

Effect of Injectable Vitamin and Trace Element Administrations During Transition Period on Fertility in Brown Swiss Dairy Cows

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ABSTRACT

This study examined the effects of injectable vitamin and trace element combination during the transition period on clinical-biochemical parameters and reproductive performance in Brown Swiss dairy cows. Sixty multiparous cows were randomly allocated two groups as trace element-vitamin treated ($n=30$) and control ($n=30$). Animals received four injections at the beginning of the dry period, 21 days before parturition, the day of parturition, and postpartum 30 ± 5 days. Energy, protein, hepatic metabolism markers, blood mineral levels concentrations were measured at -7 ± 4 days antepartum, 3 ± 2 days, and 30 ± 5 days postpartum. Genital tract examinations were performed at 30 ± 5 day postpartum. Treated cows showed lower glucose, total protein, urea, and greater NEFA concentration -7 ± 4 days relative to calving. Effect of vitamin and trace element on serum calcium and phosphorus levels were significant. The total pregnancy rate was 95.8% and 59.09%, in treatment and control groups respectively at 150 days postpartum ($P<0.05$). In conclusion, trace element and vitamin supplementation increased pregnancy rate significantly and treatment positively affected energy, ion metabolism, and hepatic function.

Keywords: Vitamin and trace element, transition period, fertility

İsviçre Esmeri ineklerde geçiş döneminde vitamin, iz element uygulamalarının fertilitete etkisinin araştırılması

ÖZ

Bu çalışmada geçiş dönemi boyunca vitamin ve iz element enjeksiyonlarının, klinik-biyokimyasal parametreler, periparturent dönem sorunları ile fertilitite parametrelerinin üzerine etkisinin araştırılması amaçlandı. Altınyıl inek, iz element ve vitamin enjeksiyonu yapılanlar ($n=30$) ve kontrol grubu ($n=30$) olarak rastgele iki gruba ayrıldı. Vitamin ve iz element enjeksiyonları kuru döneme girişte, doğumdan 21 gün önce, doğumun gerçekleştiği gün ve doğumlu izleyen (30 ± 5) günlerde gerçekleştirildi. Enerji, protein, karaciğer metabolizması belirteçleri ile mineral düzeyleri doğum öncesi 7 ± 4 gün, doğum sonrası 3 ± 2 gün ve 30 ± 5 günlerde ölçüldü. Genital organ muayeneleri doğum sonrası 30 ± 5 günde gerçekleştirildi. Uygulama grubundaki inekler kontrol grubundakilere kıyasla doğum öncesi 7 ± 4 günde daha düşük glikoz, toplam protein, üre ve daha yüksek NEFA konsantrasyonu gösterdi. Vitamin ve iz element uygulamasının serum kalsiyum ve fosfor düzeylerine etkisi önemli düzeydedi. Doğum sonrası 150. günde toplam gebelik oranı uygulama ve kontrol gruplarında sırasıyla % 95.8 ve % 59.09 olarak saptandı ($P<0.05$). Sonuç olarak, iz element ve vitamin uygulanan ineklerde toplam gebelik oranının belirgin olarak arttığı ve uygulamaların enerji, iyon metabolizması ve karaciğer fonksiyonlarını olumlu etkilediği belirlendi.

Anahtar Kelimeler: Vitamin ve iz element, geçiş dönemi, fertilitet

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INTRODUCTION

The transition period is defined as three weeks before and after parturition and the most challenging dairy cows' lactation duration. Metabolic, hormonal and immunologic alterations are the characteristic of this period (Bell 1995). During the transition period animals are susceptible to metabolic and infectious disorders due to increased demand for energy and minerals (Drackley et al. 2005, Sordillo and Aitken 2009). As a response to energy deficiency, dairy cows mobilize their body reserves (Drackley et al. 2001). Consequently, non-esterified fatty acids (NEFAs) and beta-hydroxybutyric acid (BHB) concentrations increase in blood during the transition period. Subsequently, dry matter intake and calcium ion concentration decrease which increased cortisol concentration. The high concentration of fatty acids, ketone bodies, cortisol and decreased calcium levels impairs immune response (Scalia et al. 2006, Contreras et al. 2010). Increased metabolic demands, parturition and metabolic stress alter the oxidant and antioxidant balance leading to oxidative stress (Sordillo and Aitken 2009). Oxidative stress and negative energy balance, lead to trace element and vitamin deficiencies (Hayırlı et al. 2002, Machado et al. 2013). An injectable form of trace elements and vitamins provides an alternative way of delivering extra trace elements and vitamins during the transition period. And leads an increase in tissues within several hours or days (Pogge et al. 2012). Trace elements or vitamin supplementation during the transition period minimize the stillbirth risk, retained placenta, uterus infections and mastitis. Furthermore, it increases fertility (Politis, 2012, Machado et al. 2013, Pontes et al. 2015). At the same time, they had no adverse effect on hematologic and immune parameters (Bicalho et al. 2014, Schäfers et al. 2018).

