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RESEARCH ARTICLE

Effect of Anti-Mullerian Hormone, Metabolic Profile and Mineral Levels at Transition Period On The Calving – Conception Interval in Cows

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ABSTRACT

The transition period in dairy cows is generally accepted as the period covering 3 weeks before calving and 3 weeks after calving. The aim of the study was to compare the profiles of β-hydroxybutyrate, calcium, magnesium, phosphorus, total cholesterol, total protein, triacylglycerol, free glycerol, serum lipid and serum protein with Anti-Mullerian Hormone (AMH) in dairy cows in the transition period, and to determine whether AMH to examine whether it can be used as a marker in the next insemination period. The cows whose blood samples were taken were followed up and it was determined that they became pregnant at the insemination and the study was terminated. According to the results obtained; It was determined that BHB and free glycerol, which are important markers of negative energy balance (NEB), have an effect on AMH concentration. However, it was determined that the concentration of magnesium and the ratio of cholesterol ester in serum total fat did not change much during the transition period. Our results suggest that AMH is a good biomarker of decreased follicular activity due to NEB in the transition period and that AMH can be used for herd weeding in reinsemination.

Keywords: AMH, Cow Pregnancy, Insemination, Metabolic Profile, Transition Period

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İneklerde Geçiş Döneminde Anti-Müllerian Hormon, Metabolik Profil ve Mineral Düzeylerinin Buzağılama – Gebelik Aralığına Etkisi

ÖZ

Sütçü ineklerde geçiş dönemi genel kabul ile buzağılamadan önceki 3 hafta ile buzağılamadan sonraki 3 haftatı kapsayan süreçtir. Bu çalışmanın amacı geçiş dönemindeki sütçü ineklerde negaif eneji dengesinden (NED) dolayı değişen β-hidroksibütirat, kalsiyum, magnezyum, fosfor, total kolesterol, total protein, triaçilgliserol, serbest gliserol, serum lipid ve serum protein profillerinin Anti-Müllerian Hormon (AMH) ile karşılaştırılarak AMH'nin bir sonraki tohumlama döneminde belirteç olarak kullanılıp lullanılmayacağını incelemektir. Kan örnekleri alınan inekler daha sonra takip edilerek kaçıncı tohumlamada gebe kaldıkları tespit edildi ve çalışma sonlandırıldı. Santrifüj edilerek serumları toplanan kanlar analiz edildi. Elde edilen sonuçlara göre; NED'in önemli belirteçlerinden BHB'nin ve serbest gliserolün AMH konsantrasyonu üzerine etkisi olduğu saptandı. Bununla birlikte, magnezyumun konsantrasyonunun ve serum toplam yağı içerisindeki kolesterol esteri oranının geçiş döneminde fazla değişmediği tespit edildi. Sonuçlarımız AMH'nin geçiş döneminde NED'den dolayı azalan folliküler aktivitenin iyi bir biyobelirteci olduğunu ve tekrar tohumlamada AMH'nin sürü ayıklama için kullanılabileceğini göstermektedir.

Anahtar Kelimeler: AMH, Geçiş Dönemi, İneklerde Gebelik, Metabolik Profil, Tohumlama

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INTRODUCTION

The transition period in dairy cows is defined as the period between 3 weeks before calving (close-up dry period) and 3 weeks after calving (early fresh period), during which critical physiological changes occur due to the emergence of many metabolic and infectious diseases (Drackley 1999, Grummer 1995). During this period, physiological, nutritional, metabolic and immunological changes occur in the cow, starting from late pregnancy, continuing with milk synthesis and secretion, and finally reaching a stable lactation phase (Van Saun 2016, Wankhade et al. 2017).

As a result of decreased dry matter intake during late pregnancy, energy requirement is provide by insulin resistance in adipose tissue and muscle, together with increased sensitivity to lipolytic agents (Bell 1995); These events reduce peripheral glucose uptake and facilitate the utilization of endogenous substrates, most of which are glucogenic amino acids from endogenous protein sources, and glycerol from adipose tissue mobilization (Putman et al. 2018). Inflammatory and oxidative stress experienced during parturition cause a significant increase in energy requirements with the onset of milk production (Turk et al. 2004). Meanwhile, the decreased plasma progesterone level with the transient rise in glucocorticoid and estrogen further reduces dry matter intake (Drackley et al. 2005, Ingvartsen 2006). The imbalance between energy production and consumption causes negative energy balance (NEB) (Grummer et al. 2004). Many tissues, including adipose tissue, are mobilized to produce energy in order to tolerate the imbalance.

Approximately 30-50% of dairy cows are affected by one or more metabolic or infectious diseases during the transition period (LeBlanc 2010). These include ketosis, mastitis, metritis, retention of secundinarum and abomasum displacement. Anti-mullerian hormone (AMH), a dimeric glycoprotein produced by granulosa cells of growing preantral and antral follicles, is a marker of ovarian reserve in dairy cows (La Marca et al. 2010, Rico et al. 2009). The concentration of AMH in the bloodstream is highly variable among different cows, but the measurements in the same cow are highly reproducible (Gobikrushanth et al. 2017, Ribeiro et al. 2014, Souza et al. 2015). Blood AMH concentration changes in cattle are related to nutritional condition, subspecies, breed, and lactation (Batista et al. 2014, Mossa et al. 2013) Cows with low plasma AMH concentration have a low conception rate (Gobikrushanth et al. 2018).

The aim of this study is examine the relationship between AMH, calcium, phosphorus, cholesterol (Chol), total protein, magnesium, triacylglycerol (TAG), β-Hydroxybutyrate (BHB), free glycerol levels, serum lipid and protein profiles of dairy cows from the 14th day before calving to the 21st day after calving, and the number of inseminations of the cows in the next insemination period

MATERIAL and METHODS

Experimental Animals

The animal material of the study consisted of 36 simmental dairy cows with similar body condition scores in the 2nd lactation at Nail Cinisli Dairy Farm located in Aşkale district of Erzurum province. Dairy cows were grouped as inseminated once (T1, n=13), inseminated twice (T2, n=12), and inseminated 3 or more times (T3, n=11) according to the insemination numbers at the end of the study. The study was supported by Ataturk University Scientific Research Projects Coordination Unit (project code PRJ2015/302) and the research was approved by Ataturk University Veterinary Faculty Sub-Ethics Committee (AÜVFEAK; decision dated 13.07.2015 and numbered 2015/9). The content of the ration given to the animals used in the study is given in Table 1.

Collection of Samples

Blood samples were taken from all animals in the study on the 14th day before calving, within 2 hours after calving, and on the 7th, 14th and 21st days after calving, from the vena jugularis before feeding in the morning after milking into 10 ml vacuum tubes without anticoagulant. The blood samples taken were centrifuged at 4000 rpm at +4 °C for 10 minutes in a centrifuge device and their serum was removed. The serums were portioned into eppendorf tubes and stored in a deep freezer at -80 °C until analysis.

