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Original article

Does increased immune response at early postpartum period have a relationship with metabolic markers and subsequent fertility?

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Abstract

The aim of the present study was to investigate blood parameters and subsequent fertility in cows with or without increased postpartum polymorphonuclear neutrophil activity. The study was conducted with 15 Brown Swiss cows between 1-3 lactations. Polymorphonuclear neutrophil activities were assessed at 10±4 days before and after parturition. The cows which maintained their phagocytic and oxidative burst activites compared to the prepartum period were classified as control (CON), and cows which increased phagocytic and oxidative burst activites were defined as increased cellular immune response (ICIR) cows. Energy, protein metabolism markers, hepatic enzymes, blood mineral levels and body condition scores were measured at -10±4, 3±2, 10±4 days relative to parturition. Pregnancy rates, the number of inseminations, and calving to pregnancy intervals were evaluated. The mean non-esterified fatty acid (NEFA) and beta-hydroxybutyric acid (BHB) concentrations were lower in ICIR cows. Mean serum calcium (Ca) concentrations were in subclinical hypocalcemia level at day 3±2, 10±4 days postpartum in CON cows. Postpartum immune cell functions and NEFA, BHB concentrations were negatively correlated. The calving to pregnancy interval were longer in the control cows. However, total pregnancy rates and the number of insemination in both groups were similar. In conclusion, postpartum polymorphonuclear neutrophil activity is affected by periparturient metabolic status. Postpartum energy metabolites negatively affected the postpartum cellular immune response. The increased postpartum polymorphonuclear neutrophil activity at early postpartum period is positively related with subsequent fertility in dairy cows.

Key words: cow, immunity, fertility, metabolites

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Introduction

The transition period, which is defined as 3 weeks antepartum to 3 weeks postpartum, is the most challenging time for modern dairy cows. Physiological changes in nutrition, metabolism and hormonal status affect the incidence of infectious and metabolic problems (Goff and Horst 1997). During the transition period, there is an increase in dietary energy intake due to demands of the fetus, colostrum genesis, the onset of lactation, and nutritional requirements. Thus cows suffer from a temporary negative energy balance that is characterized by decreased blood glucose concentration and an increased mobilization of body reserves. This physiological condition results in elevated non-esterified fatty acid (NEFA) and beta-hydroxybutyric acid (BHB) concentrations (Wankhade et al. 2017). Genetic adaptation capacity to transitional physiology varies in each cow and directly affects the severity of negative energy balance (Sundrum 2015).

On the other hand, peripartal polymorphonuclear cell (PMNL) capacity decreases around parturition and leads to greater susceptibility for infection (Kehrli and Goff 1989, Gilbert et al. 1993, Mallard et al. 1998). As a result of the inevitable effects of physiological disturbances, neutrophil functions have reduced chemotaxis, phagocytic and oxidative burst activities (Ingvartsen and Moyes 2013). Many hypotheses have been proposed based on endocrine or metabolic related immune dysfunction during the transition period. The increased concentration of blood non-esterified fatty acid (NEFA) during the peripartum period was found to be associated with polymorphonuclear neutrophil (PMN) function impairment (Hammon et al. 2006). However, Scalia et al. (2006) reported that high concentration of NEFA did not affect the phagocytic activity in dairy cows. Additionally, Hoeben et al. (1997) reported that subketotic concentrations of beta-hydroxybutyric acid (BHB) reduced PMN respiratory burst activity in vitro, but elevated BHB concentrations had no effect on either myeloperoxidase activity or cytochrome-c reduction. Others (Martinez et al. 2012) showed different effects of calcium concentrations on phagocytic and oxidative burst activities of bovine neutrophils. However, the exact role of blood metabolites and ions on the function of peripartal bovine immune cell functions is poorly understood.

It was hypothesized that the periparturient metabolic status might play a role on immune cell function and that early postpartum elevated immune cell functions may be related to postpartum uterine health and subsequent fertility. The aim of this study was to assess blood parameters and subsequent fertility in cows with increased postpartum immune function.