Due to the decrease of dry matter intake, an increase of growing fetus requirement and beginning of colostrogenesis and lactogenesis process resulted with trace element and vitamin insufficiency. The farming systems changed over the last few decades; thus intensive cattle breeding resulted in nutrient losses of feed due to storage problems (Tilman et al. 2002). On the other hand, trace elements associated with fiber fractions in the feedstuff and/or binding of undigested fiber to the trace minerals decreases the bioavailability. Due to immune response and oxidative status of cows altered during the transition period, the incidence of puerperal disorders increases (Sundrum 2015). The process of follicular development from the primordial pool takes 4 to 5 months to reach the ovulation phase in dairy cows (Webb et al. 2003); thus follicular growth starts from the late stage of gestation, the increased demand of trace element and vitamins might mitigate

the follicular growth or oocyte quality. It was aimed to investigate the effects of vitamin, trace element, and mineral supplementation during the dry and transition period on clinical-biochemical parameters, periparturient problems, and fertility.

MATERIALS and METHODS

All experiments on animals were carried out according to the standards approved by the Animal Welfare and Research Ethics Committee at Ankara University (2015-12-139).

Animals and Housing

This study was conducted on a farm incorporated in the Directorate General of Agricultural Enterprises in Turkey. Cows were monitored from the beginning of dry period (-60 d) until five months postpartum (150 d) for the determination of any puerperal disorders and calving as well as postpartum diseases. Serological controls (brucellosis, tuberculosis, neosporosis) and vaccinations (Bovine Herpesvirus-1, Bovine viral diarrhea virus, coronavirus, rotavirus) were performed regularly. The barns were naturally ventilated and artificially lighting. According to NRC recommendations, cows were fed total mixed rations (TMRs) ad libitum (09.00, 16.00 and 00.00 h) to meet their nutrient requirements (NRC 2001). The composition of the TMR was shown in Table 1. TMR was prepared daily by vertical mixer feeder and offered three times with diet portions equally split between three feedings.

Experimental Design and Treatments

A total of 60 pregnant multiparous Brown Swiss dairy cows between 2 to 4 lactations were enrolled in the study. Animals were randomly divided into two groups as treatment ($n=30$) and control ($n=30$) at the beginning of the dry period. Cows were randomized using the random number function in Microsoft Excel (Redmond, WA, USA) and imported into the farm's Dairy Plan (GEA®, Germany). Cows that assigned to treatment groups received four injections of trace element (Activate, Alke®, Turkey, 8 ml) and vitamin (Ademin®, Ceva, France, 10 ml) combination at 230 and 260 ± 5 days of pregnancy, around parturition and 30 ± 5 days in milk (DIM). Each trace mineral injection contains 2.5 mg Cu, 1.25 mg Se, 5 mg Mn and 5 mg Zn per ml. Each vitamin injection contains 500000 IU vitamin A, 75000 IU vitamin D3 and 50 mg vitamin E per ml. The control groups received 0.9% isotonic sodium chloride (NaCl) with the same volume. For the first three days following the parturition the treatment and control animals received calcium and phosphorus solutions parenterally.

Table 1. Integrant of diets fed during far-off (-60 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

¹Contained 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, 660kIU/ kg of vitamin D3, and 7700 IU/kg of vitamin E.