ELISA Analysis

AMH levels in serum samples obtained from the study groups were measured with the Anti-Mullerian Hormone (Elabscience Biotechnology Co., Ltd. United States) kit (Catalog No: E-EL-H0317) according to manufacturer's protocol using the Sandwich-ELISA method. In the package insert, the analytical sensitivity of the kit was reported as 56.25 $pg.ml^{-1}$ and the detection range as 93.75-6000 $pg.ml^{-1}$. β-hydroxybutyrate levels in serum samples obtained from study groups were studied with the Bovine Beta-Hydroxybutyric Acid (Sunlong Biotech Co., Ltd. China) kit (Catalog No: SL0027Bo) according to manufacturer's protocol using the Sandwich-ELISA method. In the package insert, the analytical sensitivity of the kit was reported as 0.05 $\mu g.ml^{-1}$ and the detection range as 0.3-18 $\mu g.ml^{-1}$. The terms "expression time" and "insemination*time" refer to the number of inseminations and the sampling time, respectively.

Spectrophotometric Analysis

Serum cholesterol (Catalog No: 1 1350 99 10 021), calcium (Catalog No: 1 1130 99 10 021), magnesium (Catalog No: 1 4610 99 10 021), phosphorus (Catalog No: 1 5211 99 10 021), total protein (Catalog No: 1 2311 99 10 021), triacylglycerol(Catalog No: 1 5760 99 10 021) and free glycerol(Catalog No: 1 5730 99 10 730) levels were measured using sperctometry (μQuant, Bio-Tek Instruments, USA) with commercial kits purchased from DiaSys Diagnostic Systems (Germany).

Chromatographic Analysis

Serum lipid profile analysis was performed using the High Performance Thin Layer Chromatography (HPTLC) method. 1 ml of n-hexane/iso-propanol [2:1 (v/v)] mixture was added to 1 ml of serum and centrifuged at 2000 rpm for 15 minutes at +4 °C. The shaken serum tubes were centrifuged at 8000 rpm for 10 minutes and the supernatant was loaded onto 20x10 cm "Silica Gel 60" HPTLC plates. Plates were 7 cm in a mixture of hexane: diethylether: acetic acid [80:20:2 (v/v/v)] was carried out and dried at room temperature. $CuSO_4$ 3% in H_3PO_4 8% was sprayed on these dried plates and burned in an oven at 180 °C for about 10 minutes, making the lipid bands visible (Cengiz et al. 2016).

After HPTLC plates were scanned at 600 dpi resolution in Epson Perfection V500 photo scanner, the area covered by the lipid bands of each sample was determined using Phoretix 1D (TL120) software and expressed as % of the total mixture.

Electrophoretic Analysis

In this research, only the T3 group of electrophoresis samples were analyzed. The method developed by (Laemmli 1970) was used to determine the protein profile in blood samples. For this purpose, samples were dissolved in Laemmli saample buffer and proteins were separated in 10% Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE).

After electrophoresis, the gels were stained with oriole, visualized with the GE LAS500 imaging system and analyzed with the Phoretix 1D (TL120) gel analysis program. The molecular weights of the displayed serum proteins were automatically calculated according to the above principle with the Phoretix 1D (TL 120) program available from Nonlinear Dynamics.

Statistical Analysis

All obtained data were analyzed using SAS 2009 (Statistical Analysis System, Version 9.0, 2002, Cary, NC, USA) package program. Because of the data were not categorical, they were subjected to one-way analysis of variance. The following linear model was created using the Proc. GLM (General Linear Model) procedure:

$Yij = \mu + Zi + eij$, Z = time (days) relative to the ith seeding and e = error of the i and jth value. Differences over time were determined with the LSD (Least Square Differences) option. The values of the parameters by days were presented as mean±SE (standard error) and differences were considered significant at the $p<0.05$ level.

RESULTS

ELISA Results

As serum AMH concentrations were examined, it was determined that the effect of number of insemination, sampling time and insemination*time were statistically significant ($p<0.001$). After parturition, AMH levels increased in the T1 group, whereas they decreased in the T2 and T3 groups. It was detected that it decreased until the 7th day after parturition in all 3 groups, started to increase after the 7th day in the T1 group, after the 14th day in the T2 group, and decreased until the 21st day in the T3 group.

A sharp rise after parturition followed by a gradual decline was detected in BHB concentrations, which increased until the 7th day after parturition. While the group with the highest concentration was T3, the concentrations of T1 and T2 groups were found to be similar to each other. It was determined that the effect of insemination number, sampling time an semination*time on BHB concentrations were statistically significant ($p<0.001$).

Spectrophotometric Results

The mean values of the spectrophotometrically measured parameters and their variation according to the time of parturition are presented in Table 2 and Figure 1. Accordingly, it was determined that calcium concentration decreased with parturition, but started to increase after parturition. Calcium concentration was highest in T1 group before the parturition and lowest in T3 group during parturition ($p<0.001$).

It was found that the phosphorus concentration decreased with parturition, but increased until the 14th day after parturition, after which it started to decrease again. The highest concentration was detected in the T1 group after parturition, while the lowest concentration was in the T2 group during parturition. Insemination number and sampling time were found to be statistically significant ($p<0.001$).

Total Chol amount decreased excessively with delivery but started to increase afterwards, serum total Chol levels exceeded prepartum total Chol levels on postpartunm 21st day, and the highest total Chol level was found in T3 group after parturition, the lowest the total Chol level was found to be in the T1 group during parturition ($p<0.001$).

Change in total protein concentration is limited until parturition, but decreases significantly with parturition. While the decrease in total protein level continued until the postpartum 21st day in the T3 group, T1 (7.33 ± 0.15 mg.dl^{-1}) and T2 groups started to increase from the 14th day ($p<0.001$). It was determined that the number of insemination and time-dependent sampling were statistically significant ($p<0.001$) in total protein concentrations.

In blood magnesium concentrations, T1 was the group with the highest value, while the group with the lowest value was T3. It was determined that Mg, which increased with parturition, started to decrease after parturition. While the decrease continued in the T3 group, an increase was detected in the T1 and T2 groups starting from the 14th day ($p<0.001$). While the effect of insemination*time was statistically insignificant ($p>0.05$), it was determined that there was a statistically significant difference ($p<0.001$) among

the groups when the effect of sampling time on insemination numbers was taken into account.

Serum TAG concentrations, which decreased until the 7th day after parturition, increased again after the 7th day. The highest TAG concentration was determined in T1 group 14th day before the parturition, and the lowest TAG concentration was determined in T3 group 14th after before the parturition ($p<0.001$). As serum TAG levels are examined, it is seen that the number of inseminations is statistically insignificant ($p>0.05$), while the effect of sampling time and insemination*time is statistically significant ($p<0.001$).

As the free glycerol concentrations were examined, a large increase was determined with parturition and then a gradual decrease to a value close to the prepartum concentrations was detected. The highest free glycerol concentration was detected in the T3 group during parturition, and the lowest in the T1 group before thr parturition. It was determined that the effect of insemination, sampling time and insemination*time were statistically significant ($p<0.001$) on the groups.

Chromatographic Results

The mean values of the spectrophotometrically measured parameters and their variation according to the time of parturition have been presented in Table 3 and Figure 3. In addition HPTL chromatogram of T3 group serum lipid classes have been showed in Figure 2. As the cholesterol ester (CholE) ratio in serum total fat was examined, it was determined that the ratio was increasing starting from the prepartum period until the postpartum 14th day. After the 14th day, the CholE ratio started to decrease in the T1 and T2 groups, but continued to increase in the T3 group ($p<0.001$). In the statistical evaluation, the effect of insemination number, sampling time and insemination*time was determined as ($p<0.001$).