Materials and Methods

Fifteen Brown Swiss dairy cows between one to three lactations with an average milk yield of 6000 kg were used in this prospective cohort study. The cows were from a commercial dairy farm (39°26′14.3″N 31°02′28.0″E) located in Eskisehir, Turkey. Serological controls and vaccinations were performed regularly. Only healthy cows that had not been treated for any clinical signs of diseases were included in the study. The cows were similar in terms of parity and age and were housed together three weeks before parturition and four weeks after parturition; thereafter, they were separated according to their milk yields. The cows were milked twice daily in free stall barns with natural ventilation and artificial lighting. All the cows had ad libitum access to water and fed with total mix ratio (TMR). The diets were formulated according to the NRC nutrient requirements (Table 1). TMR was prepared using a vertical mixer feeder and offered three times daily at 09.00, 16.00 and 24.00 h with diet portions equally split between the three feedings.

Experimental design

The animals were enrolled in the study 10±4 days prior to the expected parturition date, and placed into one of two groups according to the change in immune parameters at 10±4days postpartum. The cows that maintained their phagocytic and oxidative burst activities compared to prepartum period were allocated to the control group (CON, n:7). The cows with increased phagocytic and oxidative burst activities compared to prepartum period were allocated to the increased cellular immune response (ICIR, n:8) group. The cows that were affected by any puerperal disorders were recorded. Thereafter, all animals' blood ion and metabolite concentrations, and subsequent fertility parameters were evaluated.

Sample collection and processing

To determine phagocytic and oxidative burst activities of polymorphonuclear neutrophils, blood samples were taken from the coccygeal vein or artery into evacuated tubes containing lithium-heparin 10±4 day prior to expected parturition and at day 10±4 postpartum. The samples were collected at room temperature and sent to the laboratory within 2 hours. Phagocytic and oxidative activity was analyzed using a commercial kit (Phagotest, Phagoburst, Glycotope Biotechnology, Heidelberg, Germany) containing fluorescein-labeled opsonized Escherichia coli. Flow cytometric analyses was performed using a BD Accuri™ C6 Flow Cytometer (BD Biosciences) equipped with a 488-mm argon

Table 1. Integrant of diets fed during far-off (-50 to -31 d relative to expected calving), close-up (-30 d to calving), and early lactation.

Component	Far-off	Close-up	Early Lactation
Ingredient. % of DM			
Vetch hay	11.58	-	-
Alfalfa silage	-	-	9.67
Alfalfa hay	7.89	6.97	10.29
Corn silage	52.6	43.51	44.16
Wheat straw	10.52	22.25	-
Soybean meal. 48% CP	-	-	1.61
Concentrated feed	15.78	20.94	30.31
Limestone	0.82	2.25	1.57
Salt	0.30	-	0.26
Ammonium chloride	-	1.15	-
Dicalcium phosphate	0.14	0.3	0.44
Magnesium oxide	-	0.12	0.44
Magnesium sulphate	0.16	1.35	0.25
Sodium bicarbonate	-	-	0.7
Calcium sulphate	-	-	0.1
Mineral-vitamin mix[1]	0.21	0.17	0.2

[1] Contained a minimum of 4.3% Mg, 8% S, 6.1% K, 2.0% Fe, 3.0% Zn, 3.0% Mn, 5000 mg/kg Cu, 250 mg/kg of I, 40 mg/kg of Co, 150 mg/kg Se, 2200 kIU/kg of vitamin A, 660 kIU/kg of vitamin D_3, and 7700 IU/kg of vitamin E.

laser and BD Accuri™ C6 software. The parameters for phagocytic/oxidative mean fluorescence intensity (MFI) and phagocytic/oxidative index were evaluated as previously described (Yazlik et al. 2019).

