

Effect of twinship on selected hematological and biochemical parameters in late pregnant ewes

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Summary

This study aimed to determine the effect of twinship on the hematological and biochemical parameters in ewes of the Kivircik breed commonly raised in the Thrace region of Turkey. The study material consisted of 30 Kivircik ewes at days 130-150 of gestation, aged 3-5 years, weighing 55.61 ± 0.76 kg. After ultrasound examination, the ewes included in the study were divided into a single-pregnant group ($n = 15$) and a twin-pregnant group ($n = 15$), and their blood samples were taken. The levels of red blood cells (RBC), hematocrit (HCT), hemoglobin (HGB) white blood cells (WBC), lymphocytes (LYM), monocytes (MON), non-esterified fatty acid (NEFA), β -hydroxybutyrate (BHBA), glucose, cholesterol, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT) were determined in their blood samples. It was found that, although twinship did not affect hemogram values in ewes ($p > 0.05$), the biochemical parameters, BHBA, ALT, and AST values, were significantly affected by twinship ($p < 0.05$). A high positive correlation was found between BHBA and NEFA. Thus it was concluded that twin pregnancy did not cause any significant changes in hematological parameters in Kivircik ewes. However, the significantly higher BHBA in twin-pregnant ewes and the high positive correlation between BHBA and NEFA indicate that the negative energy balance started to affect these animals, and a carbohydrate deficiency emerged. Therefore, twin-pregnant ewes should be monitored closely to reduce the risk of ketosis.

Keywords: ewes, late pregnant, twinship

Sheep and sheep products are economically valuable all over the world, including Turkey. They are a significant source of animal protein, especially in human nutrition (2). Long-tailed Kivircik is a native Turkish sheep breed that accounts for 6.8% of the total sheep population in Turkey. This breed, which is widely raised in the Thrace, Marmara, and Aegean regions of Turkey, is also grown in Bulgaria and Greece, where they are known as Traki (8).

During pregnancy in sheep, the nutritional requirements of the fetus and consequently the mother increase. Since 70-80% of fetal growth occurs towards the end of pregnancy, the energy needs of late pregnant ewes are significantly greater (41). During pregnancy, maternal tissues contribute to providing the energy required by the fetus. This situation causes changes in the mother's biochemical parameters, such as glucose, cholesterol, and total protein. Through the examination and evaluation of these parameters, abnormalities in metabolism can be detected earlier. Thus, appropriate

corrections can be made to overcome metabolic diseases that may occur during pregnancy (1, 14).

Blood is one of the most crucial body fluids, and the hematological profile is a significant indicator of animal health and production. The examination of blood parameters can be used to diagnose diseases of the hemopoietic system, general metabolic disorders, as well as diseases of other systems and organs (7). The breeding period, sex, age, and season significantly influence blood parameters of farm animals. It has been reported that during pregnancy, one of physiological periods in ewes, hematological changes occur (27). These factors should therefore be considered when interpreting blood parameters (45).

The number of lambs born per ewe is a significant factor affecting profitability in sheep production. Although twin pregnancy in ewes is desirable, their health status during and after gestation also has to be considered. There are very few studies on how hematological and biochemical parameters, which are

health indicators in ewes, are affected by twin pregnancy. Therefore, the current study aimed to reveal the possible effects of twinship on hematological and biochemical parameters in Kivircik ewes.

Material and methods

The material of the study consisted of 30 Kivircik ewes at days 130-150 of gestation, aged 3-5 years, weighing 55.61 ± 0.76 kg. Ethical approval for the study was obtained from the Animal Experiments Local Ethics Committee of the Namik Kemal University (T2021-576). Each animal was fed with 1 kg of concentrated feed (containing 14% of crude protein, 15% of fiber, 3% of fat) and 2 kg of oat grass, divided into two meals per day. Drinking water and licking stone containing minerals (calcium, magnesium, phosphorus, potassium, sodium, manganese, copper, zinc, and cobalt) were always available in front of the animals. In the study, the ewes were examined with a 5 MHz ultrasonography device and divided into two groups: single-pregnant ewes ($n = 15$) and twin-pregnant ewes ($n = 15$). Also, 10 ml blood samples were taken from the external jugular vein of all ewes in the study and poured into anticoagulant (heparin) and non-anticoagulant tubes. All animals included in the study were checked every day for their health until the 72nd hour after birth. Red blood cells (RBC), hematocrit (HCT), hemoglobin (HGB), white blood cells (WBC), lymphocytes (LYM), and monocytes (MON) in anticoagulant blood samples were counted by a complete blood counter (Exigo Eos Hematology, Boule Diagnostics AB Sweden) on the same day. Non-anticoagulant blood samples were centrifuged at 3000 rpm for 15 minutes, and their serum was separated. Samples of serum were stored in Eppendorf tubes at -80°C until analysis. Non-esterified fatty acid (NEFA), β -hydroxybutyrate (BHBA), glucose, cholesterol, total protein, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels in serum were measured with an automatic biochemical analyzer (Biotechnica Instruments BT 3500, Rome, Italy).