It was determined that the TAG ratio decreased at varying rates starting from calving until the 14th day after calving. The greatest reduction was determined in the T3 group, and the lowest in the T1 group ($p<0.001$). As the effect of insemination number, sampling time and insemination*time were evaluated statistically, it was found to be significant ($p<0.001$).

As the variation of the free fatty acid (FFA) ratio according to the days at the time of calving was examined, it was determined that there was a gradual decrease from the 14th day before calving until the 14th day after calving. From the 14th day after calving, the FFA ratio increased in the T1 and T2 groups, while it continued to decrease in the T3 group. As evaluated statistically, the change between groups according to sampling time is significant ($p<0.001$).

As Chol ratio was examined, a large change was determined. There was no difference in cholesterol ratio between the groups. In the statistical evaluation, the effect of insemination number, sampling time, and insemination*time was determined as insignificant ($p>0.05$).

While diacylglycerol (DAG) ratio followed a constant course from the 14th day before calving to the 21st day after calving in the T1 and T2 groups, a gradual decrease was detected in the T3 group starting from the prepartum period. It was determined that the number of insemination, sampling time and the effect of insemination*time were statistically significant on the groups ($p<0.001$).

As the phospholipid ratio was examined, it was found that the T1 and T2 groups were close to each other, and the T3 group was higher than these two groups ($p<0.001$). It was determined that the effect of insemination number and insemination*time were statistically significant ($p<0.001$) on the groups.

Electrophoretic Results

The SDS-PAGE analysis of serum samples showed the presence of 24 proteins between 361 and 15 kDa (Figures 4 and 5 and Table 4).

Table 1. The content of the ration given to the study animals

	Dry Matter Amount, kg
Meadow Grass	2.50
Triticale Silage	4.00
Corn Silage	4.50
Triticale grain	0.89
Milk Feed	8.54
HP	14.06
Energy	2438
DM %	53.04

Table 2. ELISA and Spectrophotometer results of blood samples taken on the 14th day before parturition, at the time of parturition and on the 7th, 14th and 21st days after parturition.

	Day	T1 $\bar{x}$±SE	T2 $\bar{x}$±SE	T3 $\bar{x}$±SE	Number of Insemination	Time	Insemination *Time
AMH (pg.ml^{-1})	-14	3217±69	2835.00±100.75	2487.27±94.89			
	0	2333.08	2566.67±111.51	2125.45±103.52			
	7	2024±62	1973.33±93.30	1846.36±77.88			
	14	2036±112.01	1878.33±126.59	1694.55±111.14			
	21	2669±80.84	2086.67±83.22	1568.18±47.97			
		2456±90.11^{a}	2268.00±103.07^{b}	1944.36±87.08^{c}	<0.001	<0.001	<0.001
BHB (µg.ml^{-1})	-14	0.31±0.01	0.28±0.01	0.36±0.01			
	0	0.36±0.01	0.40±0.01	0.38±0.01			
	7	0.62±0.02	0.65±0.02	0.68±0.01			
	14	0.49±0.02	0.47±0.01	0.59±0.01			
	21	0.39±0.01	0.46±0.01	0.52±0.03			
		0.43±0.01^{b}	0.45±0.01^{b}	0.51±0.01^{a}	<0.001	<0.001	<0.001
Calcium (mg.dl^{-1})	-14	8.73±0.11	8.39±0.24	8.98±0.15			
	0	5.67±0.07	5.04±0.15	4.85±0.13			
	7	6.29±0.09	6.70±0.21	6.29±0.19			
	14	6.40±0.14	6.82±0.18	6.46±0.14			
	21	7.87±0.19	757±0.08	7.32±0.14			
		6.99±0.10^{a}	6.90±0.17^{ab}	6.78±0.15^{b}	>0.05	<0.001	<0.001
Phosphorus (mg.dl^{-1})	-14	5.79±0.20	4.91±0.33	5.51±0.13			
	0	3.94±0.12	3.43±0.21	3.76±0.13			
	7	5.55±0.18	4.67±0.31	5.57±0.26			
	14	6.19±0.24	5.72±0.18	6.00±0.14			
	21	5.97±0.29	5.45±0.15	5.75±0.14			
		5.49±0.21^{a}	4.83±0.23^{b}	5.32±0.15^{a}	<0.001	<0.001	>0.05
Magnesium (mg.dl^{-1})	-14	1.97±0.06	1.82±0.11	1.99±0.08			
	0	2.72±0.11	2.55±0.15	2.78±0.12			
	7	2.24±0.07	2.05±0.13	2.26±0.11			
	14	1.96±0.07	1.84±0.12	2.02±0.09			
	21	2.16±0.05	2.16±0.05	2.04±0.07			
		2.21±0.07^{a}	2.09±0.11^{b}	2.22±0.09^{a}	>0.05	<0.001	>0.05
Total cholesterol (mg.dl^{-1})	-14	83.46±2.79	93.58±4.31	112.91±5.36			
	0	45.88±3.04	59.58±4.20	67.75±3.21			
	7	73.86±4.51	104.40±5.18	119.32±6.31			
	14	100.50±3.86	115.58±4.95	146.09±5.86			
	21	131.63±4.38	130.45±5.06	172.33±5.07			
		87.07±3.72^{c}	100.72±4.74^{b}	123.68±5.16^{a}	<0.001	<0.001	>0.05
Total protein	-14	7.82±0.15	7.78±0.29	7.44±0.24			
	0	7.58±0.16	7.23±0.24	7.31±0.31			