(K), sodium (Na), chlorine (Cl) in blood serum (Hoedemaker et al. 2004) by using commercial kits

Blood Sampling and Metabolite, Ion Analyses
 Blood samples were collected before the morning feeding on the coccygeal artery or vein into Vacutainer tubes without anticoagulant (Hema&Tube®) using 20gauge × 2.54 cm Vacutainer needle (Vacutainer, Becton, Dickenson and Company) at 7±4 days before the expected calving date, 3±2 and 30±5 days postpartum. Samples were clotted and then placed on ice until processing. Blood samples delivered to the farm laboratory under the cold chain then were centrifuged at 1300xg, +4°C for 10 minutes in the first 15-60 minutes after collection. The serum was separated and collected into 1,5 ml tubes containing information such as ear number, administration group, blood collection date, and stored in a freezer at -20°C in the farm laboratory (Little et al. 2017). At the end of the study the serum samples placed on the ice molds in the biological sample carrying bag were delivered to the Biochemistry Laboratory of the Faculty of Veterinary Medicine, Ankara University. Serum samples were analyzed for the determination the concentration of NEFA, BHb, glutamate dehydrogenase (GLDH), glucose, albumin, total protein, urea, creatine kinase (CK), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), cholesterol, triglyceride, total bilirubin (TBIL), direct bilirubin (DBIL), calcium (Ca), phosphorus (P), magnesium (Mg), potassium

following manufacturer instructions with auto analyzer (ERBA XL 600®) equipped with spectrophotometric and ion-selective electrode (ISE) in Ankara University Faculty of Veterinary Medicine Diagnostic Laboratory. All analyses were calibrated using ERBA XL Multical® and calibration verified using two control serum (ERBA Norm® and ERBA PATH®). GLDH levels of serum samples were determined by a 96-well microplate reader device (Sunrise®, Tecan, Switzerland) with a Magellan TM data analysis software with a spectral wave range of 400-750 nm.

Reference Blood Values

The physiological upper limit for NEFA 0.3 mEq/L in the prepartum period and 0.7 mEq/L postpartum were proposed by McArt et al. (2012). The reference concentration level for BHb was below 1200 mmol/L postpartum (Ospina et al. 2010). According to Goff (2014), blood serum total Ca concentration 8.5 mg/dl and below were defined as subclinical hypocalcaemia. The physiological limit of phosphorus concentration was accepted as 4-8 mg/dl (NRC, 2001).

Body Condition Score

The animals' body condition scores were determined on the start of the dry period and days of blood collection as previously described by Ferguson et al. (1994).

Parturition and Maternity Pen

The normal-stillbirth and the sex of calf and dystocia score were recorded by using the system as previously described by Heins et al. (2006). During the postpartum period, body temperature, appetite and rumen fullness were examined and recorded twice a day for five days.

Postpartum vagina, uterus and ovary examination

Postpartum genital tract examination performed on 30 ± 5 d postpartum. The vaginal examination was performed by hand for the purpose of evaluating the vaginal discharge scoring. Before the examination, the vulva was cleaned by a paper towel. A gloved and lubricated hand inserted to the vagina. Discharges in the anterior vagina accumulated (Little et al. 2017). Vaginal discharges scored by using the system Williams et al. (2005). Animals with mucopurulent, purulent or malodorous discharge at the time of examination were diagnosed with clinical endometritis. The transrectal ultrasonography examination of the ovaries was performed using a linear probe with a frequency of 6.5-9 MHz (SIUI®, CTS 800, China). The presence of follicles with a diameter of less than 20 mm and greater than 9 mm on the ovaries were detected and the measurements were recorded (Tanaka et al. 2008). In the absence of corpus luteum in the ultrasonography examination of ovaries, the structures with dimensions of 2.5 cm or more were considered ovarian cyst (Silvia et al. 2005). The presence of corpus luteum was also investigated. Animals that had a corpus luteum recorded as cyclic. Animals with corpus luteum were treated with PGF 2α (Enzoprost-T®, CEVA, France) containing 25 mg of dinoprost. animals that did not consist a corpus luteum treated with GnRH (Ovarelin®, CEVA, France) containing 100 mcg of Gonadorelin diacetate tetrahydrate. Animals clinical endometritis were treated with the unique intrauterine treatment protocol as determined by the General Directorate of Agricultural Enterprises and other parenteral treatment protocols.

