Journal of Multidisciplinary Sciences
www.multidisciplines.com

Metabolites and hormones can predict postpartum uterine disorder during transition period of dairy cows

Mervat Sayed Hassan[1], Elham Abdelsabour Abd-Allah[2]*
[1]Department of Theriogenology, Faculty of Veterinary Medicine, New Valley University, El-Kharga, 71511, Egypt.
[2]Department of Zoology, Faculty of Science, New Valley University, El-Kharga, 72511, Egypt.
[*]Corresponding author email address: elham_abdelsabour@aun.edu.eg

Received: 25 February 2020; Accepted: 5 June 2020; Published online: 15 June 2020

Abstract. Postpartum uterine diseases in dairy cows have undesirable effects on reproductive efficiency. The current study aimed to evaluate hormones and some metabolites which can predict postpartum uterine disorder in dairy cows (Holstein Friesian) during the transition period. A total of 32 dairy cows were divided into two groups; 9 control healthy cows without any postpartum uterine disorder, and 23 cows suffered from the postpartum uterine disorder, which further subdivided into12 cows with retained fetal membranes (RFM) and 11 cows with postpartum metritis (PM). Blood samples were collected from the jugular vein of each cow weekly from 21 days' prepartum to 21 days postpartum early in the morning. Glucose (BG), triglyceride (TG), cholesterol, progesterone (P4), cortisol, calcium (Ca), phosphorus (P), magnesium (Mg), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP) and albumin, were measured. The results showed that TG, TC, P4, cortisol, Ca, P, Mg, TP, and albumin were higher at the prepartum period than the postpartum one in all dairy cows. In contrast, BG, AST, and ALT were significantly higher in all dairy cows during the postpartum period. Cows with RFM had higher serum prepartum levels of TC, P4, and cortisol. However, prepartum levels of TG, TP, AST, and ALT were significantly higher in cows with PM. A negative energy balance (NEB) and disturbance in some metabolites and hormones at prepartum could induce PM and RFM as postpartum uterine disorders during the transitional period in the dairy cows.

***Keywords**: dairy cows, postpartum disorder, hormones, metabolites.*

Cite this as: Hassan, M.S. & Abd-Allah, E.A. (2020). Egypt Metabolites and hormones can predict postpartum uterine disorder during transition period of dairy cows. J. Multidiscip. Sci. 2(1), 1-9.

1. Introduction

Negative energy balance (NEB) in dairy cows, especially in late pregnancy and early lactation periods, was associated with reduced dry matter intake (DMI), increased requirements for the development of the embryo, and synthesis of milk [1]. Reduction in minerals, proteins, and vitamins [2] leads to excessive tissue fat mobilization [3], ultimately increase susceptibility for metabolic diseases as ketosis and hypocalcemia, causing a higher incidence of postpartum disorders as puerperal metritis (PM), retained fetal membranes (RFM) and endometritis [4]. Metabolic disturbances during the prepartum period play a significant role in the puerperal period [5]. The delay in the postpartum ovarian resumption creates a severe problem for the reproduction and production of highly lactating dairy farms by lowering the reproductive efficiency and infertility in dairy cows [6].

The early postpartum periods are critical periods with a significant concern on the farm of dairy cows; RFM and PM are the prevalent disturbances in the postpartum period in dairy cows [7]. PM usually develops after RFM, but it can develop alone in many cases; a few weeks' prepartum, cows suffered from RFM showed high ketone bodies (KB) and plasma cortisol [8]. Hypoglycemia was not involved in cows with RFM or high blood KB concentrations, but it may be related to high levels of cortisol, which is considered a stress hormone responsible for RFM and PM directly or indirectly [9]. Concentrations of plasma cortisol increase 3–4 folds of baseline in cows during parturition; however, it may increase 5-7 times during postpartum disorders. Besides, dystocia and other stress factors at parturition cause elevation in the cortisol level, which has an immune suppressive effect [10].

Disturbances in the concentrations of hormones as progesterone (P4) and estrogen (E2) are also involved in postpartum disorders. Serum P4 is a helpful indicator for postpartum ovarian resumption; also, the corpus luteum is considered functionally active when the P4 level is more than 1 ng/dl [11]. Kotwica et al. [12] suggested that the reduction in oxytocin level during calving is considered a risk factor in RFM. Also, Grillo-Ardila et al. [13] found that the prostaglandins (PG) have an essential role in standard placental delivery through activation of the uterine contraction and help in the delivery of the placenta). The PG production is usually highly related to estrogen (E2); therefore, the PG level reduction in RFM can result from E2 deficiency [14]. Prostaglandin-F-2-alpha (PG-F2α) is necessary for the destruction of CL. So the low PG-F2α level act as one of the contributing factors for the development of RFM [15]. The transition period in dairy cows extends from three weeks' prepartum to three weeks postpartum, where the cows are significantly affected, especially in the lactation cycle [16]. Considerable physiological, nutritive, metabolic, and immunological variations occur within this time as the productive cycle of cow shifts from the gestational non-lactating case to the beginning of milk manufacture and production. Cows have adapted metabolically to the maximum increase in energy and nutrient requirements for milk yield in the following lactation [17].