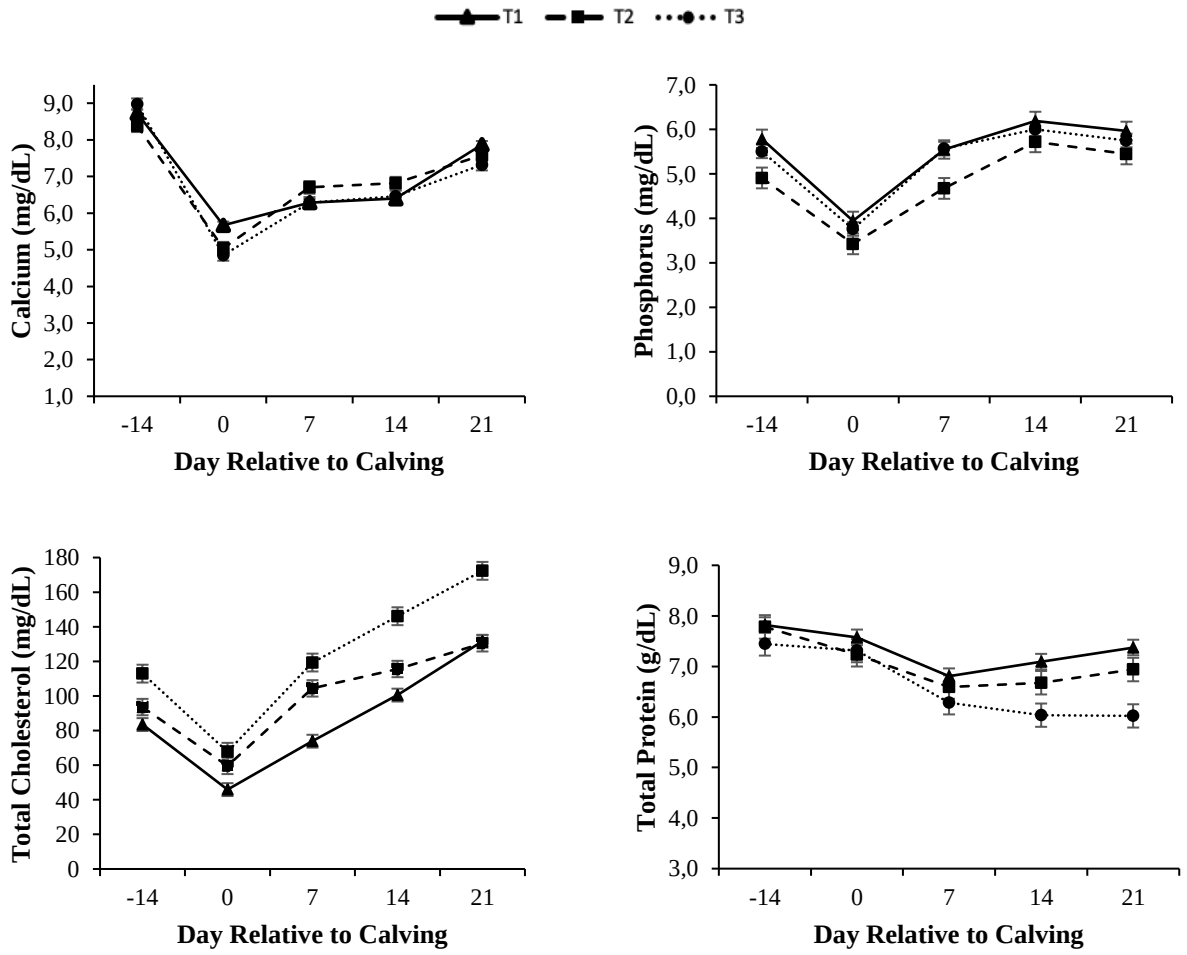
(g.dl^{-1})	7	6.81±0.21	6.59±0.28	6.28±0.27			
	14	7.09±0.13	6.68±0.20	6.04±0.15			
	21	7.37±0.11	6.94±0.15	6.02±0.17			
		7.33±0.15^{a}	7.04±0.23^{b}	6.62±0.23^{c}	<0.001	<0.001	>0.05
Triacylglycerol (mg.dl^{-1})	-14	32.62±2.29	28.92±2.28	23.64±1.38			
	0	19.69±1.64	16.25±1.69	12.91±1.21			
	7	14.17±1.28	13.29±1.58	11.91±1.35			
	14	16.05±1.53	13.09±1.09	11.41±1.01			
	21	19.85±1.98	16.00±1.50	13.27±1.30			
		20.47±1.82^{a}	17.51±1.63^{b}	14.63±1.25^{c}	<0.001	<0.001	>0.05
Free glycerol (mg.dl^{-1})	-14	91.88±0.57	84.56±1.15	11284±238			
	0	295.35±408	312.10±3.33	32434±2.72			
	7	222.03±1.55	226.61±5.03	247.29±4.71			
	14	144.78±2.94	164.55±5.01	194.14±5.10			
	21	103.96±2.61	126.85±4.64	159.26±4.62			
		171.60±2.35^{c}	183.53±3.83^{b}	207.58±3.91^{a}	<0.001	<0.001	<0.001

* Different exponential letters in the same line indicate the difference between groups ($p<0.05$).

T1: Inseminated once in the next insemination period

T2: Inseminated 2 times in the next insemination period

T3: Inseminated 3 or more times in the next insemination period



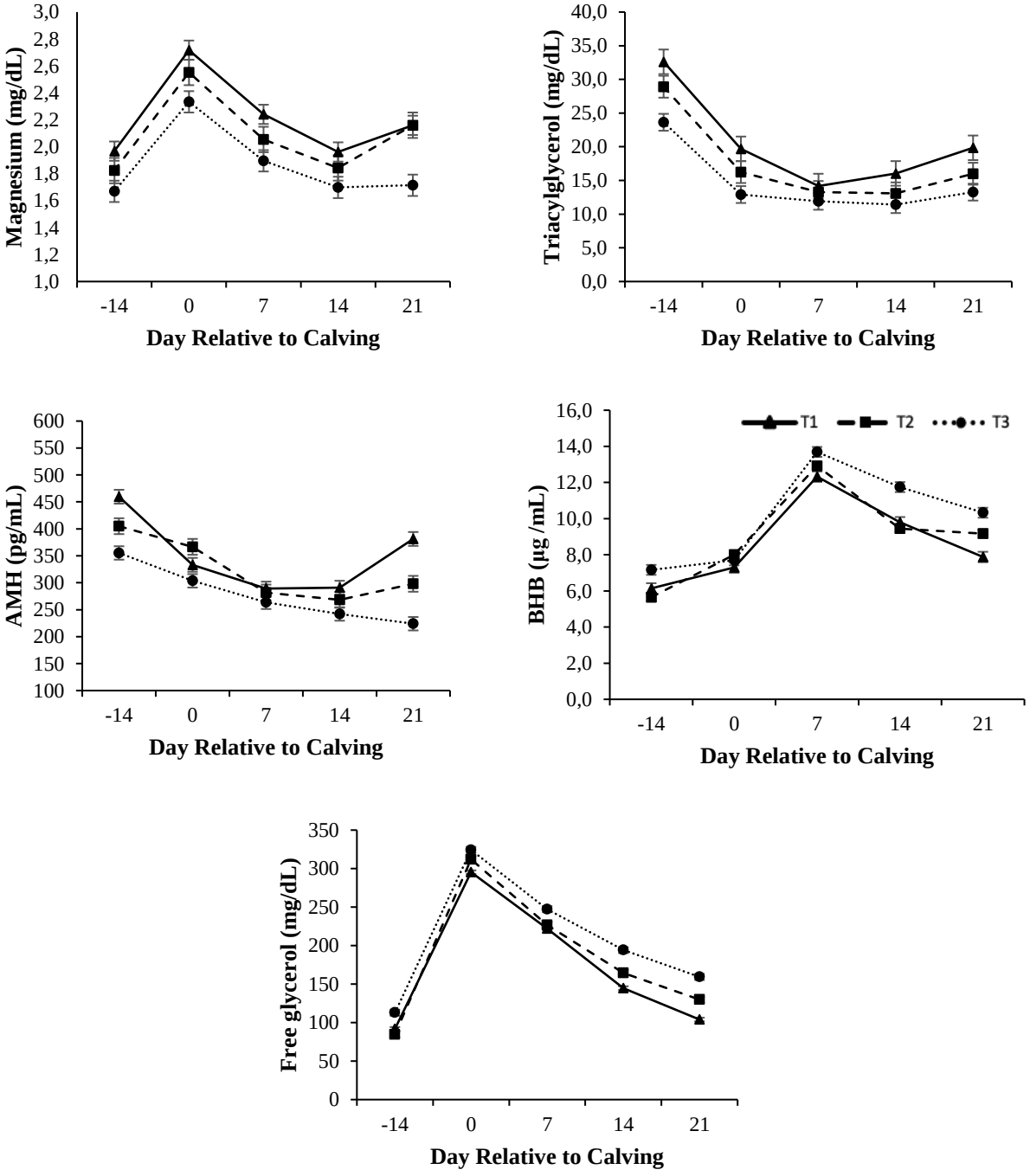


Figure 1. Variation of spectrophotometrically measured parameters according to day relative to calving

