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Examination of Blood Cortisol and Some Parameters at Parturition and On 30th Day Postpartum In Single and Twin - Pregnant Ewes

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Abstract

Lambs gain about 70 % of their birth weight in the last 6 weeks of pregnancy. In this period, many physiological events occur in ewe metabolism. The increase in the number of fetuses also affects these events. The aim of this study was to determine the effect of single and twin pregnancy on serum cortisol, glucose, triglyceride, HDL-cholesterol, total protein, total cholesterol, calcium and phosphorus levels at birth and postpartum days. This study was conducted on 2 - 3 aged single (n = 11) and twin (n = 8) pregnant Anatolian Merino ewes which housed the Department of Animal Breeding and Animal Production of Konya Bahri Dağdaş International Agricultural Research Institute in Turkey. Blood samples were collected from the jugular vein immediately after parturition and on 30th day postpartum in single and twin pregnant ewes. According to the data obtained, the differences between single and twin pregnant ewes of serum cortisol and phosphorus values at parturition were found statistically significant. The differences between parturition and 30th day postpartum were found statistically significant for the cortisol and glucose values in single pregnant ewes. The differences between the parturition and 30th day postpartum for glucose, triglyceride and phosphorus values were found statistically significant in twin pregnant ewes. In conclusion, it can be said that cortisol and some blood parameters in ewes are affected by pregnancy and the number of fetuses.

Introduction

Pregnancy is an event that causes changes in cardiovascular, respiratory, gastrointestinal, urogenital central nervous system, blood parameters and immunological functions in animals (Özyurtlu *et al*., 2007). Pregnant ewes are exposed to severe distress on the metabolism, especially on ewes with more than one fetus. It is known that the fetus number affects some blood parameters (Siggurdson, 1988). Lambs gain approximately 70 % of their birth weight in the last 6 weeks of pregnancy (Siggurdson, 1988). Pregnancy can cause physiological stress that increases maternal plasma cortisol (Keller-Wood, 1996; Medan *et al*., 2015). Cortisol is a glucocorticoid hormone that produced by the adrenal cortex in response to stress (Medan *et al*., 2015). In sheep, lambing causes a sharp release of cortisol from the fetal adrenal glands. Cortisol increases estrogen concentration and decreases progesterone concentration. Thus, by stimulating PGF2α release, the level of oxytocin which provides myometrial contractions increases (Nagel *et al*., 2019). Thus, it is

thought that the cortisol level at parturition may be affected in the number of fetus.

Fetal development in pregnant animals causes some metabolic changes due to increased requirements of nutrition (Ismaeel *et al*. 2019). The metabolism of mineral substances plays an important role in the regulation of physiological functions of the periparturient and puerperal period (Atakişi *et al*., 2009). Metabolic problems in ewes generally occur in the periparturient period and these problems affect lipid metabolism (Nazifi *et al*., 2002). In ewes, pregnancy toxemia is the most common metabolic disease in late gestational period. In this period, the increase in the energy needs of the fetus can cause negative energy balance and trigger lipid mobilization (Xue *et al*. 2019). Some studies have reported that the reproductive status in ewes affects blood parameters. (Antunović et al., 2002; Karapehlivan *et al*., 2007; Balıkçı *et al*., 2007). In addition to the reproductive status, the number of fetuses may also affect to blood parameters, especially cortisol. In addition, blood mineral levels are affected in pregnant ewes due to the mineral needs of the fetus (Yıldız *et al*. 2005). In this study, we aimed to determine the effect of fetus number on serum cortisol, glucose, triglyceride, HDL-cholesterol, total protein, total cholesterol, calcium and phosphorus levels in Anatolian Merino ewes at parturition and on the 30th day of postpartum.