Evaluation of some blood metabolites at the prepartum period helps indicate the nutritional, metabolic, and health status of dairy cows, which helps predict postpartum metabolic and uterine disorders [15]. Moreover, the changes in the levels of blood metabolites in healthy cows versus diseased ones may give insight into the possible risk factors behind disease occurrence [17]. Metabolic changes that occur 2 weeks before calving significantly affect dairy cows' reproductive activity after calving [18]. Therefore, the present study aimed to evaluate some hormones and blood metabolites to use these parameters to predict some postpartum uterine disorders in dairy cows during the transition period.

2. Materials and methods

2.1. Animals and experimental design

The present study was carried out using 32 dairy cows (Holstein Friesian, 2-6 parity, 20-23 Kg/day average milk production) were selected from two dairy farms with the same nutritional and environmental conditions, in New Assuit city, at Assiut governorate, Egypt from December 2016 to March 2017. These cows were examined during prepartum (-21 days) and postpartum (+21 days) periods. They were divided into two groups control (n=9), which consisted of healthy cows without any postpartum uterine disorder, and uterine disorder group (n=23), which were subdivided into cows that had retained fetal membrane (RFM; n=12), and those had puerperal metritis (PM; n=11). Gynecological examination through rectally and vaginal examination was performed considering the following parameters: a) RFM: when fetal membranes were not released within 24 h postpartum [19]. b) PM: when cows had enlarged uterus and exhibited offensive vaginal discharge, they were usually watery red-brown within the first 10 days postpartum and the presence of systemic illness as fever [20].

2.2. Blood samples collection and analysis

Blood samples were collected from the jugular vein of each cow weekly from 21 days prepartum to 21 days postpartum early in the morning. They were collected in two Wisterman tubes; the first one containing sodium fluoride and potassium oxalates anticoagulants for BG estimation, while the second one was a plain tube for analysis of other biochemical parameters. Blood samples were centrifuged at 3000 rpm for 15 minutes, and the obtained plasma and serum were stored at -80°C at a deep freezer until the measurement of biochemical and hormonal parameters.

2.3. Measurements of biochemical parameters

Total protein (TP), blood glucose (BG), cholesterol (TC), and triglyceride (TG) calometeric kits were obtained from Diamond (Egypt). Calometric albumin and kinetic alanine aminotransferase (ALT) and aspartate aminotransferase (AST) kits were purchased from the Human Company (Germany). Calcium (Ca^{2+}) and magnesium (Mg^{2+}) kits were obtained from Spectrum Company (Germany). Based on the manufacturer's instructions, phosphorus (P) (Catalog no. 294001) was estimated by a commercially available kit (Egyptian Company for Biotechnology, Cairo, Egypt). Progesterone (P4) level was measured using a linked immunosorbent assay kit provided by Bio Check, Inc., South San Francisco, USA (Catalog number; BC-1113). According to manufacturer instructions, cortisol level was determined using cortisol ELISA kit (Diagnostics Biochem. Canada Inc., Canada, Catalog no. CAN-C-270).

2.4. Statistical analysis

The analysis was carried out using a standard statistical software program (SPSS version 16, SPSS Inc, Chicago). The data were expressed as means ± standard error of the mean (SEM). The paired sample T-test estimated the differences between the groups. Differences were considered statistically significant at $P \leq 0.05$.

3. Results and discussion

3.1. Blood Glucose, Triglyceride, and Total Cholesterol levels

TG and TC were significantly ($P \leq 0.001$) elevated in all cows at the prepartum period compared with the control ones. At the postpartum period, TG was raised while TC decreased compared with the control cows (Figure 1). BG levels were markedly reduced in cows who suffered from postpartum disorders (PM+RFM) at the two periods (pre and postpartum) compared to the control cows. Comparing dairy cows who suffered from RFM and PM during the two periods, we found that BG, TG, and TC were significantly lowered in the postpartum period than in the prepartum period. The early predictions of postpartum uterine problems are critical for optimizing dairy animals' productive and reproductive performance**.** Postpartum uterine disorder remains a significant challenge affecting fertility, overall reproductive efficiency, and dairy industry [21]. Glucose is a primary metabolic fuel essential for the functions of vital organs, fetal growth, and milk synthesis [18]. This explains our results concerning significant hypoglycemia were noted in our results mainly at the first week postpartum than prepartum period; these findings may be the same line with Mohebbi-Fani et al. [22], who noted that BG levels decreased significantly in dairy cows 3-5 days postpartum as compared with that found during 3-5 days' prepartum. Doepel et al. [23] reported a temporary reduction in the BG in the first week after calving secondary to increased lactose synthesis and decreased gluconeogenesis. This decrease in the BG levels may be due to the diet's energy restriction, mainly at early lactation periods, along with high glucose utilization in the mammary gland [24].