Fertility Parameters

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

Statistical Analysis

Before performing the statistical analysis, 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 \pm standard error of the mean". Chi-square test was used to determine the difference between treatment and control groups following measures, presence of corpus luteum, pregnancy rates, and uterus infection. The difference between calving to first AI, calving to conception interval, number of inseminations and lactations parameters, Mann-Whitney U test was performed.

The effect of group, day of sampling and their interaction on NEFA, BHB, GLDH, glucose, albumin, total protein, urea, CK, ALT, ALP, AST, GGT, LDH, cholesterol, triglyceride, TBIL, DBIL, Ca, P, Mg, K, Na, and Cl were analyzed using MIXED procedure of SPSS (V22.0; SPSS Inc. Chicago, IL, USA), the following model with repeated measures

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

Where Y_{ijk} was the dependent variable, μ was the overall mean, G_i was the effect of the group ($i =$ Treatment and control), Z_j was the effect of day of sampling ($j = -7 \pm 4, 3 \pm 2$, and 30 ± 5 d), ($G \times Z$) $_{ij}$ represented interaction between group i and day of sampling j , and e_{ijk} was the residual error.

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

RESULTS

Six animals from the treatment and eight animals from the control group were excluded from the study as per management policies. There was no significant difference between mean lactation numbers among groups. Trace element and vitamin supplementation did not affect BCS in cows. The BCS was influenced by time only, showing a drop toward 30 ± 5 DIM.

Postpartum Period and Fertility Parameters

Body temperature and time of rumination in 5 days postpartum were similar in both groups. The number of cases for retained placenta were not affected by treatment. Treatment did not affect on the incidence of clinical endometritis. The 66.6% of cows were considered cyclic at 30 ± 5 DIM. There was no effect of treatment on cyclicity ($P > 0.05$). Also, treated cows showed similar calving to conception interval, calving to first AI interval compared to control cows. Additionally, the number of inseminations were similar among groups. The pregnancy rate at first and third service did not differ between treatment and control cows. However, the odds of second insemination and total pregnancy were greater in the treatment group

than the control group at 150 days postpartum ($P<0.05$, Table 2).

Energy and Protein Metabolism Markers

The main treatment and interactive effects on blood energy and protein biomarkers are presented in Table 3. Supplementation with trace elements and vitamins did not affect the serum concentration of BHB ($P>0.05$). NEFA, glucose, total protein, albumin, and urea concentration changed over time. Also, these parameters affected by the interaction between treatment and time. The glucose concentration showed most significant decrease around parturition in control cows ($P<0.05$). NEFA concentration was greater however, serum glucose, total protein, albumin and urea concentration were lower in the treatment group at prepartum period ($P<0.05$). Treated cows had increased urea levels after parturition compared to the levels at pre-calving sampling levels and showed similar urea concentrations during the postpartum period. The total proteins marginally decreased in control cows in the early postpartum period ($P<0.05$). Although treated cows showed greater NEFA, glucose and urea concentrations, they did not reach the pathological threshold levels after parturition (Table 3).

Table 2. Findings of genital tract examination on 30 ± 5 days postpartum and fertility parameters.

Items	Animals		P
	Treatment (n=24)	Control (n=22)	
Clinical Endometritis	10/24	6/22	0.210
Presence of CL	16/24	10/22	0.092
Ovarian Cyst	1/24	1/22	-
Number of animals that have follicle within 12-20 mm in the absence of CL	4/24	7/22	-
Calving to first AI interval (days) ($\bar{x}\pm SEM$)	91.84 ± 9.65	105.48 ± 7.40	0.300
Calving to conception interval (days) ($\bar{x}\pm SEM$)	121.69 ± 13.28	111.27 ± 11.7	0.527
Number of inseminations ($\bar{x}\pm SEM$)	1.65 ± 0.15	1.87 ± 0.19	0.564
Pregnancy rate after 1st AI (%)	41.18	28	0.374
Pregnancy rate after 2nd AI (%)	80 ^{B,a}	21.43 ^A	0.005
Pregnancy rate after 3rd AI (%)	100	83.33	0.659
Total pregnancy rate (%)	95.8 ^{B,a}	59.09 ^A	0.003

Means within a row (a-c) and column (A-B) with differ superscript letters differ significantly ($P<0.05$).