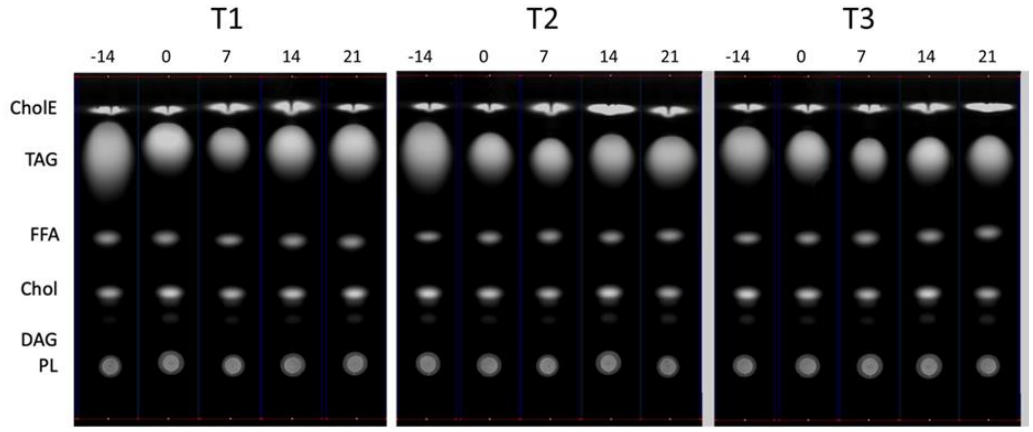


Figure 2. HPTL chromatogram of T1, T2, and T3 group serum lipid classes.

Table 3. HPTLC analyzes of blood samples taken on the 14th day before parturition, at the time of parturition and on the 7th, 14th and 21st days after parturition.

	Day	T1 x̄±SE	T2 x̄±SE	T3 x̄±SE	Number of Insemination	Time	Insemination *Time
Cholesterol ester, %	-14	23.79±0.41	23.44±0.46	24.07±0.42			
	0	27.24±0.35	27.77±0.27	30.19±0.34			
	7	29.63±0.40	31.53±0.29	33.82±0.38			
	14	31.13±0.43	32.87±0.38	35.67±0.31			
	21	29.03±0.36	30.35±0.28	35.94±0.31			
		28.16±0.80^{c}	29.09±1.02^{b}	31.94±1.39^{a}	<0.001	<0.001	<0.001
TAG, %	-14	24.90±0.43	23.98±0.40	24.18±0.47			
	0	20.83±0.30	20.01±0.34	18.46±0.39			
	7	18.74±0.29	17.00±0.31	14.10±0.24			
	14	17.75±0.30	15.85±0.34	12.72±0.28			
	21	19.22±0.34	17.85±0.32	12.21±0.28			
		20.29±0.77^{a}	18.94±0.90^{b}	16.33±1.41^{c}	<0.001	<0.001	<0.001
FFA, %	-14	10.35±0.19	10.42±0.23	10.09±0.25			
	0	8.92±0.13	9.77±0.15	9.54±0.16			
	7	8.92±0.10	9.47±0.14	8.67±0.15			
	14	8.78±0.10	8.91±0.17	8.83±0.19			
	21	9.08±0.15	9.34±0.15	8.45±0.19			
		9.21±0.21^{a}	9.58±0.22^{b}	9.12±0.26^{c}	<0.001	<0.001	<0.001
Free Cholesterol, %	-14	13.26±0.21	13.52±0.26	12.89±0.23			
	0	14.70±0.21	13.47±0.22	14.66±0.21			
	7	14.63±0.20	14.75±0.24	14.37±0.20			
	14	14.73±0.23	14.86±0.18	14.21±0.23			
	21	14.71±0.15	14.23±0.20	14.37±0.23			
		14.41±0.25^{a}	14.16±0.27^{ab}	14.10±0.27^{b}	>0.05	<0.001	>0.05
DAG, %	-14	2.47±0.04	2.79±0.05	2.56±0.05			
	0	2.45±0.04	2.39±0.03	2.45±0.04			
	7	2.42±0.03	2.40±0.03	1.89±0.03			
	14	2.51±0.04	2.55±0.04	1.50±0.04			
	21	2.48±0.03	2.56±0.05	1.32±0.03			
		2.47±0.04^{b}	2.54±0.06^{a}	1.94±0.16^{c}	<0.001	<0.001	<0.001
Phospholipid, %	-14	25.22±0.40	25.85±0.34	25.40±0.46			
	0	25.87±0.37	26.59±0.29	24.69±0.30			
	7	25.66±0.34	24.85±0.27	27.16±0.33			
	14	25.09±0.31	25.09±0.32	27.06±0.35			
	21	25.48±0.33	25.67±0.28	27.70±0.35			
		25.47±0.35^{b}	25.61±0.34^{b}	26.40±0.49^{a}	<0.001	>0.05	<0.001

* Different exponential letters in the same line indicate the difference between groups ($p<0.05$).