Our results revealed significantly lower BG levels in cows suffering from postpartum uterine disorders than those in the healthy cows, in agreement with the findings of Galvao et al. [25]. They suggested that glucose reduction in the dairy cows during the transition period is a predisposing factor for postpartum uterine disorders (RFM, PM, and clinical endometritis). Moreover, Mili et al. [26] found a negative relation between BG level and postpartum uterine disorders in buffaloes; this may be due to NEB during the early lactation period besides immune suppression, which enhances the development of postpartum infections.

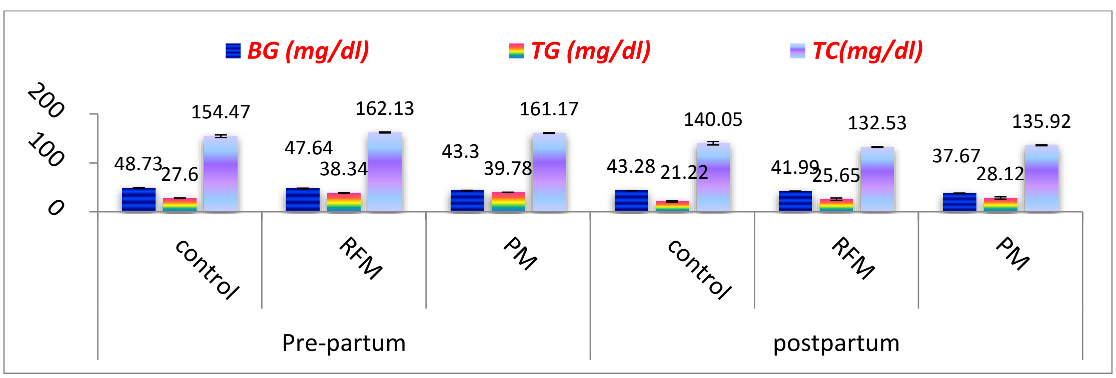

Figure 1. BG, TG, and TC levels (mean ± SE) in control dairy cows versus diseased ones suffered from postpartum uterine disorders during the transition period.

According to our findings, BG levels were slightly lower in cows suffered from RFM than healthy ones during the prepartum period in harmony with that observed by Doepel et al. [23]. RFM in cows can cause uterine inflammation and bacterial growth, especially during the early puerperium, resulting in subsequent endometrium, cervical, or vaginal inflammation. Moreover, our result revealed a significant decrease in the BG level in cows suffered from PM than those who suffered from REF. This BG reduction may have considered a risk factor for PM [4]. TC levels were significantly higher at prepartum than postpartum, in our findings were in the same line with Kim and Suh [27]. They showed that energy deficiency at early lactation was associated with a reduction in the cholesterol level, indicating that TC level may be helpful in the prediction of energy status during this period and may be related to NEB and decreased DMI after calving. Besides, reduced food intake and increased cortisol subsequently decreased cholesterol synthesis [28]. Moreover, cholesterol is known as an ovarian steroidogenic precursor that stimulates P4 synthesis in the luteal tissue. Higher prepartum TC level in cows suffered from RFM, as denoted in our study, was similar to Quiroz-Rocha et al. [29], who reported that RFM risk was higher with increased prepartum cholesterol levels.

Significantly higher TG levels at prepartum than postpartum in the present work and the presence of TG at the highest levels during prepartum followed by reduction at parturition and postpartum and declared that this increase might be due to elevation of mammary lipoprotein lipase activity. Due to NEB and decreased DMI, fat mobilization occurs, linked to immunosuppression [30].

3.2. Plasma progesterone (P4) and Cortisol levels

At the prepartum period, dairy cows suffered from RFM, and PM had significantly higher (P≤0.05-0.01) P4 and serum cortisol levels compared with control ones. The same results were obtained from caws in the postpartum period (Figure 2). When comparing caws with postpartum disorders, P4 and serum cortisol levels were markedly decreased in the postpartum period comparing with a prepartum period. Higher P4 and cortisol levels in cows suffered from RFM during the prepartum period, as noted in our results, were in harmony with Kaczmarowski et al. [6]. The latter denoted that elevated P4 inhibits collagenase activity, leading to RFM and delayed uterine involution. During parturition as a stressful condition, glucocorticoids released stimulating P4 production in placental tissue and blocked proteolytic activities, impairing placental delivery. LeBlance [18] mentioned that cows suffered from RFM had higher serum cortisol levels for several days before calving, which inhibited neutrophil function.