Blood samples for serum ion and metabolite concentrations were collected between 09.00 h and 10.00 h from the coccygeal vein or artery in evacuated tubes without anticoagulant at -10±4, 3±2, and 10±4 days relative to parturition. Samples for biochemical analyses were immediately stored on ice through the collection process. Within 1 h of collection, blood samples were centrifuged (3000 g 15°C, 15 min) and the serum was transferred to a new tube for storage at -20°C until analysis. Superoxide dismutase (SOD) activity, NEFA, calcium (Ca), phosphorus (P), aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), creatinine kinase (CK), gammaglutamyl transferase (GGT), lactate dehydrogenase (LDH), total bilirubin (T-BIL), direct bilirubin (D-BIL) cholesterol, glucose, urea, albumin, total protein, and triglyceride concentrations were determined at pre-and postpartum sampling days to assess ion status and energy status of the animals. Serum BHB concentration was measured at days 3±2 and 10±4 post-calving.

The samples were analyzed by using an auto analyzer (ERBA XL 600). Commercial kits were used for the determination of serum NEFA (NEFA-C Kit; Wako Diagnostics Inc.) and BHB (Wako Autokit 3-HB; Wako Diagnostic Inc.) concentrations. Serum SOD activity was assessed using a Superoxide Dismutase Assay Kit (Cayman Chemical Company).

Reference blood values

The physiological upper limit for NEFA of 0.3 mEq/L in the prepartum period and 0.7 mEq/L in postpartum was proposed by McArt et al. (2012). The reference concentration level for BHB was below 1,200 mmol/L postpartum (Ospina et al. 2010). According to Goff (2014), blood serum total Ca concentrations of 8.5 mg/dl and below were defined as subclinical hypocalcemia. The physiological limit of phosphorus concentration was accepted as 4 to 8 mg/dl (NRC, 2001).

Body condition score

Changes in body condition scores (BCS) of the animals were determined on the day of blood collection by the inspection method at intervals of 0.25 units from 1.0 to 5.0 as described by Ferguson et al. (1994).

Environment data

Temperature and relative humidity in the barns were recorded daily during the study period. Maximum and minimum temperatures, and the humidity index (THI) were calculated as,

$$(t,rh)=(1.8xt+32)-(0.55-0.0055xrh)x(1.8xt-26)$$

Table 2. The mean percentage of phagocytic and oxidative burst activities of neutrophils, the mean fluorescence intensity (MFI) of phagocytic neutrophils and oxidative burst, and the mean phagocytic and oxidative index of neutrophils in control (CON) and increased cellular immune response (ICIR) cows at day -10±4 prepartum and day 10±4 postpartum.

Parameters		Days		P value		
		Pre	Post	Group	Time	Group*Time
Phagocytic Activity (%)						
	CON	52±3	46±3^{B}	0.003	0.036	<0.001
	ICIR	49±3^{a}	70 ± 3^{A,b}			
Phagocytic MFI						
	CON	83200.81±13469.01	50479.02±13469.01	0.854	0.423	0.137
	ICIR	59228.33±14398.98	69256.74±14398.98			
Phagocytic Index						
	CON	45.07±8.9	23.53±8.93	0.493	0.784	0.055
	ICIR	32.52±9.55	48.94±9.55			
Oxidative Burst Activity (%)						
	CON	17±2	15±2^{B}	<0.001	0.061	0.017
	ICIR	23.5±3.1^{b}	37±3$^{a, A}$			
Oxidative Burst MFI						
	CON	10060.52±1440.18	7813.07±1440.18	0.357	0.547	0.378
	ICIR	7324.64±1539.62	7753.69±1539.62			
Oxidative Burst Index						
	CON	1.78±0.4	1.34±0.4	0.132	0.444	0.080
	ICIR	1.76±0.43	2.81±0.43			

MFI (x1000) = Mean fluorescence intensity
Phagocytic Index = [(% of positive Phagocytic activity)x(MFI)]/100)
Oxidative Burst Index = [(% of positive oxidative burst activity)x(MFI)]/100)
Mean values within a row (a-b) and column (A-B) with different superscript letters differ significantly ($p<0.05$).

where t is temperature in degrees Celsius and rh is relative humidity as percentage (Aguilar et al. 2010). A THI <72 was indicative of no heat stress.