Statistical analysis. SPSS 24 and Minitab 17 package programs were used to prepare statistical data. Findings were entered as mean value \pm standard deviation. The Shapiro-Wilk test ($n < 50$) was used to examine whether the continuous variables in the study were normally distributed or not. It was seen that the variables were not normally distributed, and nonparametric tests were applied. The Mann-Whitney U test was performed to compare biochemistry and hemogram analyses. Spear-

man correlation coefficients were calculated to examine the relationships between biochemical parameters in sheep carrying a twin pregnancy.

In the calculations, the statistical significance level was set at 5% ($p < 0.05$).

Results and discussion

In the late pregnant ewes, BHBA was significantly higher in twin-pregnant ewes than in single-pregnant ewes, whereas ALT and AST values were significantly lower ($p < 0.05$). On the other hand, no statistically significant difference between twin- and single-pregnant groups was observed in glucose, cholesterol, t. protein, and NEFA levels ($p > 0.05$) (Tab. 1).

Correlations between biochemical parameters in twin-pregnant ewes are presented in Table 2. There was a significant positive correlation between NEFA and BHBA, as well as between AST and ALT, and a significant negative correlation between glucose and BHBA, as well as between total protein and glucose ($p < 0.05$) (Tab. 2).

In the hematological parameters (RBC, HTC, WBC, HGB, LYM, MON), no statistically significant difference was observed between the twin- and single-pregnant groups ($p > 0.05$). The values of these parameters in the two groups were similar (Tab. 3).

It has been reported that measuring the serum BHBA concentration can be an effective method for monitoring the energy status of pregnant ewes, and values between 0.80 and 1.60 mmol/L show a negative energy balance (12, 18). The blood BHBA concentration in

Tab. 1. Selected biochemical parameters in single- and twin-pregnant ewes

Parameters	Single (n: 15)	Twin (n: 15)	p-values	Reference*
BHBA (mmol/L)	0.474 ± 0.127	0.926 ± 0.490	0.034*	0.30-0.38
Glucose (mg/dl)	63.70 ± 7.573	58.50 ± 20.69	0.63	50-80
Cholesterol (mg/dl)	49.20 ± 9.796	54.60 ± 7.863	0.289	52-76
T. Protein (g/dl)	7.064 ± 0.688	6.464 ± 0.470	0.059	6.0-7.9
ALT (U/L)	27.50 ± 11.769	18.40 ± 7.427	0.037*	22-38
AST (U/L)	137.0 ± 37.318	98.40 ± 32.793	0.019*	60-280
NEFA (mmol/L)	0.370 ± 0.219	0.597 ± 0.261	0.061	0.14-0.28

Explanations: statistics are presented as mean \pm SD; statistical results were considered significant at $p < 0.05$. NEFA – non-esterified fatty acids, BHBA – β -hydroxybutyrate, AST – aspartate aminotransferase, ALT – alanine aminotransferase. *Kaneko et al. (21), Radostits et al. (30)

Tab. 2. Correlations between biochemical parameters in twin-pregnant ewes

		BHBA	Glucose	Cholesterol	T. Protein	ALT	AST	NEFA
Glucose	r	-0.777*						
Cholesterol	r	0.206	0.003					
T. Protein	r	0.477	-0.701*	0.271				
ALT	r	-0.009	-0.305	-0.408	0.223			
AST	r	0.034	-0.378	-0.376	0.267	0.960*		
NEFA	r	0.796*	-0.580	-0.071	0.211	0.009	0.079	

Explanations: statistical results were considered significant at $p < 0.05$; r – Spearman correlation coefficient