Moreover, significantly higher postpartum P4 and cortisol levels in cows affected by RFM and PM than the healthy cows in the present data were similar to the findings of Kimura et al. [31], who found that RFM and PM cows had higher P4 and cortisol levels in comparison to the control ones between 5th and 8th days postpartum. They also reported that the high postpartum P4 levels cause immunosuppression that may be responsible for postpartum uterine infections, reduced leukocytic activity, and increased RFM and PM incidence. Scheibl and Zerbe [32] found that the high P4 level in the uterus postpartum causes immunosuppression as P4 causes decreased polymorph nuclear cells (PMN) migration gravid uterus, bacterial persistence, and the accumulation of the immunosuppressive protein in the uterine lumen. Therefore, the uterus becomes more susceptible to bacterial infection and persistence.

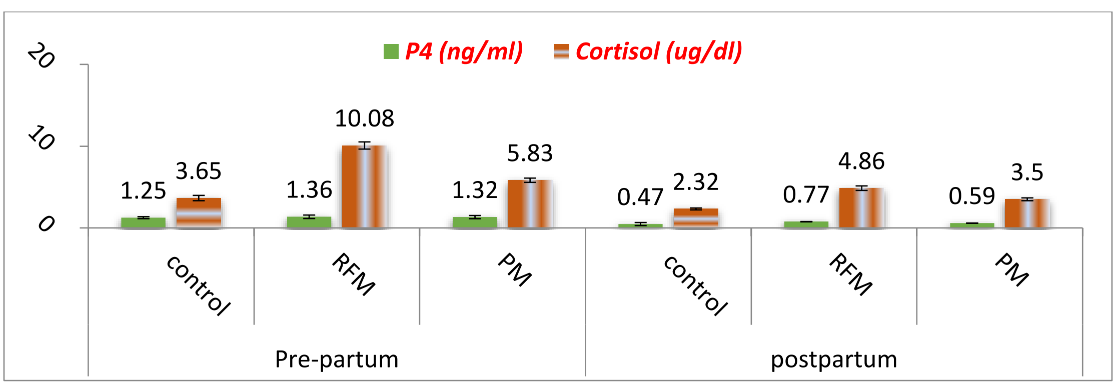

Figure 2. Serum P4 and Cortisol levels (mean ± SE) in control dairy cows versus diseased ones suffered from postpartum uterine disorders during the transition period.

3.3. Serum minerals levels (Ca, P and Mg)

Concerning mineral profile, in the prepartum period, Ca, P, and Mg levels in all dairy cows were significantly decreased (p≤0.05- 0.01) than control cows (Figure 3). It was noted that the Ca, P, and Mg levels in all dairy cows at the postpartum period were significantly decreased (p≤0.05- 0.01) than other cows at prepartum. Minerals are essential for fetal growth, lactation, and fertility in dairy animals, and any alteration in Ca-P-Mg homeostasis may lead to the occurrence of reproductive problems [33]. The significantly low levels of Ca and P in the postpartum dairy cows in the current work were similar [34]. In contrast, a very high P level impairs Ca homeostasis. Moderate P reduction may be due to its needs in the colostrum synthesis, insufficient feed supply, and prolonged anorexia [35]. Our results agreed with Sheldon et al. [19], who noted a relationship between low serum Ca and P and the development of uterine diseases in lactating cows. Ca is essential in normal muscle contraction, sensitization of female genitalia for the oxytocin hormone. Therefore, Ca deficiency may be a predisposing factor in uterine inertia, leading to dystocia, RFM, and PM [36]. Also, Lower P may contribute to the reduction of uterine muscle contraction.

Moreover, they added that cows with low Ca, P, and Mg values during the prepartum and the post-calving periods were more predispose to RFM. Furthermore, hypocalcemia is associated with immunosuppression and uterine diseases such as RFM and PM [37]. Cows with RFM have a reduction in neutrophil count and phagocytic function, leading to PM occurrence. Mg plays a vital role during the metabolism of carbohydrates, proteins, and lipids [31]. In our study, postpartum hypomagnesemia was similar to Masoero et al. [38], who demonstrated that Mg reduction frequently occurs in postpartum cows. This reduction may be due to its participation in the regulation of bone resorption.