Fertility parameters

Reproductive tract examinations were performed at 30±4 DIM and a vaginal discharge score was calculated based on a scoring system established as follows (Williams et al., 2005): 0: clear or translucent mucus; 1: mucus containing flecks of white or off-white pus; 2: containing ≤50% white or off-white mucopurulent material and 3: discharge containing >50% purulent material and ≥1 was diagnosed as clinical endometritis.

Oestrus symptoms were detected visually by experienced farm personnel from the 50th day of the postpartum. All the animals were examined before the inseminations. Each cow was inseminated until 150 days postpartum. Transrectal ultrasonography examinations were performed to determine whether the animals were pregnant or non-pregnant at 30±2 days post insemination. Pregnancy rates, the number of inseminations, and calving to pregnancy interval were evaluated (Hoedemaker et al. 2009).

Statistical analyses

The data were examined with the Shapiro-Wilk test for normality and Levene test for homogeneity of variances as parametric test assumptions. Descriptive statistics for each variable were calculated and presented as "Mean ± Standard Error of Mean." The Spearman correlation coefficient was used to determine the correlation between phagocytic activity, oxidative burst activity, concentrations of NEFA, BHB and calcium for each sampling period. A Chi-square test was used to investigate the differences between groups in terms of the retention of fetal membranes, endometritis, and pregnancy. A Mann-Whitney U test was used to evaluate the differences between groups for pregnancy and parturition interval and number of inseminations.

All data were analyzed using MIXED procedure of SPSS (V22.0; SPSS Inc., Chicago, IL, USA). The effect of group, day of sampling, and their interaction on BCS, concentration of SOD, NEFA, BHB, Ca, P, AST, ALP, ALT, CK, GGT, LDH, T-BIL, D-BIL, cholesterol, glucose, urea, albumin, total protein, and triglyceride were analyzed by using the following model with repeated measures:

Table 3. Changes in serum concentrations of cholesterol, P, Urea, Albumin, Ca, NEFA, BHB, and SOD in control (CON) and increased cellular immune response (ICIR) cows at at day -10±4 prepartum, 3±2 and 10±4 days postpartum.

Parameters		Days			P value		
		-10±4	3±2	10±4	Group	Time	Group*Time
Cholesterol (mg/dL)							
	CON	88.6±6.4^{a}	67.3±6.4^{ab}	52.1± 6.4$^{b, B}$	0.026	0.024	0.018
	ICIR	84.7±6.8	74.2±6.8	86.8±6.8^{A}			
P (mg/dL)							
	CON	5.8±0.4	5.6±0.4	5.5±0.4	0.961	0.017	0.041
	ICIR	6.6±0.4^{a}	4.6±0.4^{b}	5.7±0.4^{ab}			
Urea (mg/dL)							
	CON	21.4±3.6^{b}	41.9±3.6$^{a, A}$	23.6±3.6^{b}	0.579	0.018	0.010
	ICIR	24.6±3.9	26.2±3.9^{B}	30.5±3.9			
Albumin (g/dL)							
	CON	3.2±0.1^{a}	3.3±0.1^{a}	2.8±0.1$^{b, B}$	0.017	0.078	0.023
	ICIR	3.3±0.1	3.4±0.1	3.4±0.1^{A}			
Ca (mg/dL)							
	CON	8.5±0.3	8.1±0.3	8.4±0.3	0.057	0.523	0.970
	ICIR	9.2±0.4	8.8±0.4	9.3±0.4			
NEFA (mEq/L)							
	CON	0.6±0.1	0.5±0.1	0.5±0.1	0.044	0.504	0.405
	ICIR	0.1±0.1	0.4±0.1	0.2±0.1			
BHB (µmol/L)							
	CON		1166.9±118.2	1117.6±118.2	<0.001	0.697	0.989
	ICIR		588.8±126.4	543.02±126.4			
SOD (U/ml)							
	CON	0.2±0.05	0.3±0.05	0.342 ± 0.053	0.725	0.138	0.418
	ICIR	0.2±0.06	0.3±0.06	0.2±0.06			

