enzyme-linked immunosorbent analysis was used. The serum standard of omentin-1 is showed in graphic 1. The sensitivity of the kit was 2.358 ng/ml, and the assay range was 2.5-750 ng/ml. The intra-assay coefficient of variation was <10%, and the inter-assay coefficient of variation was <12%.

Serum glucose and TG levels were determined witha BIOLABO colorimetric measurement kit (BIOLABO, France).

Serum NEFA levels were measured with an EFFA-100 colorimetric EnzyChrom™ Free Fatty Acid Assay Kit (BioAssay Systems, Hayward, CA, USA).

Serum BHB levels were established with a colorimetric enzymatic measurement kit (Cayman Chemical, Ann Arbor, MI, USA).



Graph 1. Standard graph for omentin. The standard graph was generated based on the formula $Y=(A-D)/(1+(X/C)^B)+D$. The findings were as follows: (A:0.038, B:1.41, C:331, D:1.92) and $R^2:1$

Statistical analyses

In this study of cows in their periparturient period, the SPSS Windows 18.0 packaged software was used for the statistical analyses of data obtained from the studied parameters. Repeated measures were used in determining the differences between the PreP30, PreP14, P, PostP14, and PostP30 periods for omen-

tin-1, glucose, NEFA, TG, and BHB. For identifying the relationship between parameters, a first normality test was executed, followed by Pearson's correlation analysis. Results are provided as mean±standard error ($X\pm SEM$). $P<0.05$ was accepted as statistically significant.

RESULTS

In this study of cows in their periparturient period, the serum omentin-1 level was higher ($P<0.001$) in P than in PreP30, PostP14, and PostP30 periods (Figure 1). The serum glucose level was higher ($P<0.001$) in PreP30 than P and PostP14 periods and in PreP14 than P, PostP14, and PostP30 periods (Figure 2). The serum NEFA level was higher ($P<0.001$) in P than in PreP14 and PostP30 periods (Figure 3). The serum TG level was higher ($P<0.001$) in PreP30 than P, PostP14, and PostP30 periods and in PreP14 than P and PostP14 periods (Figure 4). The serum BHB level was higher ($P<0.001$) in P than PreP30 and PostP30 periods (Figure 5).

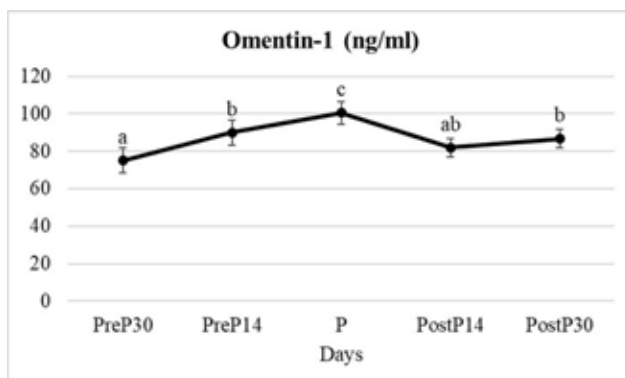


Figure 1. The change in omentin-1 concentration according to periparturient sampling periods (abc: present statistical differences between groups)

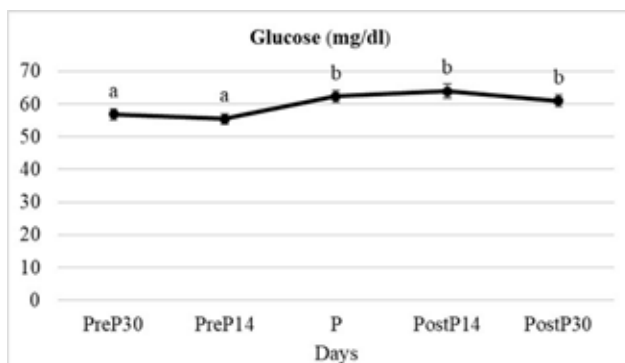


Figure 2. The change in glucose concentration according to periparturient sampling periods (abc: present statistical differences between groups)

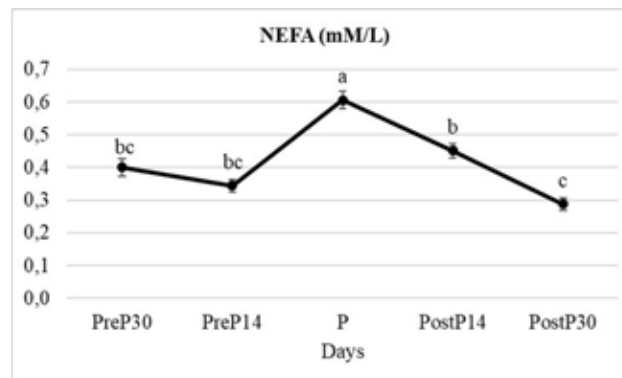


Figure 3. The change in NEFA concentration according to periparturient sampling periods (abc: present statistical differences between groups)