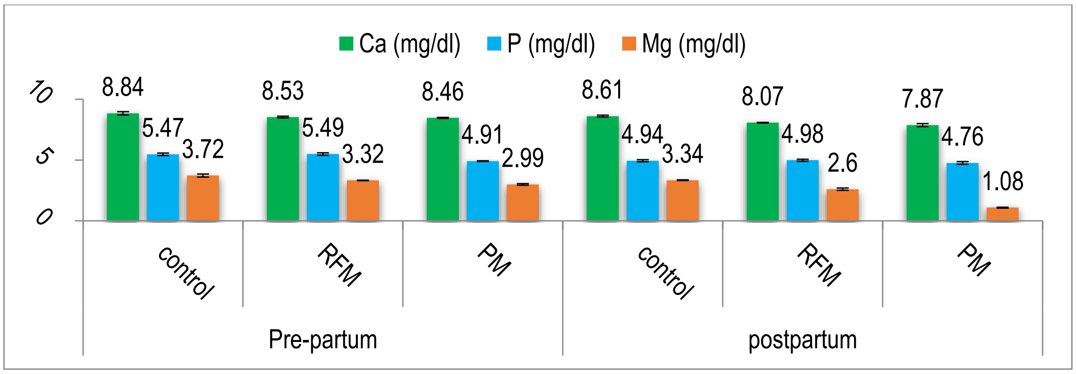

Figure 3. Serum calcium, phosphorus, and magnesium levels (mean ± SE) in control dairy cows versus diseased ones suffered from postpartum uterine disorders during the transition period.

3.4. Liver enzymes activities (AST and ALT)

The changes in the activities of liver enzymes (AST and ALT) at prepartum and postpartum periods in the control of dairy cows and those suffering from uterine disorders were shown in (Figure 4). Their activities were significantly increased in cows affected by uterine disorders than the control ones in both pre and postpartum.

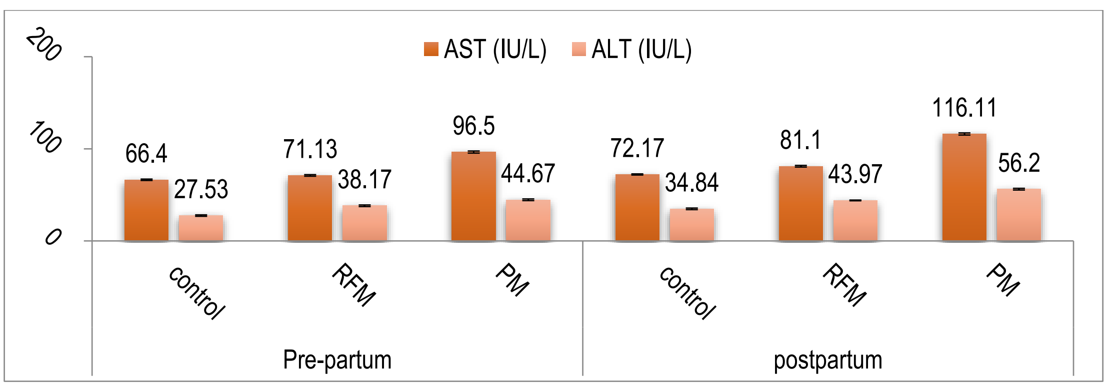

Figure 4. Serum AST and ALT levels (mean ± SE) in control dairy cows versus diseased ones suffered from postpartum uterine disorders during the transition period.

Their activities were significantly higher (P≤0.05) during the postpartum period than in the prepartum period in all dairy cows. Regarding the hepatic enzymes, the increases in AST and ALT activities in dairy cows postpartum than prepartum, as demonstrated in our study, were in agreement with Djokovic et al. [39], who found higher AST activities at calving and postpartum than prepartum. Our study revealed higher enzyme activities in cows affected by uterine disorders than the healthy cows. Sahinduran et al. [40] showed that high liver enzyme activity mainly occurred in diseases accompanied by hepatic damage. Our results pointed to a significant increase in AST and ALT activities in cows affected by PM in agreement with Cui et al. [41], who declared that inflammation leads to increased permeability of cell membrane and leakage of liver enzymes into the bloodstream. Goff [42] suggested that in PM had NEB, excessive consumption of body fat for energy supply during postpartum resulted in accumulation of TG within the hepatocytes and impaired liver function.

3.5. Total protein (TP) and albumin levels

TP concentrations were significantly decreased (p≤0.001) in diseased cows than control ones in the two periods. The TP and albumin levels significantly lower (P≤0.01) during postpartum than prepartum (Figure 5). Additionally, cows that suffered from RFM showed significantly (P≤0.05) lower TP than PM. In comparison, cows with PM show a reduction in albumin levels than those suffered from RFM. Based on our findings, lower TP and albumin levels during postpartum were similar to a previous result, Roussel et al. [35] found a reduction in their concentrations with the progression of lactation. TP reduction may be due to the requirements of proteins for milking and the formation of immunoglobulins. Xu et al. [43] declared that this reduction is highly attributed to the abnormality in the liver function of diseased cows. Our results also denoted that RFM cows had significantly lowered TP levels than the healthy or PM cows. Magnus and Lali [44] found that buffaloes with RFM had significantly low TP levels four weeks postpartum. Otherwise, Van Saun [45] demonstrated that cows with low serum albumin concentrations in dry cows resulted in a higher risk for postpartum problems. Albumin is considered a diagnostic indicator of liver function, and he also reported a low serum albumin level in severe hepatic fatty infiltration associated with a reduction in albumin synthesis by the liver [46]. Also, NEB and energy deficiency in the diet at the prepartum period can lead to the decreased serum level of glucose and insulin concentrations, resulting in excess lipolysis of body fat and elevated serum Non-Esterified Fatty Acids (NEFA) levels. Increased the hepatic outfit of fatty acids, the elevation of their esterification may be several times higher than average, leading to the pathological deposition in the liver in the form of triglycerides (TG), resulting in the fatty liver formation and ketosis [47]. The reduction of the NEB is substantial for the prevention of fatty liver formation. This can be carried out by preventing unbalanced feeds, fat cattle, and environmental stress [48].