Materials and Methods

Animal

This study was conducted on 2 - 3 aged single (n = 11) and twin (n = 8) pregnant Anatolian Merino ewes which housed the Department of Animal Breeding and Animal Production of Konya Bahri Dağdaş International Agricultural Research Institute in Turkey. Except for enterotoxemia, vaccinations and antiparasitic applications are mostly completed before breeding season. Enterotoxemia vaccine administered in the third month of pregnancy. Fetus numbers were determined by ultrasonographic examination at 60^{th} day of pregnancy (DP 50 VET, Mindray Ltd. China). Live weight after shearing of ewes were about 60-65 kg. During the last 45 days of pregnancy, concentrated feed (750 gr) containing 16 % crude protein and 2500 kcal metabolizable energy were used in addition to 500 gr dry alfalfa grass, 250 gr wheat stem, 500 gr corn silage daily for ewes. After parturition, approximately 1000 gr concentrated feed were given in addition to 500 gr dry alfalfa grass, 250 gr wheat stem, 500 gr corn silage. The births occurred in a normal course and there was no dystocia. There was no negativity (trauma, illness, death, unfavorable environmental conditions) until the 30th day postpartum. The lambs stayed in separate compartments with their mothers for 30 days on the postpartum and during this period they were fed only colostrum-breast milk. After this period, in addition to breast milk, ad libitum concentrate feed and roughage were given for lambs and ewes was never milked. This study was approved by the Konya Bahri Dağdaş International Agricultural Research Institute Animal Experiments Local Ethics Committee (30.04.2020-106)

Blood Samples

Blood samples were collected from the jugular vein immediately after parturition (the sheep usually gave birth between 22:00 and 05:00) and on 30th day postpartum (at 14:00) in single and twin pregnant ewes. Blood samples (5 ml) were centrifuged at 5000 g for 5 minutes to obtain serum and stored at - 20 for analysis. Glucose, triglyceride, HDL, total protein, cholesterol, calcium and phosphorus analyzes were performed using Abbott Architect C 8000 brand autoanalyzer (ABD) using colorimetric method. Cortisol analysis was performed with immunoassay analyzer (Abbott Architect i2000, ABD) using the CLIA method (Darwish, 2019). CLIA kits (Abbott Architect-8D15, ABD) were used for analysis. The intra- and interassay CV were less than 10 %.

Statistical Analysis

SPSS 23 statistics program was used to evaluate the obtained data. Paired T-Test was used to compare serum parameters levels between the same ewes data at parturition and postpartum 30th day. Independent-Sample T-Test was used to compare serum parameters of single and twin pregnancies at parturition and postpartum 30th day.

Results

Serum cortisol, glucose, triglyceride, HDL-cholesterol, total protein, total cholesterol, calcium, and phosphorus values of the single and twin pregnant Anatolian merino ewes at parturition and on 30th day postpartum are presented in Table 1. According to these data, the differences of serum cortisol and phosphorus values between single pregnant and twin pregnant obtained at parturition were found statistically significant ($P < 0.05$).

In single pregnant ewes, differences between parturition and 30th day postpartum of cortisol ($P < 0.01$) and glucose ($P < 0.01$) were found statistically significant. In twin pregnant ewes, differences between parturition and 30th day postpartum of glucose ($P < 0.05$), triglyceride ($P < 0.01$) and phosphorus ($P < 0.01$) were found statistically significant.

Discussion

There are important differences in biochemical parameters of animals before and after parturition due to changing physiology (Gürgöze *et al*., 2009). Pregnancy

Table 1. According to parturition and 30th day postpartum serum values in single and twin pregnant ewes

Parameters	Parturition		30th day Postpartum		P values			
					Part.	Post.	Single	Twin
	Single (n = 11)	Twin (n = 8)	Single (n =11)	Twin (n = 8)	S/T	S/T	Part./Post.	Part./Post.
Cortisol (µg / dl)	2.45 ± 1.23	1.05 ± 0.72	0.62 ± 0.82	0.65 ± 0.46	***0.011****	0.920	***0.004****	0.184
Glucose (mg / dl)	129.90 ± 49.1	106.25 ± 57.5	52.81 ± 14.64	58.50 ± 13.16	0.348	0.397	***0.001****	***0.045****
Triglycerid (mg / dl)	17.72 ± 6.00	16.50 ± 4.95	13.00 ± 4.31	12.12 ± 2.23	0.643	0.608	0.074	***0.009****
Cholesterol (mg / dl)	38.73 ± 15.37	32.25 ± 6.67	39.27 ± 8.17	38.00 ± 14.90	0.233	0.814	0.925	0.372
HDL (mg / dl)	21.90 ± 7.63	17.37 ± 3.24	20.63 ± 4.15	21.62 ± 8.65	0.099	0.744	0.648	0.245
Total Protein (g / dl)	4.50 ± 1.78	3.80 ± 1.18	4.75 ± 0.91	4.86 ± 1.21	0.345	0.827	0.730	0.075
Phosphorus (mg / dl)	2.86 ± 0.99	2.06 ± 0.32	3.85 ± 0.72	3.77 ± 0.73	***0.027****	0.818	0.055	***0.001****
Calcium (mg / dl)	6.51 ± 2.18	5.71 ± 1.52	6.35 ± 1.11	7.03 ± 1.35	0.385	0.244	0.849	0.080