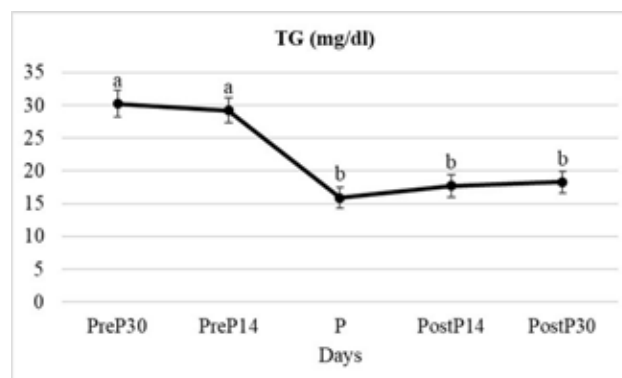


Figure 4. The change in TG concentration according to periparturient sampling periods (abc: present statistical differences between groups)

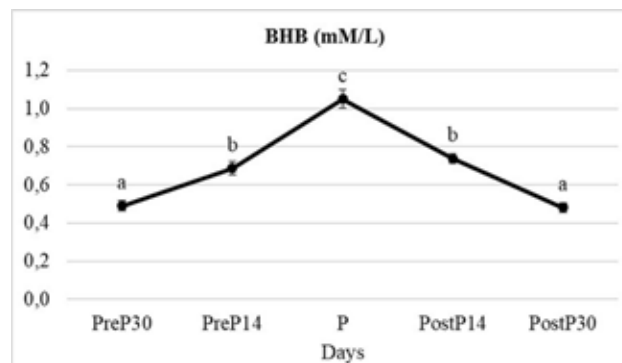


Figure 5. The change in BHB concentration according to periparturient sampling periods (abc: present statistical differences between groups)

In the correlation tests, a positive correlation was found between serum omentin-1 and serum glucose ($r^2=0.768$), serum NEFA ($r^2=0.155$), and serum BHB ($r^2=0.431$). A negative correlation was found between serum omentin-1 and serum TG ($r^2=-0.253$). A positive correlation was found between serum glucose,

serum NEFA ($r^2=0.149$), and serum BHB ($r^2=0.217$). A negative correlation was found between serum glucose and serum TG ($r^2=-0.391$) and a negative correlation was found between serum NEFA and serum TG ($r^2=-0.203$). A positive correlation was found between serum NEFA and serum BHB ($r^2=0.722$). Additionally, the serum TG level was negatively correlated with serum BHB ($r^2=-0.260$) (Table 1).

Table 1. The correlation of omentin-1, glucose, NEFA, TG and BHB concentrations according to periparturient sampling periods

		Omentin-1	Glucose	NEFA	TG
Glucose	r	0.768			
	P	0.01			
NEFA	r	0.155	0.149		
	P	NS	NS		
TG	r	-0.253	-0.391	-0.203	
	P	0.05	0.01	NS	
BHB	r	0.431	0.217	0.722	-0.260
	P	0.01	NS	0.01	NS

DISCUSSION

In cows, the periparturient period is associated with many metabolic failures related to energy metabolism. In the present study, it has been revealed that omentin-1 was studied for the first time in the periparturient period of cows based on the literature research. Therefore, we had to discuss our findings on omentin-1 with other species. It was reported that, serum omentin-1 levels were found to be lower in rats on the 21st day of pregnancy compared to levels in non-pregnant rats. In the same study, omentin-1 levels, measured in women during the three trimesters (early, mid, and late pregnancy), were observed to be significantly lower in mid and late pregnancy compared to the levels in non-pregnant women. Similarly, in pregnant rats and women, omentin-1 levels were low by the end of pregnancy (Garces et al., 2015). On the contrary, in the present study conducted on cows, omentin-1 levels started to increase in the serum on the 14th day before parturition and the level was found to be significantly high during parturition (Figure 1), decreasing again by the 14th day after parturition. In another study, omentin-1 was determined to be synthesized in fetal or maternal tissues and the concentration was discovered to have a positive correlation between these two tissues. Thus, the adipocytokines are considered to be carried by way of transplacental passage. Additionally, omentin-1 concentrations were

found to be significantly high in umbilical serum samples. As a result of high concentrations found in the fetus, omentin-1 is claimed to encourage fetal growth (Briana et al., 2011). Omentin-1 should be expected to increase in pregnancy since it is particularly synthesized in fetal and maternal tissues. Moreover, an increase in omentin-1 synthesis may be expected with the growing fetus as a result of its increasing glucose needs, which is closely related to energy metabolism. Therefore, an increased amount of omentin-1 in the serum near the end of parturition may be expected.