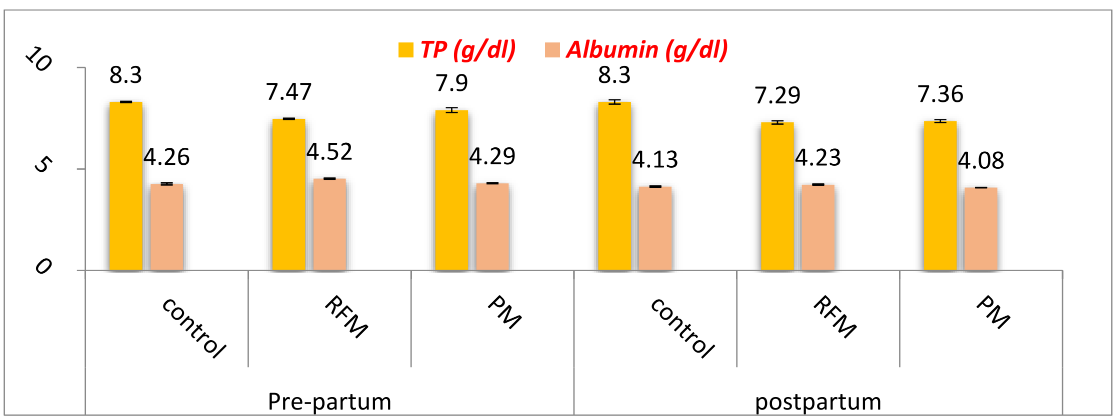

Figure 5. Serum TP and Albumin levels (mean ± SE) in control dairy cows versus diseased ones suffered from postpartum uterine disorders during the transition period.

4. Conclusion

Deficiency of essential metabolites and hormones at the prepartum period, besides fetus and milk production, acts as a stress factor that may contribute to PM and RFM as a postpartum uterine disorder the transition period in the dairy cows. Therefore, it is highly recommended to improve the nutritional and immune health status of dairy animals at the prepartum and early lactation time, which most probably significantly affects animals at these periods.

Conflicts of interest. The authors declare that there is no conflict of interest.

ORCID
Mervat Sayed Hassan: http://orcid.org/0000-0002-6151-8570
Elham Abdelsabour Abd-Allah: http://orcid.org/0000-0001-9762-4220

References

[1] LeBlanc, S.J. (2013). Managing transition period health for reproductive performance in dairy cows. Cattle Pract. 21, 209-215.

[2] Paiano, R.B., Lahr, F.C., Poit, D.A.S., Costa, A.G.B.V.B., Birgel, D.B., Junior, E.H.B. (2018). Biochemical profile in dairy cows with artificial induction of lactation. Pesqui. Vet. Bras. 38, 2289-2292.

[3] Paiano, R.B., Birgel, D.B., Junior, E.H.B. (2019). Uterine involution and reproductive performance in dairy cows with metabolic diseases. Animals 9(3), 93.

[4] Bicalho, M.L.S., Marques, E.C., Gilbert, R.O., Bicalho, R.C. (2017). The association of plasma glucose, BHBA, and NEFA with postpartum uterine diseases, fertility, and milk production of Holstein dairy cows. Theriogenology 88, 270-282.

[5] Kaczmarowski, M., Malinowski, E., Markiewicz, H. (2008). Some hormonal and biochemical blood indices in cows with retained placenta and puerperal metritis. Bull. Vet. Inst. Pulawy. 50, 89-92.

[6] Dawod, A., Mostafa, I., El-Baz, H., Abdel-Hamid, T., Fathala MM. (2015). Risks of some postpartum uterine affection on reproduction and milk yield of high yielding dairy cows. J. Veterinar. Sci. Technol. 6, 4.

[7] Rohidas, U.A., Pande, V.V., Girme, A., Kulkarni, N.D., Bhalke, R.D. (2018). Physiology and causes of retention of fetal membrane in dairy cows: An Overview. IAJPS. 05(03), 1962-1968.

[8] Huzzey, J.M., Nydam, D.V., Grant, R.J., Overton, T.R. (2011). Associations of prepartum plasma cortisol, haptoglobin, fecal cortisol metabolites, and nonesterified fatty acids with postpartum health status in Holstein dairy cows. J. Dairy Sci. 94, 5878-5889.