Treatment: Cows supplemented with trace element and Vitamin Combination

Control: Control group

(\bar{x}): Average

SEM: Standart Error Mean

CL= Corpus Luteum

Table 3. The effect of trace element and vitamin treatment on blood serum metabolites during the pre-, post-partum period ($\bar{x}\pm SEM$).

Enzyme and Liver Function Markers

GLDH, AST, LDH, cholesterol, BIT and BID concentration changed over time significantly ($P<0.05$). There was only an interaction noted between time and treatment for ALT and cholesterol levels. Prepartum decreased ALT concentration was shown in the treatment group ($P<0.05$). Cholesterol concentration decreased in the treatment group at 30 ± 5 days postpartum (Table 3).

Mineral Concentrations

All mineral panel parameters changed over time, but, there was only interaction between treatment and time for calcium and phosphorus concentration. Chlorine, sodium, potassium and magnesium concentrations were similar among groups. Prepartum calcium and phosphorus concentration were lower in the treatment group ($P<0.05$). Mean calcium concentration was in subclinical hypocalcaemia levels in control cows after parturition (Table 3). Treatment with trace elements and vitamins during the transition period did not affect pre-postpartum Mg, Na, K, Cl levels.

	Prepartum		Postpartum		P		
	-7±4 d	3±2 d	30±5 d	Treatment	Time	Treatment*Time	
Energy and Protein Metabolism Markers							
NEFA (mEq/L)							
Treatment	0.48 ± 0.11 ^{ab, A}	0.58 ± 0.05 ^a _A	0.30 ± 0.03 ^b	0.000	0.004		0.007
Control	0.11 ± 0.003 ^{b, B}	0.32 ± 0.04 ^a _B	0.27 ± 0.03 ^{ab}				
Urea (mg/dl)							
Treatment	24.99±1.30 ^{b, B}	37.88±3.34 ^{a, A}	32.60±3.26 ^a	0.476	0.008		0.010
Control	29.26±1.34 ^A	29.18±2.66 ^B	31.37±1.79				
Glucose (mg/dl)							
Treatment	60.17±1.65 ^B	61.96±3.20 ^A	57.13±2.29	0.740	0.014		0.005
Control	67.69±1.71 ^{a, A}	52.44±2.78 ^{b, B}	57.44±2.25 ^b				
T. Protein (g/dl)							
Treatment	6.38 ± 0.18 ^{b, B}	6.68 ± 0.17 ^{ab}	7.31 ± 0.26 ^a	0.121	0.000		0.001
Control	7.61 ± 0.14 ^{a, A}	6.27 ± 0.29 ^b	7.48 ± 0.25 ^a				
Albumin (g/dl)							
Treatment	3.19 ± 0.06 ^b	3.47 ± 0.06 ^a	2.95 ± 0.08 ^{b, B}	0.369	0.003		0.003
Control	3.35 ± 0.07	3.24 ± 0.1	3.23 ± 0.09 ^A				
Enzymes and Hepatic Function Markers							
ALT (IU/L)							
Treatment	19.70±1.04 ^B	20.53±1.23	21.90±1.67	0.139	0.077		0.003
Control	27.08±2.23 ^{a, A}	20.63±1.35 ^b	21.40±1.06 ^b				
Cholesterol (mg/dl)							
Treatment	84.17±4.23 ^b	76.38±4.93 ^b	104.54±6.32 ^{a, B}	0.267	0.000		0.000
Control	82.85±3.38 ^b	64.85±4.86 ^c	136.35±7.43 ^{a, A}				
Minerals							
Ca (mg/dl)							
Treatment	8.20 ± 0.21 ^{b, B}	8.54 ± 0.21 ^{ab}	9.16 ± 0.13 ^a	0.073	0.000		0.000
Control	9.93 ± 0.09 ^{a, A}	8.07 ± 0.33 ^c	8.92 ± 0.20 ^b				
Means within a P (mg/dl)							
Treatment	6.08±0.20 ^B	5.88±0.27 ^A	6.70±0.49	0.714	0.000		0.005
Control	7.12±0.21 ^{a, A}	4.50±0.22 ^{b, B}	6.67±0.60 ^a				

row (a-c) and column (A-B) with differ superscript letters differ significantly ($P<0.05$).