While serum glucose level was significantly low at the prenatal 30th and 14th days in the present study, it significantly increased at parturition and on the postnatal 14th and 30th days (Figure 2). These findings display similarities with the control group that did not receive any treatment in the study of Markantonatos and Varga (2017), conducted on cows during their transition periods. However, studies determining plasma glucose concentrations in cows in their transition periods are not completely consistent. Some studies state that glucose concentrations do not change in the transition period (Asl et al., 2011; Weber et al., 2016; Jaakson et al., 2018), while others studies indicate that glucose concentrations are low before parturition and high after parturition (Markantonatos and Varga, 2017), and other state glucose concentrations are high before parturition and low after parturition (De Koster et al., 2015; Bicalho et al., 2017; Zarrin et al., 2017; Salin et al., 2017). Increasing glucose demands during lactation for lactose synthesis and consequently increasing milk synthesis are claimed to cause plasma glucose concentrations to decrease in cows in early lactation (Zarrin et al., 2017). Nonetheless, blood glucose levels are thought to increase as a result of the glucose required to be synthesized from glucose precursors together with milk synthesis. Zachut et al. (2013) reported prepartum insulin levels to be significantly higher than postpartum levels which could explain the reason why blood glucose levels would be higher in the postpartum period. Zachut et al. (2013) examined mRNA levels of gluconeogenesis enzymes in the liver and ascertained them to be higher in the postpartum period compared to the prepartum period. Higher gluconeogenesis and lower insulin levels in the postpartum period can explain the glucose increase in postpartum periods compared to prepartum periods in the present study. Omentin-1 increases glucose uptake and decreases insulin resistance. High glucose levels and high omentin-1 levels are claimed to be related in gestational diabetes. Additionally, the

upregulation of omentin-1 expression in gestational diabetes is thought to form a new mechanism of placental glucose homeostasis in pregnancy (Mast et al., 2012). A negative correlation has been found between plasma glucose levels and omentin-1, indicating that omentin-1 insufficiency may contribute to the development of insulin resistance and type 2 diabetes mellitus (Pan et al., 2010). Conversely, a significantly positive correlation ($P < 0.01$) in the present study was found between serum glucose and omentin-1 levels (Table 1). Since the energy metabolism of ruminants and monogastrics are different, it is believed that further studies are necessary to examine the relationship between omentin-1 and glucose in the serum of cows. In the present study, while the serum TG concentration was high before parturition, it decreased at the moment of parturition similar to the findings of Schuermann et al. (2019). In our study, it remained low until the 30th day (Figure 4). Similarly, Schuermann et al. (2019) found it to remain low until the pre-breeding period (4th week after parturition). In human studies, generally, a negative correlation is found between serum omentin-1 levels and serum TG levels similar to our findings (Yan et al., 2011; Garces et al., 2015; Koryany et al., 2018).

In cows, ketone bodies and especially BHB are known to cause subclinical and clinical ketosis, suppress feed intake, and have a negative impact on reproduction (Duffield et al., 2009; Ospina et al., 2010; Zarrin et al., 2013; Raboisson et al., 2014; Zarrin et al., 2017). While NEFA and BHB levels were low before parturition in our study similar to other studies (Weber et al., 2015; McCarthy et al., 2015), they reached their highest levels at parturition (Figs. 3 and 5). Nevertheless, different results have been obtained in different studies, such as similar BHB concentrations in the two weeks before parturition and two weeks after parturition (Duffield et al., 2009; Ospina et al., 2010; Zarrin et al., 2013; Raboisson et al., 2014; Zarrin et al., 2017), BHB values peaking between the first and third weeks after parturition (Zhang et al., 2016), high

NEFA amounts in cows before parturition (De Koster et al., 2015), and serum NEFA amounts decreasing after parturition (Hausmann et al., 2017). McCarthy et al. (2015) found a negative correlation between NEFA and BHB levels in cows studied during the transition period. They attributed this result to the carbon source necessary for BHB synthesis provided from other compounds such as lactate and ketogenic amino acids, instead of NEFA. However, a significant positive correlation ($P < 0.01$) between serum NEFA and BHB was found in our study (Table 1). NEFA molecules transform into BHB through Acetyl-coA as their levels increase in blood and they are degraded to be used as energy. Because of this mechanism, a positive correlation between them is natural. In the present study, a statistically significant relationship between NEFA levels and TG, glucose, and omentin-1 levels could not be determined. While a significant relationship between BHB levels and TG and glucose did not exist, a significant positive correlation ($P < 0.01$) was found between omentin-1 and BHB levels (Table 1).

CONCLUSION

In conclusion, findings of the present study shows that omentin-1 may play an important role in the periparturient period. A positive correlation of omentin-1 with energy fuels NEFA, glucose, and BHB suggests that omentin-1 plays a role in energy metabolism like other adipokines. The fact that omentin-1 levels increase during delivery, when the fetus needs energy most, supports this hypothesis.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

All authors declare that there is no potential conflict of interest.

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