*P < 0.05 **P < 0.01 Part.= Parturition; Post.= 30th day Postpartum; S/T= Single/Twin

can increase the level of maternal cortisol by causing physiological stress. In sheep, lambing causes a sharp release of cortisol from the fetal adrenal glands. Cortisol enhances estrogen concentration and decreases progesterone concentration. Thus, by stimulating PGF2α release, the level of oxytocin which provides myometrial contractions increases (Nagel *et al*., 2019). Some studies are reported that there were no significant differences in serum cortisol levels in the prepartum period between single and twin pregnant ewes (Medan *et al*., 2015) before and after pregnancy (Brunet and Sebastian, 1991) in ewes. Drost *et al*. (1973) found that serum cortisol levels of parturition higher than the levels of prepartum period in ewes. In this study, serum cortisol levels obtained from blood taken immediately after parturition were found as 2.45 µg / dl in single-bearing ewes and 1.05 µg / dl in twin-bearing ewes. On the 30th day postpartum serum cortisol levels were found as 0.62 µg / dl for single-bearing ewes and 0.65 µg / dl for twin-bearing ewes. According to these results, serum cortisol levels obtained immediately after parturition were found to be statistically significant ($P < 0.05$) in single-bearing ewes compared to twin-bearing ewes. Besides, the difference between serum cortisol levels at parturition and on 30th day postpartum in single-bearing ewes was found to be statistically significant ($P < 0.01$). The birth weights of single and twin lambs were found as 5.14 kg and 4.25 kg, respectively in this study. In this case, it can be said that the birth weights of single-born lambs are higher than those of twin-born (Roubles *et al*., 2003) and the single-bearing ewes have been exposed to more stress. Thus, the effect of fetal number on maternal cortisol levels can be evaluated as important at parturition.

Blood glucose levels are affected by increasing energy needs during pregnancy (Atakişi *et al*., 2009). Fetal growth and energy consumption of the fetus are mostly related to glucose (Clapp, 2006; Chlumbohm and Harmeyer, 2008). Chlumbohm and Harmeyer (2008) found that serum glucose levels of single-pregnant ewes were higher than twin-pregnant ewes. Fırat (1994) found that glucose levels at parturition were higher statistically than at pregnancy. Kaya (2004) reported that there was no significant fluctuation in serum glucose levels in the late pregnancy, but increased by 109 % vertically at parturition. In our study, serum glucose levels were found in single and twin - pregnant ewes at parturition as 129.90 mg / dl and 106.25 mg / dl, respectively. On the 30th day postpartum, serum glucose levels were found in single and twin - bearing ewes as 52.81 mg / dl and 58.50 mg / dl, respectively. These results are similar to those of Fırat (1994) and Kaya (2004). The increase in plasma glucose level at parturition can be explained by the sudden release of ewes from the hexose requirement for the fetus and the increase in the glucose level or it may reflect an urgent requirement of the ewe for readily available energy (Fırat, 1994).

In pregnant ewes, triglyceride levels are reported as lower in postpartum measurements than in the last three days of pregnancy (Kaya, 2004; Karadaş, 2008). Toker (2004) reported that ewes suffered a significant loss in terms of cholesterol during the last week of pregnancy. Kaya (2004) found that plasma triglyceride levels followed a linear course until parturition and showed a significant decrease postpartum period in their study in ewes. In the other study, it was found that total cholesterol levels decreased to the lowest level on