T1: Inseminated once in the next insemination period

T2: Inseminated 2 times in the next insemination period

T3: Inseminated 3 or more times in the next insemination period

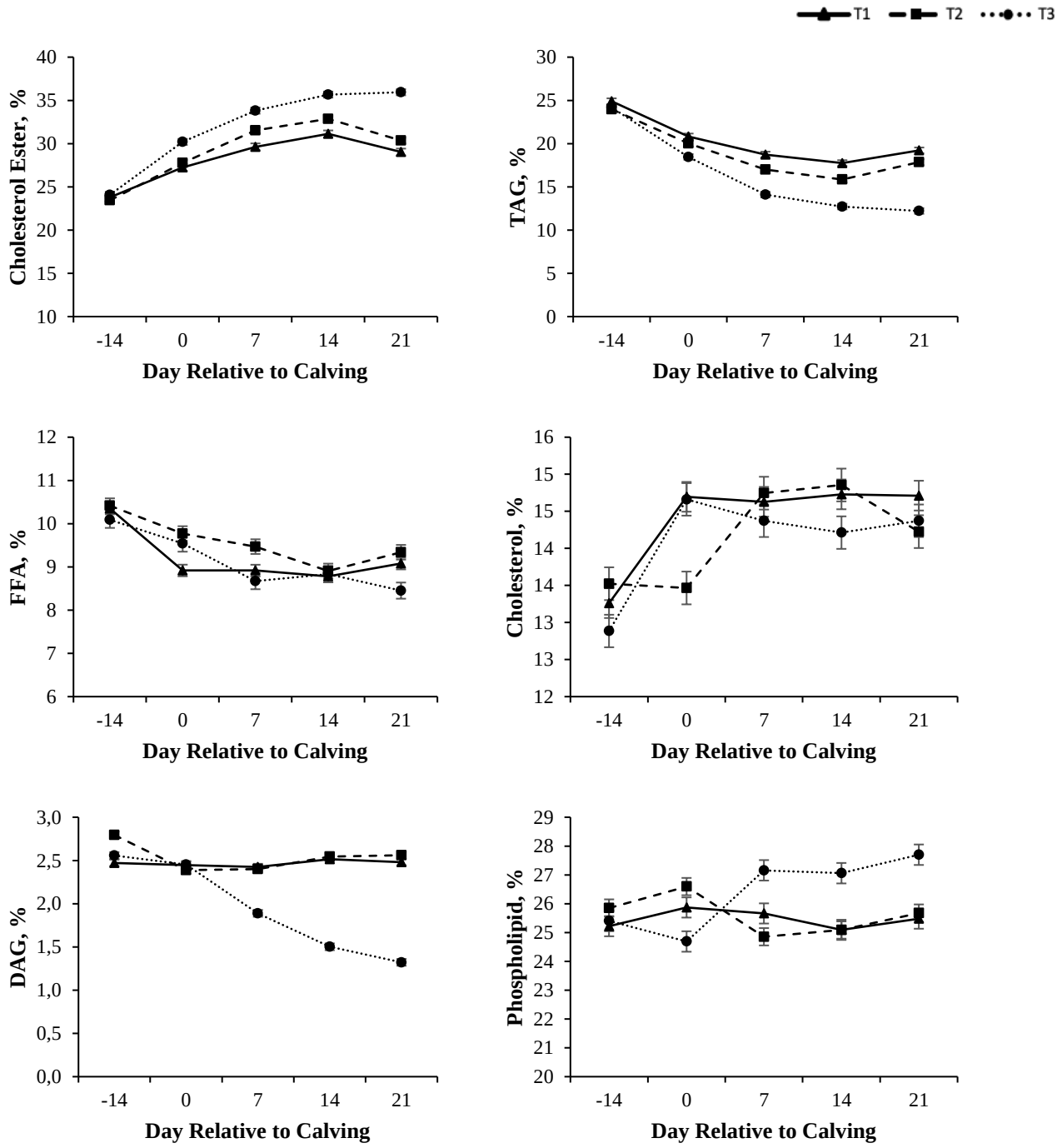


Figure 3. Variation of chromatographically measured parameters according to day relative to calving

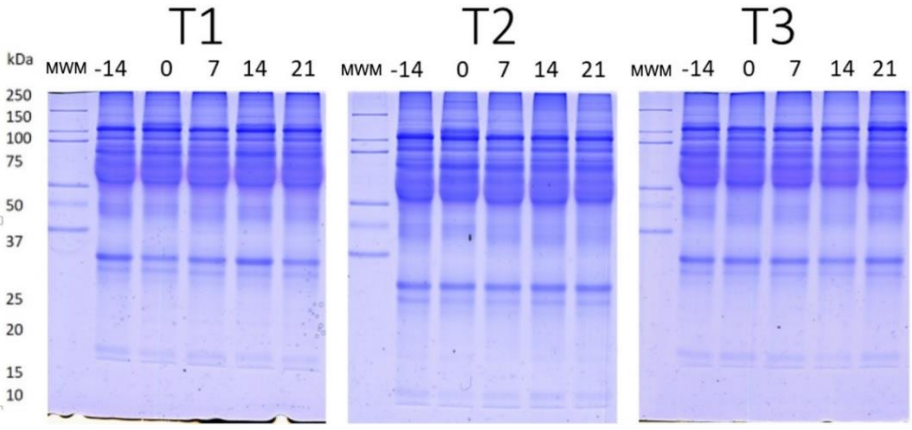
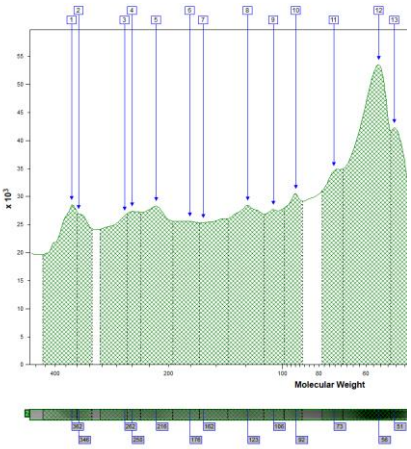


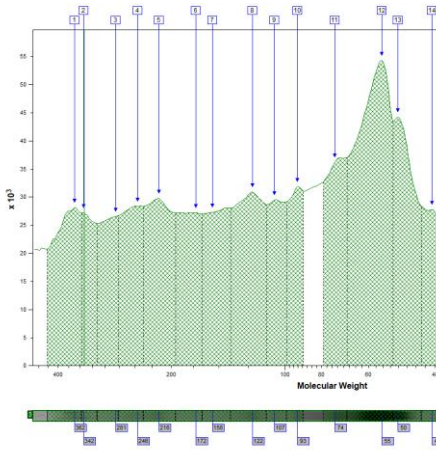
Figure 4. SDS-PAG electrophoretogram of T1, T2, and T3 group serum protein classes.

Table 4. Quantities (volume) of individual proteins in the T3 group serum total protein.

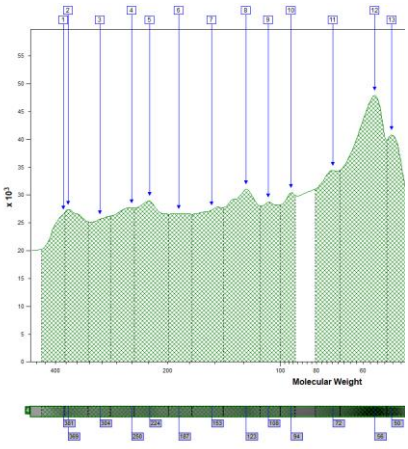
	Day									
MA	-14		0		7		14		21	
(kDa)	Volum	Band %	Volum	Band %	Volum	Band %	Volum	Band %	Volum	Band %
361	501	4.6	485	4.00	253	2.18	283	2.51	188	1.76
346	212	1.72	194	1.60	323	2.79	135	1.20	159	1.49
261	305	2.47	238	1.97	260	2.25	291	2.58	237	2.22
250	138	1.12	255	2.10	249	2.16	237	2.10	279	2.62
215	276	2.24	264	2.18	256	2.22	224	1.99	240	2.25
176	207	1.68	200	1.65	195	1.69	166	1.47	178	1.67
162	233	1.89	228	1.88	248	2.15	292	2.59	202	1.89
123	444	3.60	435	3.59	431	3.73	388	3.44	398	3.73
105	360	2.92	348	2.87	339	2.93	332	2.94	303	2.84
92	356	2.88	310	2.56	273	2.36	297	2.64	246	2.30
73	510	4.14	556	4.59	582	5.03	492	4.37	472	4.42
55(Albümin)	2502	20.28	2297	18.94	2052	17.75	2189	19.42	1963	18.39
50	1551	12.57	1504	12.4	1426	12.33	1458	12.94	1500	14.05
41	750	6.08	772	6.36	738	6.38	691	6.13	599	5.62
36	423	3.43	462	3.81	414	3.58	496	4.40	391	3.67
33	407	3.30	415	3.42	470	4.06	347	3.08	372	3.48
29	465	3.77	495	4.08	435	3.76	387	3.43	403	3.77
26	1005	8.14	922	7.60	890	7.69	896	7.95	899	8.42
24	369	2.99	467	3.85	372	3.21	408	3.62	340	3.18
20	510	4.13	449	3.70	578	5.00	579	5.13	578	5.41
18	532	4.31	541	4.46	485	4.19	429	3.81	413	3.87
15	282	2.28	291	2.40	296	2.56	255	2.26	314	2.94
Total *100000	12337	100	12127	100	11564	100	11271	100	10674	100