Tab. 3. Selected hematological parameters in twin- and single-pregnant ewes

Parameters	Single (n: 15)	Twin (n: 15)	p-values	Reference*
RBC ($\times 10^{12}/L$)	8.04 \pm 0.54	7.82 \pm 0.36	0.101	9-15
HCT (%)	21.41 \pm 2.23	20.34 \pm 1.56	0.088	27-45
HGB (g/L)	109.1 \pm 10.8	105.1 \pm 7.8	0.129	90-150
WBC ($\times 10^9/L$)	11.80 \pm 3.42	12.21 \pm 3.53	0.755	4.0-12.0
LYM ($\times 10^9/L$)	6.77 \pm 2.60	7.11 \pm 2.66	0.662	2.0-9.0
MON ($\times 10^9/L$)	1.13 \pm 0.35	1.29 \pm 0.31	0.086	0.0-0.75

Explanations: statistics are presented as mean \pm SD; statistical results were considered significant at $p < 0.05$. RBC – red blood cells, HCT – haematocrit, HGB – haemoglobin, WBC – white blood cells, LYM – lymphocytes, MON – monocytes. *Meyer and Harvey (25), Radostits et al. (30)

late pregnant and lactating ewes is higher than it is in non-pregnant and non-lactating ewes (36). Raoofi et al. (32) have reported that the blood BHBA concentration was significantly higher in twin-pregnant ewes than in single-pregnant ewes, and there was a negative correlation between BHBA and glucose concentrations. Moallem et al. (26) have reported that, as the number of lambs increases, the BHBA concentration increases. In the current study, the blood BHBA level in twin-pregnant ewes was significantly higher than in single-pregnant ewes ($p < 0.05$). In addition, there was a significant positive correlation between BHBA and NEFA in twin-pregnant ewes ($p < 0.05$) and a significant negative correlation between BHBA and glucose ($p < 0.05$). These results are consistent with those of Anoushepour et al. (3), Duehlmeier et al. (11), and Raoofi et al. (32). Increased BHBA concentrations in twin pregnancy suggest that the animal enters a negative energy balance, carbohydrate deficiency occurs, and the animal is a candidate for ketosis disease.

It has been demonstrated that a negative energy balance causes a severe lipid metabolism impairment in the liver of pregnant ewes, leading to a significant accumulation of NEFA and BHBA in the liver and blood (44). It has been reported that mean NEFA concentrations in the blood of pregnant ewes increase as the birth approaches, and they rise more if the number of offspring is larger (26). It has been reported that the rise in blood NEFA concentrations in the last four weeks of gestation in twin-pregnant ewes is significantly higher than it is in single-pregnant ewes (13). Similarly, in the current study, the blood NEFA level was higher in twin-pregnant ewes than in single-pregnant ewes, although the difference was not significant ($p > 0.05$). Increased NEFA concentrations in ewes carrying a twin pregnancy might be associated with a decreased feed intake due to increased stress and hormonal changes. A negative energy balance changes the insulin/glucagon ratio and destroys fat tissues in the body because of energy production. The mobilization of adipose tissues increases the blood NEFA concentration (32).

Ismael et al. (20) and Taleb et al. (42) have reported that serum glucose levels decrease significantly in late pregnant ewes. On the other hand, while some researchers (15) found no significant difference in

blood glucose levels between ewes in different reproductive periods, some other researchers (19, 23) have reported high glucose levels in late pregnancy. In the present study, the blood glucose level was higher in ewes carrying a twin pregnancy than it was in those bearing a single pregnancy ($p > 0.05$). A significant negative correlation

($p < 0.05$) was found between glucose and total protein in twin-pregnant ewes. It has been reported that hypoglycemia at the end of pregnancy in twin-pregnant ewes was due to sensitivity to a stress-related decrease in the glucose production rate (37). Low glucose levels in twin-pregnant ewes indicate that these animals have a high demand for energy and glucose.

It has been reported that the serum cholesterol level in late pregnancy is significantly higher in twin-pregnant ewes than in those carrying a single pregnancy (5, 31, 35). It has been demonstrated that this increase is due to the decreasing amount of insulin, which plays a direct role in fat metabolism, and a decreased insulin sensitivity of target tissues (38). Some researchers, however, have reported a significant decrease in cholesterol levels in late pregnant ewes (28). Moallem et al. (26) have reported a significant decline in cholesterol levels in twin-pregnant ewes. In the current study, although the cholesterol levels were higher in twin-pregnant ewes compared to those carrying a single pregnancy, the difference was insignificant ($p > 0.05$). Differences between studies might have resulted from differences in nutrition, to which these parameters are directly related.