the 1st day postpartum while the highest was on the 3rd day prepartum (Karadaş, 2008). Nazifi *et al*. (2002) reported that triglyceride, total cholesterol, HDL-cholesterol and VLDL-cholesterol levels were higher in the last week of pregnancy than in other periods of pregnancy in ewes. In our study, serum total cholesterol levels were found in single and twin - bearing ewes at parturition as 38.73 mg / dl and 32.25 mg / dl and in 30th day of postpartum, 39.27 mg / dl and 38.00 mg / dl respectively. Also, serum triglyceride levels were found in single and twin - bearing ewes at parturition as 17.72 mg / dl and 16.50 mg / dl and in 30th postpartum day, 13.00 mg / dl and 12.12 mg / dl respectively. At parturition and in 30th day of postpartum, the difference of serum total cholesterol levels between single and twin - bearing ewes was nonsignificant. There were no significant differences between single and twin - bearing ewes at parturition, whereas differences between serum triglyceride levels between parturition and 30th day postpartum were found significant in twin births. ($P < 0.01$). Karadaş (2008) reported that serum HDL-cholesterol levels fluctuated throughout pregnancy, tended to fall towards the end of pregnancy, and increased again in the postpartum period. In our study, serum HDL-cholesterol levels were found in single and twin - bearing ewes as 21.90 mg / dl, 17.37 mg / dl respectively at parturition, and as 17.37 mg / dl and 21.62 mg/dl respectively in 30th day postpartum. In this study, lower serum HDL-cholesterol levels were obtained than other studies (Nazifi *et al*., 2002; Karadaş, 2008) and there was no significant difference between the groups.

The fetus synthesizes its proteins from the amino acids derived from the mother; proteins are used mainly for synthesis rather than oxidation or gluconeogenesis (Jainudee and Hafez 2000). Balıkçı *et al*. (2007) found a decrease in serum total protein levels on the 150th day of pregnancy, compared to another period of pregnancy in both single and twin pregnant ewes. In the same study, differences in serum total protein values between single and twin - bearing ewes on 45th day postpartum were found statistically insignificant. Kaya (2004) reported that total protein levels in single and twin pregnant ewes were 5.9 g / dl and 5.6 g / dl respectively at parturition. Altiner (2006) found that total protein level as 6.3 g/dl at parturition, while he found 7.1 g / dl in 15th day of postpartum. In our study, at parturition serum total protein levels were found to be 4.50 g/dl for single pregnant ewes and 3.80 g / dl for twin pregnant ewes, besides in 30th day of postpartum were found as 4.75 g / dl and 4.86 g / dl respectively. These values are lower than the other studies (Kaya, 2004; Altiner, 2006).

Retention of calcium, phosphorus and iron increases according to fetal body weight during pregnancy. The fetus has a unique ability to consume the maternal skeleton (Jainudee and Hafez 2000). Roubles *et al*. (2003) reported that serum calcium (Ca) values decreased markedly in the late period of pregnancy, reached the lowest level at parturition and continued to decrease until the 3rd week postpartum in the single and twin - bearing goat. Gürgöze *et al*. (2009) stated that serum Ca values were significantly higher in the postpartum period compared to the peripartum period. On the 150th day of pregnancy, Yıldız *et al*. (2005) found that Ca and P values of single and twin pregnant ewes lower than values of 45th day postpartum. In our study, Ca levels of single and twin pregnant ewes were found to be 6.51 mg / dl and 5.71 mg / dl at parturition, while Ca levels of 30th day postpartum was found to be 6.35 mg / dl and 7.03 mg / dl respectively. As in our study, Özyurtlu *et al*., (2007) found that there was a small increase in serum Ca levels of postpartum compared to the prepartum, but statistically nonsignificant. Phosphorus levels of single and twin pregnant ewes were found to be 3.85 mg / dl and 3.77 mg / dl at parturition, P values of 30th day postpartum were found to be 2.86 mg / dl and 2.06 mg / dl respectively. While the Ca and P levels of parturition were found to be lower than levels of the 30th day postpartum similar to some studies (Roubles *et al*., 2003; Özyurtlu *et al*., 2007), differences of P values between the parturition and 30th days postpartum was found statistically significant ($P < 0.01$) only in twin pregnant ewes. This situation may be an indication that twin pregnant sheep need more calcium and phosphorus during pregnancy.

Conclusion

In conclusion, it can be said that cortisol and some blood parameters in ewes are affected by pregnancy and the number of fetuses. This situation depends on the various mineral needs of the fetus, the energy needs and the stress which the mother is exposed to at parturition. Cortisol levels are higher in ewes carrying single fetus compared to twins. In addition, glucose levels are higher in ewes at parturition compared to the 30th day of postpartum due to the energy need. Phosphorus levels of sheep are affected by the number of fetuses.

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