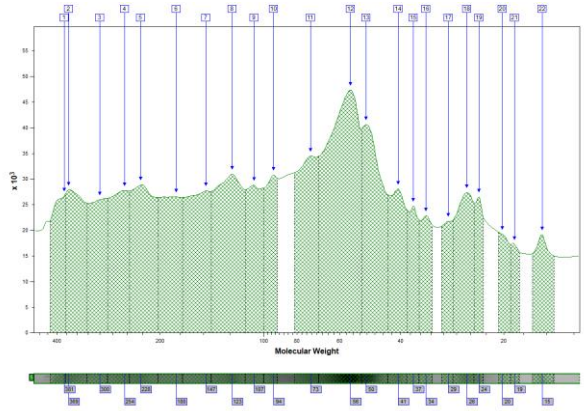
SDS-PAGE densitogram of T3 group serum protein classes 14 days before the parturition



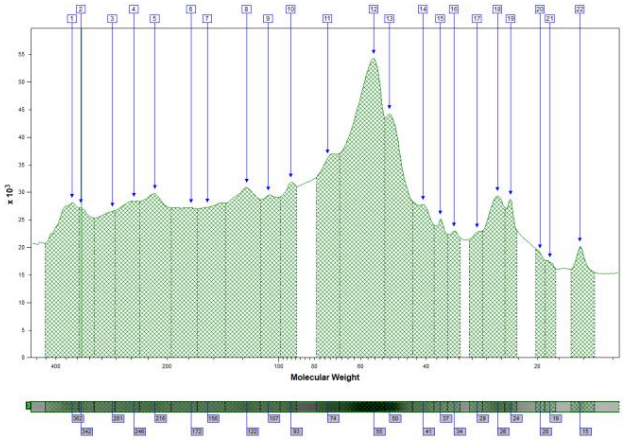
SDS-PAGE densitogram of parturition time T3 group serum protein classes



SDS-PAGE densitogram of T3 group serum protein classes 7 days after parturition



SDS-PAGE densitogram of T3 group serum protein classes 14 days after parturition



SDS-PAGE densitogram of T3 group serum protein classes 21 days after parturition

Figure 5. SDS-PAGE densitogram of T3 group serum protein classes.

Table 5. The correlation between parameters.

	CholE. %	TAG.%	FFA.%	Chol.%	DAG.%	Pl.%	Ca (mg.dl^{-1})	TP (g.dl^{-1})	TAG (mg.dl^{-1})	P (mg.dl^{-1})	Mg (mg.dl^{-1})	Ghol (mg.dl^{-1})	AMH (pg.ml^{-1})	BHB (µg.ml^{-1})	F. Glycerol (mg.dl^{-1})
CholE	1	-0.942**	-0.596**	0.317**	-0.670**	0.184*	0.012	-0.246**	-0.297**	-0.034	-0.096	0.462**	-0.323**	0.111	-0.007
TAG. %		1	0.601**	-0.407**	0.702**	-0.336**	-0.015	0.274**	0.301**	0.035	0.078	-0.478**	0.352**	-0.148*	-0.016
FFA. %			1	-0.253**	0.464**	-0.366**	0.047	0.250**	0.252**	-0.097	0.000	-0.333**	0.320**	-0.179*	-0.007
Chol. %				1	-0.077	-0.194**	-0.023	-0.120	-0.163*	-0.018	-0.091	0.150*	-0.173*	0.077	0.010
DAG. %					1	-0.490**	0.010	0.221**	0.116	-0.034	0.015	-0.329**	0.214**	-0.067	-0.015
Pl. %						1	-0.008	-0.133	-0.079	-0.002	0.093	0.127	-0.154*	0.080	0.058
Ca (mg.dl^{-1})							1	0.243**	0.494**	0.440**	-0.360**	0.376**	0.409**	-0.315**	-0.845**
TP (g.dl^{-1})								1	0.376**	-0.117	0.114	-0.297**	0.528**	-0.493**	-0.097
TAG (mg.dl^{-1})									1	0.042	-0.121	-0.126	0.571**	-0.508**	-0.435**
P (mg.dl^{-1})										1	-0.376**	0.553**	-0.122	0.164*	-0.566**
Mg (mg.dl^{-1})											1	-0.327**	0.009	-0.058	0.569**
Ghol (mg.dl^{-1})												1	-0.334**	0.283**	-0.469**
AMH (pg.ml^{-1})													1	-0.599**	-0.277**
BHB (µg.dl^{-1})														1	0.278**
F. Glycerol (mg.dl^{-1})															1

** Significance p<0.01

* Significance p<0.05

DISCUSSION

Transition health is an important indicator of the production and reproductive performance of dairy animals in later periods. In the periparturient period, deep endocrine changes occur to provide the energy need (Goff and Horst 1997). The main purpose of this study is to evaluate the correlation between the transition period serum concentration of AMH, which is one of the main factors in the regulation of follicle development, and the rate of pregnancy.

Anti-Müllerian hormone inhibits early primordial follicle activation in females. Deficiency of this hormone has been shown to cause early menopause in mice (Pereira et al. 2013). Individual AMH levels measured in serum and/or plasma have been shown to be consistent across oestrus cycles and correlated with FSH levels (Wang et al. 2009). As examined at the population level, AMH shows quite different levels among individuals with different ovarian potential (Ireland et al. 2008). Ovarian reserve estimated by AMH measurement generally reflects the total amount of oocytes present in the ovary (Ireland et al. 2011). In a previous study, AMH has been shown to be accurate when measured in serum at any stage of the oestrus cycle, regardless of the concentrations of other endocrine and paracrine reproductive hormones (La Marca et al. 2009).

In addition, AMH is a good prognostic tool for fertility and can be used to weed cattle to obtain a good dairy herd (Ireland et al. 2007). In this study, it was determined that the effect of serum AMH concentrations on the number of insemination, sampling time and insemination*time were statistically significant ($p<0.001$). Based on these results, it can be said that cows that can reach postpartum AMH concentrations faster and higher in negative energy balance can become pregnant more easily in the next insemination.