Mean values within a row (a-b) and column (A-B) with different superscript letters differ significantly ($p<0.05$).

$$Y_{ijk} = \mu + G_i + D_j + (G \times D)_{ij} + e_{ijk}$$

Where, Y_{ijk}, dependent variable; μ, overall mean; G_i, effect of the group (i = Cows that allocated their immune response (CON) and increased cellular immune response (ICIR) at postpartum period); , effect of day of sampling (j = -10±4, 3±2 and 10±4 d); $(G \times D)_{ij}$, interaction between group and day of sampling j; and e_{ijk}, residual error.

Another model was evaluated for phagocytic activity, phagocytic MFI, phagocytic index, oxidative burst activity, oxidative burst MFI and oxidative burst index, to analyze the effect of period, group and their interactions:.

$$Y_{ijk} = \mu + G_i + P_j + (G \times P)_{ij} + e_{ijk}$$

Where, Y_{ijk}, dependent variable; μ, overall mean; G_i, effect of the group (i = Cows that allocated their immune response (CON) and increased cellular immune response (ICIR) at postpartum period); D_j, effect of period (j = prepartum and postpartum); $(G \times P)_{ij}$, interaction between group and period j; and e_{ijk}, residual error.

Animals within group were assessed as a random effect, while group, period or day of sampling and their interaction were assessed as fixed effects. $P<0.05$ was considered as significant in all analyses. When a significant difference was revealed, any significant terms were compared by Simple effect analysis with Bonferroni adjustment.

Results

The average minimum and maximum THI during the study period were 59.91 (±8.23) and 61.71 (±7.44), respectively. During the study period, none of the cows experienced Max THI >72.

Increased cellular immune response cows showed greater phagocytic and oxidative burst activities at post-

Table 4. Correlations of cellular immune response and some blood metabolites at pre-and postpartum period (Significant correlations indicated * p< 0.05 and ** p<0.001; ns: not significant).

	Prepartum Phagocytic Activity	Prepartum Oxidative Burst Activity	Postpartum Phagocytic Activity	Postpartum Oxidative Burst Activity	Prepartum Ca	Ca at day 3±2	Ca at day 10±4	Prepartum NEFA	NEFA at day 3±2	NEFA at day 10±4	BHB at day 3±2	BHB at day 10±4
Prepartum Phagocytic Activity		ns	ns	ns	ns	ns	ns	0.61*	ns	ns	ns	ns
Prepartum Oxidative Burst Activity			ns	ns	ns	0.56*	ns	ns	ns	ns	ns	ns
Postpartum Phagocytic Activity				0.77**	0.57*	ns	ns	ns	-0.54*	ns	-0.81**	-0.57*
Postpartum Oxidative Burst Activity					ns	ns	ns	ns	ns	-0.54*	-0.52*	-0.63*
Prepartum Ca Concentration						ns	ns	ns	-0.68**	ns	-0.76**	ns
Ca concentration at day 3±2							ns	ns	ns	ns	ns	ns
Ca concentration at day 10±4								ns	ns	-0.55*	ns	ns
Prepartum NEFA Concentration									ns	ns	ns	ns
NEFA concentration at day 3±2										ns	ns	ns
BHB concentration at day 3±2											ns	0.64**
BHB concentration at day 10±4												ns

partum when compared to prepartum period. There was an interaction noted for group, time, and group x time for phagocytic activity. But, there was no interaction noted for phagocytic MFI and phagocytic index. In oxidative burst activity, only group and group x time interaction was noted (Table 2).

Although there were no significant difference among groups for the rate of retained placenta (p=0.310) and clinical endometritis (p=0.205), the CON cows had numerically higher retained placenta cases (n=3) and clinical endometritis (n=6). The number of days open during the first 150 days of lactation were lower in ICIR cows. However, the number of inseminations (p=0.983) and pregnancy (p=0.200) were similar in both groups.