[9] Weber, P.S.D., Preisler, M.T., Kizilkaya, K., Burton, J. (2000). Cortisol correlates with neutrophil CD62L mRNA abundance at parturition. Proceedings of International Symposium on Immunology of Ruminant Mammary Gland. Stresa Italy. 171-173.

[10] Civelek, T., Celik, H.A., Avci, G., Cingi, C.C. (2008). Effects of dystocia on plasma cortisol and cholesterol levels in Holstein heifers and their newborn calves. Bull. Vet. Inst. Pulavy. 52, 649-654.

[11] Mojtaba, C.R., Thatcher, W.W., Clark, J.H. (2012). Relation between ovarian activity and energy status during the early postpartum period of high producing dairy cows. J. Dairy Sci. 73, 938-947.

[12] Kotwica, G., Janowski, T., Zduńczyk, S., Raś, A. (1990). Oxytocin plasma levels in cows with normal parturition or dystocia and with placental retention. Exp. Clin. Endocrinol. 95, 203-209.

[13] Grillo-Ardila, C.F., Amaya-Guio, J., Ruiz-Parra, A.I., Amaya- Restrepo, J.C. (2018). Systematic review of prostaglandin analogues for retained placenta. Int. J. Gynaecol. Obstet. 143(1), 19-23.

[14] Yasuhara, T., Koyama, K., Sakumoto, R., Fujii, T., Naito, A., Moriyasu, S., Kageyama, S., Hirayama, H. (2019). Enhanced glucocorticoid exposure facilitates the expression of genes involved in prostaglandin and estrogen syntheses in bovine placentomes at induced parturition. Theriogenology 139, 1-7.

[15] Parmar, S.C., Parmar, C.P., Patel, J.A. (2016). Use of PGF 2α in ovarian and uterine pathological conditions of bovine: a therapeutic approach. Explor. Anim. Med. Res. 6(2), 132-2141.

[16] Sordillo, L.M., Raphael, W. (2013). Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. Vet. Clin. North Am. Food Anim. Pract. 29, 267-278.

[17] Ametaj, B.N. (2014). Metabolic disorders of dairy cattle. In Encyclopedia of Life Support Systems (EOLSS): Veterinary Science, Eolss Publishers Paris, France.

[18] LeBlance, S. (2010). Monitoring metabolic health of dairy cattle in the transition period. J. Reprod. Develop. 56, S29-S35.

[19] Sheldon, I.M., Cronin, J., Goetze, L., Donofrio, G., Schuberth, H.J. (2009). Defining postpartum uterine disease and the mechanisms of infection and immunity in the female reproductive tract in cattle. Biol. Reprod. 81(1), 1025-1032.

[20] Sheldon, I.M., Lewis, G.S., Leblanc, S., Gilbert, R.O. (2006). Defining postpartum uterine disease in cattle. Theriogenology 65 (8), 1516-1530.

[21] Joshua, O. (2014). Cow postpartum uterine infection: A review of risk factors, prevention and the overall impact. Vet. Res. Int. (2), 18-32.

[22] Mohebbi-Fani, M., Naifi, S., Rowghani, E., Bahrami, S., Jamshidi, O. (2009). Thyroid hormones and their correlations with serum glucose, beta hydroxybutyrate, nonesterified fatty acids, cholesterol, and lipoproteins of high yielding dairy cows at different stages of lactation cycle. Comp. Clin. Path. 18(3), 211-216.

[23] Doepel, L., Lapierre, H., Kenneky, J.J. (2002). Peripartum performance and metabolism of dairy cows in response to prepartum energy and protein intake. J. Dairy Sci. 85, 2315-2334.

[24] Nazifi, S., Mohebbi-Fani, M., Rowghani, E., Behbood, M.R. (2008). Studies on the relationship between subclinical ketosis and liver injuries with first two months of lactation period in high producing Iranian Holstein cows. J. Dairy Sci. 3(1), 29-35.

[25] Galvao, K.N., Flaminio, M.J.B., Brittin, S.B., Sper, R., Fraga, M., Caixeta, L. (2010). Association between uterine disease and indicators of neutrophil and systemic energy status in lactating Holstein cows. J. Dairy Sci. 93, 2926-2937.

[26] Mili, B., Pandita, S., Bharath Kumar, B.S. (2016). Association of blood metabolites with reproductive disorders in postpartum murrah buffaloes. Buffalo Bull. 35 (4), 643-651.

[27] Kim, I.H., Suh, G.H. (2003). Effect of the amount of body condition loss from the dry to near calving periods on the subsequent body condition change, occurrence of postpartum diseases, metabolic parameters and reproductive performance in Holstein dairy cows. Theriogenology 60, 1445-1456.