β-hydroxybutyrate is the most stable ketone body that can be easily measured. Comparably FFA, BHB reflects the NEB level in dairy cows in the transition period. It has been reported that an increase in the level of BHB in the blood to 1.4 $mmol.l^{-1}$ is associated with an increased risk of metabolic disorders, while a level higher than 2.0 $mmol.l^{-1}$ is associated with reduced milk yield (Duffield 2000). In addition, it has been suggested that BHB concentration is the golden marker for the diagnosis of ketosis in cattle (Kaneko et al. 2008). In a previous study, it was shown that serum BHB concentration between 1.0 and 3.0 $mmol.l^{-1}$ is generally detected in dairy cows with subclinical ketosis, and concentrations less than 1 $mmol.l^{-1}$ are normal (Li et al. 2016). It was determined that while the BHB levels of T1 and T2 of the groups in this study were at normal levels, the T3 group was in ketosis and the BHB level affected the number of inseminations.

Cholesterol levels may change due to the limitation of both lactation period and nutrient intake due to the demand for a large amount of biomolecules in colostrum-milk production with birth (Gross et al. 2015). In this study, lipid parameters of transitional cows were investigated both spectrophotometrically and chromatographically. As the lipid classes that make up the serum total fat are examined, it was found that the ratio of CholE in serum total fat increased continuously from the 14th day before birth until the 14th day after birth, it started to decrease from the 14th day after parturition in the T1 and T2 groups, but the increase continued in the T3 group. On the other hand, it was detected that the ratio of free Chol in serum total fat did not change during the transition period, but the total amount of Chol increased with birth in the T1 and T3 groups, and on the 7th postnatal day in the T2 group. In the presented study, it was determined that the free Chol ratio did not change relatively, but the total Chol level increased chromatographically. It suggests that the decrease in TAG ratio in serum total fat suppresses the decrease in serum free Chol ratio,

and that the increase in serum total Chol level may be caused by excessive CholE ratio, not free Chol.

Free fatty acid concentration is less than 0.2 mM on average in dairy cows during early pregnancy and late lactation and reaches a concentration above 0.7 mM by 10 days postpartum (Adewuyi et al. 2005). FFA concentration in the transition period was higher than after the transition period. In the present study, it was determined that the TAG ratio in serum total fat decreased continuously from the 14th day before calving until the 14th day after calving, it started to increase from the 14th day in the T1 and T2 groups, but the decrease continued in the T3 group, and the lowest TAG level was in the T3 group. It was determined that the DAG ratio of the lipolysis product remained relatively unchanged in the T1 and T2 groups, and decreased in the T3 group until the postpartum 21st day, while the PL ratio fluctuated according to the groups and time. It was determined that the rate of FFA, another lipolysis product, decreased with calving, there was no postpartum decrease in the T1 group, the decrease stopped on the 7th postparum day in the T2 and T3 groups, and started to decrease again on the 14th postpartum day in the T3 group. Contrary to the general literature, consistent with (Van der Drift et al. 2012) the decreased FFA concentration we detected in the transition period suggested that it may be due to increased intake, FFAs are rapidly removed from the circulation to meet the excess energy need by β-oxidation, taken up by the peripheral tissues and/or produced by the mammary gland for milk fat synthesis. In our study, we found that the PL ratio in serum total fat increased contrary to the literature. This increase suggests that the excessive decrease in TAG ratio in serum total fat masks the decrease in serum PL ratio due to the pressure on lipolysis and/or lipogenesis, as in free Chol.

The homeostatic mechanisms controlling the blood calcium concentration are generally not able to respond quickly enough to provide the sudden increase in calcium demand with calving in periparturient dairy cows. As a result, clinical and subclinical hypocalcemia occurs in dairy cows. Some researchers found no association between clinical hypocalcemia and reproductive outcomes in line with our study (Eicker et al. 1996). Other studies have shown lower reproductive outcomes in cows with clinical hypocalcemia, including a prolonged interval to first ovulation, a longer luteal phase after first ovulation, impaired ovarian cyclicity, and increased times to both first insemination and attachment (Risco et al. 1994, Whiteford and Sheldon 2005).

Hypophosphatemia is commonly encountered in the transition period of dairy cows (Macrae et al. 2006). The clinical significance of this electrolyte imbalance remains unclear and has been discussed for many years. Empirical evidence suggests that hypophosphatemia or phosphorus deficiency in young cows can cause or at least contribute to diseases such as underlying cow syndrome or postpartum hemoglobinuria in dairy cows (Grünberg 2014, Ménard and Thompson 2007). Although hypophosphatemia has been investigated in transitional cows, its effects on fertility are still not understood. In our study, it was determined that phosphorus concentration decreased until calving and returned to its former levels with parturition. As a result of the statistics, it was determined that this phosphorus change contributed significantly to the next insemination period of the cows ($p<0.001$).

Low Mg levels in plasma affect Ca metabolism by reducing parathormone (PTH) secretion or tissue sensitivity in response to hypocalcemia. Magnesium concentration in colostrum is approximately three times higher than in normal milk and has been shown to rapidly cause hypomagnesemia in lactating cows if the extracellular magnesium used for milk production is not resupplied (Goff 2006, Tsioulpas et al. 2007). Our results reveal that transitional serum magnesium

concentrations are associated with reproductive performance in dairy cows. Thus, it is thought that the serum magnesium concentration during the transition period may serve as a biomarker for re-insemination in dairy cows. Preeclampsia has been associated with impaired maternal mineral homeostasis, particularly related to magnesium. However, it is likely that low serum magnesium concentrations in the peripartum period will increase the risk of fetal and placental involvement, since magnesium deficiency can lead to hyperexcitability caused by a decrease in nerve resting membrane potential.

Albumin and globulins all make up "total serum protein". In a study, it was reported that the amount of total protein decreased slightly calving the 4th day after birth, but increased until the 60th day after parturition (Gaona et al. 2012). On the other hand, it was stated that albumin concentration decreased during the transition period (Trevisi et al. 2015). In addition, (Djokovic et al. 2015) reported that serum albumin level indicates the synthetic capacity of the liver and tends to decrease in animals in early lactation. Similarly, one study reported low albumin levels, a high incidence of fatty liver - negative energy balance in transitional cows, indicating impaired liver function (Montagner et al. 2016). In our study, it was determined that the total protein was low until calving, but decreased significantly until the 7th day after calving, then increased in the T1 and T2 groups, whereas in the T3 group, the decrease stopped at the postnatal 14th day and did not increase even on the 21st postpartum day. As serum protein electrophoretograms were examined, it was determined that this decrease in protein level was the result of decreased expression of many serum proteins, including albumin, at different levels.

According to the data obtained, it was seen that serum AMH concentration was closely related to the parameters that reflect the energy balance of the cow - TP, TAG, BHB levels rather than mineral levels. It was determined that cows with high prepartum AMH level and cows that came out of negative energy balance earlier were pregnant with less insemination number. In addition, in parallel with these results, it was concluded that the individual AMH levels of the cows in the herd can help the veterinarian about the insemination strategy, and may also be an indicator for making a decision about an animal in weeding.

Conflict of Interest: The authors declared that there is no conflict of interest.

Authors Contribution Rate: Mustafa İLERİTÜRK: %70, Özgür KAYNAR:%30

Ethical Approval: Tis study was approved by Ataturk University Veterinary Faculty Sub-Ethics Committee (AÜVFEAK; decision dated 13.07.2015 and numbered 2015/9).

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