Body condition score, D-BIL, T-BIL, AST, cholesterol, P, glucose, LDH, total protein, triglyceride concentrations changed over time in both groups. However, group x time interaction were noted for cholesterol, urea, P, and albumin concentrations. The BCS were similar in both groups at transition period. The cholesterol concentration were greater in ICIR cows at 10±4 day postpartum. The concentration of urea was significantly higher in CON cows at day 3±2 and urea concentration changed over time in CON cows. Phosphorus concentration were significantly lower in ICIR cows at day 3±2. A negative acute phase protein called albumin changed over time in CON cows and decreased at day 10±4 postpartum. NEFA concentrations were in the physiological range at postpartum period. The mean NEFA concentration were greater in CON cows and prepartum NEFA concentration in CON cows reached pathological levels. The group interaction effect was noted for BHB. The mean concentration of BHB was greater in CON cows. Mean Ca concentrations were in subclinical hypocalcemia level at day 3±2 and 10±4 postpartum in CON cows (Table 3).

The relationship between serum metabolites and ion concentrations, and cellular immune response were evaluated (Table 4). The prepartum phagocytic activity was positively correlated with prepartum NEFA concentrations (r=0.610, p=0.016) and postpartum phagocytic activity was positively correlated with prepartum Ca concentrations (r=0.574, p=0.025). At the same time, prepartum oxidative burst activity was also positively correlated with day 3±2 Ca concentrations (r=0.563, p=0.029). A positive strong correlation was observed between postpartum phagocytic and oxidative burst activity (r=0.775, p=0.001). The relationship between postpartum cellular immune response and NEFA and BHB concentrations was negatively correlated. Postpartum phagocytic activity was negatively correlated with serum NEFA at day 3±2 (r=-0.542, p=0.037), BHB at day 3±2 (r=-0.811, p<0.001), and BHB at day 10±4 (r=-0.571, p=0.026) postpartum. Postpartum oxidative burst activity was negatively correlated with NEFA concentrations

at day 10±4 (r=0.546, p=0.035), and BHB concentrations at day 3±2 (r=-0.525, p=0.044) and 10±4 (r=-0.632, p=0.011). Ca and NEFA (r=-0.554, p=0.033) were negatively correlated, while BHB and NEFA (r=0.649, p=0.009) concentrations were positively correlated at 10±4 days postpartum (Table 5).

Discussion

In this study, the function of blood neutrophils, as tested in an in vitro functional assay, was evaluated among groups that maintained or increased phagocytic and oxidative burst activities at day 10±4 before and after parturition.

Polymorph nuclear neutrophil leukocytes are the first line defense (Paape et al. 2002). In dairy cows, PMN functions decrease gradually beginning 2 to 3 weeks before parturition and recover 2 to 4 weeks postpartum (Kehrli and Goff 1989, Kimura et al. 2014). However, there are also different reports about phagocytic and oxidative burst activities during the periparturient period (Kimura et al. 2002). Meglia et al. (2005) reported that cows had no difference in oxidative burst activity, whereas others (Kehrli et al. 1989, Piccini et al. 2004). observed a decrease during the periparturient period Kehrli et al. (1989) reported increased phacocytic activity at the time of calving, whereas others reported a decrease (Tan et al. 2012) or maintained (Sander et al. 2011, Graugnard et al. 2012). The possible reasons for varying results among these studies might be due to differences in experimental designs or assays (Kimura et al. 2014). On the other hand, it should be noted that the increased cellular immune response may not necessarily be a positive finding, in fact, it may highlight an elevated inflammatory status of animals. Interestingly, increases in cholesterol concentrations might support this idea because an increase is linked to the presence of systemic infection (Ridker et al. 2000).