Treatment: Cows supplemented with trace element and Vitamin Combination

Control: Control group

(× ¯): Average

SEM: Standart Error Mean

DISCUSSION

This study evaluated the effect of injecting a trace mineral and vitamin complex during the transition period on reproductive outcomes and serum metabolite and ion concentrations in multiparous Brown Swiss cows. We hypothesized that trace element and vitamin injections at the beginning of the dry period, during the last three weeks of gestation, after parturition and 30±5 days postpartum would increase the reproductive parameters. When considering all cows' data, pregnancy rates at first or third services did not differ between treatments. However, the second service pregnancy rate and odds of total pregnancy were greater in the treatment group than in the control. The literature review showed

inconsistent results regarding the role of trace mineral and vitamin injections on fertility. Campbell et al. (1999) showed that trace element administration positively impacted fertility by decreasing calving to first oestrus interval. Griffiths et al. (2007) and Pontes et al. (2015) also reported that vitamin administrations increased pregnancy rate in dairy cows. As well as trace element injections increased the success rate of embryo transfer studies (Sales et al. 2011). However, Machado et al. (2013) and Bach et al. (2015) did not find any positive effect of trace element supplementation on fertility. Furthermore, the trace mineral supplementation increased the risk of metritis (Machado et al. 2013). Additionally, Bach et al. (2015) stated that the exact mechanism by which trace elements affect reproduction is unknown. There are

several theories exist on how the trace element and vitamin affect reproductive parameters. Trace elements and vitamins have a role in enzymatic and metabolic pathways that interact with steroidogenesis, small follicle cell proliferation, and embryo development (Griffiths et al. 2007, Faes et al. 2009). The period from the primordial follicle pool to the selection and ovulation of the follicles is approximately 4-5 months (Webb et al. 2003). The higher total pregnancy rate in the treatment group may have increased the quality of follicle and oocyte developing from the primordial follicle pool by trace element and vitamin applications since the beginning of the dry period, and thus higher pregnancy may be achieved. Besides, trace element supplementation decreases the number of bacteria species in the microbiota of early postpartum uterus. Trace elements and vitamin administrations influence innate and humoral immune systems; thus, uterine health might be protected at the molecular level.

On the other hand, those compounds are assigned on the antioxidant system, thereby the negative effect of reactive oxygen species which produced by immune and somatic cells might be prevented on follicle as well as testis level (Wintergerst et al. 2007, Tafuri et al. 2015). Galvão et al. (2010) detected a greater negative energy balance in cows with endometritis. Bicalho et al. (2014) reported that endometritic cows had lower blood calcium level. These findings coincided with the present study and NEFA concentration was greater, with decreased Ca concentration in the treatment group at the prepartum period. Numerical differences may be observed due to metabolic inflammation, which resulted from an increase in NEFA concentration at the prepartum period.

Body condition score is a simple method to assess the energy status in dairy cows. Because there is a strong relationship between BCS and energy balance. There is evidence that trace element supplementation does not impact BCS (Sales et al. 2011, Bicalho et al. 2014, Machado et al. 2014). Similarly, in our study, trace element and vitamin administration did not affect the BCS. Colostrogenesis, accelerated foetal growth, hormonal disturbances, parturition and beginning of lactation during the transition period resulted in increased metabolic demands. Furthermore, these physiological changes decreased dry matter intake and resulted in negative energy balance. As a result of negative energy balance, gluconeogenesis and lipomobilization rate alters. This change triggers the severity of negative energy balance (Joksimović Todorović and Davidović 2012). Even so, trace element and vitamin concentration did not affect glucose concentration (Avci and Kızıl 2013), manganese had a negative role on carbohydrate metabolism (Ömür et al. 2016), thus supplementation might decrease the prepartum glucose concentration. However, the glucose concentration increased after parturition in treated cows and was greater. This result consistent with Nayyar et al. (2003). The possible

mechanism of the increase in glucose concentration might be the influence of treatment. Because selenium shows insulin-like activity, it had a role in glycolysis and gluconeogenesis pathways (Stapleton 2000).