[28] Alvarenga, E.A., Moreira, G.H.F.A., Filho, E.J.F., Leme, F.O.P., Coelho, S.G., Molina, L.R. (2015). Avaliação do perfilMetabólico de vacas da raça holandesa durante o período de transição. Pesqui. Vet. Bras. 35(3), 281-290.

[29] Quiroz-Rocha, G.F., LeBlance, S.J., Duffield, T.F., Wood, D., Leslie, K.E., Jacobs, R.M. (2009). Reference limits for biochemical and hematological analytes of dairy cows one week before and one week after parturition. Can. Vet. J. 50, 383-388.

[30] Imhasly, S., Bieli, C., Naegeli, H., Nystrom, L., Ruetten, M., Gerspach, C. (2015). Blood plasma lipidome profile of dairy cows during the transition period, BMC Vet. Res. 11, 252.

[31] Saqib, M.N., Qureshi, M.S., Khan, R.U. (2018). Changes in postpartum metabolites and resumption of ovarian cyclicity in primiparous and multiparous dairy cows. Appl. Biol. Chem. 61(1), 107-111.

[32] Kimura, K., Goff, J.P., Kehreli, M.E.Jr., Reinhardt TA (2002). Decreased neutrophil function as a cause of retained placenta in dairy cattle. J. Dairy Sci. 85, 544-550.

[33] Scheibl, P., Zerbe, H. (2000). Effect of progesterone on the immune system in consideration of bovine placental retention. DTW. Deut. Tierärztl. Woch. 107(6), 221-227.

[34] Ahuja, A., Parmar, D. (2017). Role of minerals in reproductive health of dairy cattle: A review. Int. J. Livest. Res. 7(10), 16-26.

[35] Goff, J.P. (2004). Macromineral disorders of the transition cow. Vet. Clin. North Am. Food Anim. Pract. 20(3), 471-494.

[36] Youssef, M.A., El-Khodery, S.A., El-deeb, W.M., Abou El-Amaiem, W.E. (2010). Ketosis in buffalo (Bubalusbubalis): clinical findings and the associated oxidative stress level. Trop. Anim. Health Prod. 42(8), 1771-1777.

[37] Martinez, N.C.A., Risco, F.S., Lima, R.S., Bisinotto, L.F., Greco, E.S., Ribeiro, F., Maunsell, F., Galvão, K., Santos, J.E. (2012). Evaluation of peripartal calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. J. Dairy Sci. 95, 7158-7172.

[38] Tillard, E., Humbolt, P., Faye, B., Lecomte, P., Dohoo, I., Bocquier, F. (2008). Post calving factors affecting conception risk in Holstein dairy cows in tropical and subtropical conditions. Theriogenology 69(4), 443-445.

[39] Masoero, F., Moschini, M., Pulimeno, A.M. (2016). Serum calcium and magnesium level in dairy cows at calving. Ital. J. Anim. Sci. 2(1), 172-174.

[40] Djokovic, R., Cincovic, M., Ilic, Z., *Kurcubic, V., Andjelic, B., Petrovic, M.,* Lalic N., Jasovic B. (2019). Estimation of metabolic status in high yielding dairy cows during transition period and full lactation. Acta Sci. Vet. 47, 1667.

[41] Sahinduran, S., Sezer, K., Buyukoglu, T., Albay, M.K., Karakurum, M.C. (2010). Evaluation of some haematological and biochemical parameters before and after treatment in cows with ketosis and comparison of different treatment methods. J. Anim. Ve. Adv. 9, 266-271.

[42] Cui, L., Wang, H., Ding, Y., Li, J., Li, J. (2019). Changes in the blood routine, biochemical indexes and the pro-inflammatory cytokine expressions of peripheral leukocytes in postpartum dairy cows with metritis. BMC Vet. Res. 15, 157.

[43] Goff, J.P. (2008). Immune suppression around the time of calving and the impact of metabolic disease. XXV Jubilee World Buiatrics Congress Budapest, Hungry. Hungarian Vet. J. 130 (1), 39-42.

[44] Xu, C., Shu, S., Xia, C., Wang, B., Zhang, H.Y., Jun, B. (2014). Investigation on the relationship of insulin resistance and ketosis in dairy cows. J. Vet. Sci. Technol. 5, 162.
[45] Magnus, P.K., Lali, F.A. (2009). Serum biochemical profile of postpartum metritic cow. Vet. World 2 (1), 27-28.
[46] Van Saun, R.J. (2004a). Metabolic profiling and health risk in transition cows. Proc. Am. Assoc. Bovine Prac. 37, 212-213.
[47] Wathers, D.C. (2010). Interactions between energy balance, the immune system ends the reproductive tract with influence on dairy cow fertility. Cattle Pract. 18, 19-26.
[48] Mordak, R., Nicpoń, J., Josef, I. (2017). Metabolic and mineral conditions of retained placenta in highly productive dairy cows: pathogenesis, diagnostics and prevention: A review. Acta Vet. Brno. 86, 239-248.