However, it is known that some metabolic indicators have direct role in immune cell functions. The activation of PMN depends directly on Ca^{2+} entry into the cell. Intracellular calcium concentration is related with blood calcium levels and needed for the initiation of phagocytic activity (Sayeed 2000). In the present study, prepartum Ca concentrations were positively correlated with postpartum phagocytic activity. Also, postpartum serum Ca concentrations were positively correlated with oxidative burst activity of bovine neutrophils. Not only for the initiation of phagocytic activity, but also for Cytosolic Ca^{2+} that is required to control the fusion process of secondary granules to phagosomal membrane during bactericidal activity (Jaconi et al. 1990). The uptake of Ca^{2+} to the cell is better in the presence of cholesterol (Kannan et al. 2007). Our results support this explanation because cows with increased cellular immune response have greater cholesterol level at postpartum period. Subclinical hypocalcemic cows showed decreased phagocytic activity and killing capacity (Martinez et al. 2012). The present study supports that finding, the mean Ca concentrations were at subclinical hypocalcemia levels in the control cows and phagocytic and oxidative burst activities were lower at the postpartum period

This study also shows different effects of NEFA on immune function. Dairy cows are temporarily affected by negative energy balance due to energy demand and energy differences in the ration (Goff and Horst 1997). Negative energy balance is characterized by decreased glucose concentration, lipomobilization and increased concentration of NEFA that may lead to the ketotic state (Roberts et al. 1981). Energy related blood metabolites cause immunosuppression (Ster et al. 2012) and the high concentration of NEFA and BHB can adversely affect the bovine neutrophil functions (Suriyasathaporn et al. 2000, Scalia et al. 2006). As a result, the maintained immune response might be due to postpartum increased energy related blood metabolites.

On the other hand, glucose metabolism promotes mortality during bactericidal infection, however, BHB concentrations reduce glucose availability and prevents infection-related losses in mice (Wang et al. 2016). A similar mechanism may have role on transition cow. The postpartum overwhelming increase in BHB concentration might be a response to the aseptic inflammation status, which is supported by hypoalbuminemia (Gabay and Kushner 1999). Thus, the infection associated losses may be prevented. In the present study, high BHB concentrations were observed in cows that maintained their immune response after parturition. Also, statistically similar infection rates for retained placenta and clinical endometritis were observed in both groups. However, as a side effect of increased concentrations of BHB, some fertility parameters may have been reduced.

Prepartum or postpartum metabolic indicator levels or periparturient disorders might alter the immune function status of dairy cows. It is documented that the important periparturient disorder retained placenta did not affect prepartum cellular immune response or, in other words, retained placenta has no prolonged effect on immune function alteration (Yazlik et al. 2019). The present study supports the finding that periparturient cows had similar rates of retained placenta in the early postpartum period. One of the most common subfertility and infertility problem in dairy cows is clinical endometritis. Mateus et al. (2002) have

reported that blood leucocyte activity is not relevant to establish the presence of endometritis. However, the same research group reported an impaired oxidative burst activity in cows with endometritis. They surmised that a decrease in oxidative burst activity might be a cause of the establishment of endometritis. This information is partially supported by the present study. The increased cellular immune response cows showed greater oxidative burst activity also and had a numerically lower number of endometritis.

To ensure a successful pregnancy, reproductive disorders should be minimized and the resumption of cyclicity should be maintained as early as possible. In the present study, an increase in the cellular immune response in cows showed shorter calving to pregnancy interval; however, pregnancy rates were similar among groups., Postpartum increased immune response might numerically decrease the rate of uterine infections. As well cows that allocated their cellular immune response suffered from subclinical hypocalcemia in the postpartum period. Cows that suffered from subclinical hypocalcemia at the early stage of postpartum, had delayed resumption of ovarian activity (Kamgarpour et al. 1999). Caixetia et al. (2015) reported that cows with normocalcemia in the first 3 days after parturition had higher progesterone levels and resumed ovarian activity earlier than hypocalcemic cows. In this study, the cows that maintained their immune response showed greater calving to pregnancy interval.

In conclusion, postpartum phagocytic and oxidative burst activities are affected by periparturient metabolic status. The increased cellular immune response at early postpartum period is positively related with subsequent fertility in dairy cows. However, the exact mechanism of bovine neutrophil functions on fertility requires further research.

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