The increased concentration of NEFA during the prepartum period ($\geq 0,4$ mEq/L) is a risk factor for retained placenta, metritis and ketosis (LeBlanc 2006). González et al. (2011) reported that postpartum NEFA concentration had no relationship with subclinical ketosis, mostly it is related to postpartum lipomobilization. The present study showed that trace element and vitamin treatment might increase the severity of lipomobilization at prepartum period. However, NEFA concentration was in physiological level in both groups at the postpartum period. Notwithstanding, treated cows had higher prepartum NEFA concentration, both groups had similar metabolic and infectious problems. Thus, trace element and vitamin injection during the transition period might suppress the negative effect of prepartum lipomobilization and decrease the incidence of peripartum problems. NEFA is converted to BHB by the liver and used by brain, heart, liver, and mammary glands in ruminants (Drackley and Andersen 2006). Machado et al. (2013) reported that trace element injections during the transition period decreased BHB concentration. However, in the present study, BHB concentration was not affected by treatment. The possible difference might be the weak correlation between NEFA and BHB (Cavestany et al. 2005).

The role of cholesterol on ruminant energy metabolism unknown exactly. In one theory, increased concentration of cholesterol better for energy balance, on the other hand, Bruss et al. (1997) reported that increased cholesterol results from energy deficiency in dairy cows. Chandra et al. (2018) observed increased cholesterol concentration after trace element and vitamin supplementation, similar to the present study.

Urea concentration directly related to hepatic function and composition of ration (Strang et al. 1998). In comparison with Chandra et al. (2018), the urea concentration was increased after parturition in treated cows. This changes possibly related to prepartum increased NEFA and decreased glucose concentration, resulting in the usage of protein reserves in the body and increased urea concentration. Also, ration composition changes affect the urea concentration, but the same ration is offered to the control and treatment groups. A similar mechanism might be effective for decreased total protein concentration in the prepartum period. We observed an increase in total protein and albumin concentration after parturition in treated cows while it remained similar or showed a decrease in control cows. Hence, similar to Ömür et al. (2016), trace element and vitamin combination had a positive role in protein metabolism.

Liver enzymes activities and hepatic function markers help determine liver status in transition cows (Bertoni and Trevisi 2013). Like Schäfers et al. (2018) AST, GGT, GLDH concentrations were in

physiological ranges in treatment and control groups. Moreover, increased NEFA concentration negatively affects hepatic function markers, but in the present study, liver enzymes were not affected (within the reference ranges) by greater NEFA concentration. Thus, possibly, trace element and vitamin combination might suppress the negative effect of increased NEFA concentration.

Calcium is an ion that direct relationship with immune parameters and muscle cell functions. It might be a marker for determining immune cell functions during the transition period (Martinez et al. 2012). Decreased calcium concentration increase the cortisol level and chemotactic and bactericidal activities of immune cells are suppressed (Salak-Johnson and McGlone 2007). Also, cytosolic Ca²⁺ concentration affected by total calcium levels. Cytosolic Ca²⁺ has no direct relation with phagocytosis; it has a role at the beginning of the activity (Sayeed 2000). With decreased glucose and increased NEFA, the level of low calcium might be the result of depression of dry matter intake. Adipocytes stimulated by calcium-sensitive receptor agonists decrease the basal lipolysis; however, excessive lipolysis decreases the calcium level (Cifuentes et al. 2005). Another role of calcium is in energy metabolism as a seconder messenger. Thus, directly affect the cycle of tricarboxylic acid. The decrease in calcium concentration resulted in alteration in carbohydrate metabolism and tricarboxylic acid cycle, which resulted in severe negative energy balance (Chamberlin et al. 2013). We considered that trace element and vitamin supplementation and calcium and phosphorus injection after parturition might suppress the severity of negative energy balance.

CONCLUSION

The present study showed that cows injected with trace element and vitamin supplementation at 230 or 260 days of gestation, around parturition and 30±5 days postpartum had greater odds of becoming pregnant than control cows. Combined injectable trace elements and vitamins increase the quality of inseminable follicles leading to ovulation in the postpartum period. Also, trace element and vitamin administration positively affect energy, hepatic and ion metabolism. However, the exact mechanism by which trace elements and vitamins affect bovine fertility requires further research.

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Conflict of interest: The authors declare that there is no actual, potential or perceived conflict of interest for this article.

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