Significant decreases in serum total protein levels have been reported in late pregnant ewes (14, 20). El-Tarabany (14) has reported a significant decrease in total protein levels of twin-pregnant ewes compared to single-pregnant ewes. Although the difference was not significant, the total protein concentration was lower in twin-pregnant ewes than in single-pregnant ewes in the current study ($p > 0.05$). These results are consistent with the findings of Balıkcı et al. (5). The decrease in serum total protein concentrations in late pregnancy suggests that all proteins required for the rapid growth and muscle development of the offspring during this period are synthesized from the mother's amino acids (34).

It has been reported that as pregnancy progresses in ewes, serum ALT and AST values increase (9, 14). It has been suggested that the increase in these enzymes indicates an increase in liver metabolism and may have resulted from a decrease in dry matter intake in late pregnancy (9). Taghipour et al. (43) have reported that serum AST levels in sheep reach the lowest levels

depending on high corticosteroid levels during late pregnancy and delivery. Lotfollahzadeh et al. (24) and El-Tarabany (14) have reported that ALT and AST values were significantly higher in twin-pregnant ewes than in single-pregnant ewes. On the other hand, some authors have reported that ALT and AST values measured at days 20 and 140 of gestation were statistically insignificantly lower in twin-pregnant ewes than in single-pregnant ewes (6). In the current study, ALT and AST values in both groups were within normal reference ranges (30). ALT and AST values were significantly lower in twin-pregnant ewes than in single-pregnant ewes ($p < 0.05$). These results are different from those of El-Tarabany (14) and Lotfollahzadeh et al. (24) and similar to those of Berlinguer et al. (6). It is thought that the differences between the results might be related to nutrition.

The measurement of hematological parameters is important for determining the physiological and health status of livestock (4). Monitoring the hematological parameters of ewes during pregnancy and lactation improves diagnosis, prognosis, and treatment in sheep breeding and reproduction (33).

Sharma et al. (39) and Soliman (40) have reported that erythrocytes and hemoglobin increase significantly in late pregnancy because of increased oxygen demand and increased metabolic rate. Similarly, Cihan et al. (10) have reported that erythrocyte, hemoglobin, hematocrit, and MCV values increased in the last week of pregnancy. Habibu et al. (17) have reported that hematopoietic tissues produced more erythrocytes to provide oxygen to fetuses in twin pregnancies and that erythrocyte counts were higher in twin-pregnant goats than in single-pregnant ones. Panousis et al. (27) have reported that pregnancy did not alter the parameters except for monocyte and eosinophil values. Khalif et al. (22) have demonstrated that erythrocyte, hemoglobin, and PCV (%) were higher in ewes giving twin births than in those giving single births. Although in the current study, RBC, HGB, and HCT values were lower in twin-pregnant ewes than in single-pregnant ewes, the difference between them was insignificant ($p > 0.05$). The RBC, HGB, and HCT values obtained in the present study were consistent with those found in late pregnant ewes by Cihan et al. (10), but lower than values reported by Khalif et al. (22) and Habibu et al. (17). It is thought that the differences between these studies might have resulted from breed and nutritional differences (10, 39, 40). In addition, the lower numbers of erythrocytes in twin-pregnant ewes compared to single-pregnant ewes might be due to the higher hemodilution occurring in twin-pregnant ewes in late pregnancy.

Bezerra et al. (7) have reported that the total leukocyte count was significantly lower in late-pregnant ewes, while eosinophil, basophil, and monocyte counts remained the same, and lymphocyte numbers decreased. Greguła-Kania et al. (16) have reported

decreasing WBC and lymphocyte counts in ewes due to metabolic stress in advanced pregnancy. Preisler et al. (29) have suggested that a down-regulation in glucocorticoid receptors of lymphocytes and monocytes in the blood is due to increased adrenal cortisol secretion in multiparous cows during calving time, which affects the mechanisms suppressing immunity and increasing susceptibility. Khalif et al. (22) have stated that WBC, as well as lymphocyte, monocyte, and granulocyte counts were higher in ewes giving twins than those with a single birth, but there was no statistical difference between them. WBC, lymphocyte, and monocyte counts in the present study were high in twin-pregnant ewes, similar to those reported by Khalif et al. (22), but there was no statistical difference between the two groups ($p > 0.05$).

In conclusion, we found that twin pregnancy in ewes does not cause significant changes in hematological parameters. However, the high positive correlation between BHBA and NEFA values in twin-pregnant ewes indicates that the negative energy balance has started to affect these animals, and a carbohydrate deficiency has emerged. Therefore, twin-pregnant ewes should be monitored closely for ketosis.

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