Veterinary Integrative Sciences 2022; 20(3): 625-633 DOI; 10.12982/VIS.2022.047

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Influence of litter size on the hematologic profile of Awassi ewes during gestation and lactation

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Abstract

Physiological status and litter size affect ewes' hematological parameters, which serve as health indicators. These parameters reflect changes in an animal's physiological condition. Therefore, this study examined the hematological profiles of Awassi ewes with single and twin pregnancies during pregnancy and postpartum. A total of 232 sexually mature and healthy ewes (123 with single pregnancies and 109 with twin pregnancies) between the ages of 3 and 4 years were included in the current study. The sheep's blood was collected and hematological tests were conducted immediately on the samples. Results revealed variation in hematological parameters between ewes during pregnancy based on litter size. Twin-pregnant ewes had significantly higher amounts of RBC, PCV %, MCH, and MCHC during pregnancy months as compared to single-pregnant ewes. Compared to single pregnancies, Awassi ewes with twin pregnancies had higher leukocytes, lymphocytes, granulocytes, lymphocytes %, and granulocytes % than those with a singleton. Logistic regression confirmed that litter size contributes to the increase in hematological parameters. In conclusion, Awassi ewes with twin pregnancies exhibited an increase in the counts of leukocytes and erythrocytes constituents. This study provided valuable information about the association of twin-pregnant ewes with hematological parameters that could be used in sheep breeding and reproduction to improve diagnosis, prognosis, and treatment in the field.

Keywords: Blood profile, Birth type, Pregnancy, Sheep

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Article history; *received manuscript: 23 June 2022,*
revised manuscript: 8 August 2022,
accepted manuscript: 25 August 2022,
published online: 30 August 2022

Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Litter size is the most important factor in determining sheep production economics (Sodiq et al., 2011; Ajafar et al., 2022). A ewe's productivity is measured by litter size, which represents the majority of the income from sheep production (Ekiz et al., 2005; Al-Thuwaini and Kareem, 2022). Bezerra et al. (2017) and Khalif et al. (2020) revealed that the litter size and reproductive stages could affect the hematological profile of non-pregnant ewes and Santa Inês and Morada Nova ewes in a semi-arid environment. Furthermore, hematological characteristics could be used to extract information about the health and physiological status of sheep as well as other animals (Owusu et al., 2016; Al-Thuwaini, 2021); and could be used to determine their physiological condition (Pieragostini et al., 2010; Islam et al., 2018). The physiological status (sex, pregnancy, and lactation) and clinically normal and healthy status of an animal without hematological parameters can have low accuracy and reliability (Al-Samarai and Al-Jbory, 2017). Hematological parameters in sheep and goats show considerable variability depending on the breed, age, sex, physiological state, and genotype of the animal (Oramari et al., 2014; Arfuso et al., 2016; Ahmed et al., 2018; Seixas et al., 2021). Santarosa et al. (2021) discuss the effects of pregnancy-associated physiological changes on maternal hematopoiesis. These changes during late pregnancy affect the birth weight and neonatal viability of the fetus. Ewes in the fourth and fifth months of their pregnancy represent affected periods that have the greatest impact on lamb growth. Kenyon et al. (2019) report that the last 50 days of pregnancy exhibit rapid fetal growth with distinct differences in feed demand among pregnancy ranks. Besides, Pesántez-Pacheco et al. (2019) revealed that the number of fetuses affects dam physiological conditions during late pregnancy. Twin pregnancy affects the hematological and biochemical parameters of ewes (Yenilmez et al., 2021). Habibu et al. (2014) have demonstrated that the hematopoietic system produces more erythrocytes in in twin-pregnant goats to supply oxygen to the fetuses. Khalif et al. (2020) observed that the constitutes of erythrocytes and leukocytes are different according to litter size in non-pregnant ewes.

To the best of our knowledge, the hematological profiles of Awassi ewes during pregnancy and postpartum have not been studied, nor has their fetus number been analyzed. Due to the importance of the Awassi breed for the sheep industry, this study compared the hematological parameters in Awassi ewes during gestation and postpartum between single and twin pregnancies.

MATERIALS AND METHODS

Animals, blood sampling, and hematological examination

The study was conducted on Awassi ewes from July 2021 to April 2022 at the College of Agriculture/Department of Animal Production at Al-Qasim Green University according to international guidelines of animal care and use (Agri, No. 015,7,20). A total of 232 healthy, sexually mature, multiparous ewes aged between 3 and 4 years participated in the study. Expert veterinarians confirmed that the ewes were clinically healthy. Ewes were collected randomly from two stations for raising sheep (Babylon and Karbala), including (123 ewes with single pregnancies and 109 ewes with twin pregnancies) with weights ranging from 40-60 kg. Study participants included ewes in the fourth and fifth months of pregnancy and the month following birth. A single or twin-bearing ewe was identified by ultrasound on day 40 of gestation. Selected ewes were raised under similar management conditions in two stations for raising sheep. Seasonal green grass was provided for the sheep in spring and autumn and housed indoors in winter, and concentrated feed was given to them two times daily in proportion to 2.5% of their weight. Where fitted feed the center of barley with 59% and 40% bran, and salt 1%. Green alfalfa and straw were provided per animal in amounts of 3 kg green alfalfa and 1 kg straw. Drinking water was available all the time. Lambs had left all daytime with their dams for suckling to the weaning age at three months. Ewes during pregnancy and postpartum were weighed in the morning before the supply of feed, and their body condition scores (BCS) were assessed on a scale of 1 (Emaciated) to 5 (Obese).

Blood samples were collected from the external jugular vein of each sheep using a disposable 18-gauge sterile needle and vacutainer tubes containing EDTA. The blood samples were drawn (before morning meal administration) at a single timepoint at 120 and 150 days of gestation and one month after lambing. Immediately after collection, blood samples were cooled at 4 °C and were subjected to a hematology evaluation. Hematological parameters, including erythrocytes and leukocytes, were analyzed in the laboratory using a hematology analyzer (vet.18, mythic company).

Statistical analysis

Statistical programs were used to analyze the data (SPSS for Windows, version 23.0, SPSS Inc., Chicago, IL) using ANOVA-repeated measures to compare the measurements by time. The following model was used; $Yijk = \mu + Li + Pj + pk(j) + eijk$, where μ is the overall mean, Li is the main effect of litter size (single, twin) (fixed w/$\sum$i Li=0), Pj is the main effect of the physiological stage (gestation and postpartum) (fixed w/$\sum$j Pj =0), $pk(j)$ is the main effect of subjects N $(0, \sigma^2 p)$, and eijk is random error assumed $eijk \sim N(0; \sigma^2)$. Comparing means for significant main factors was done using Tukey-Kramer tests. A P-value of 0.05 was used to determine significance. Logistic regression analysis was used to examine the relationship between litter size and hematological parameters. The potential effects of the factor interaction, season (autumn, winter, and spring), and the station were evaluated and discarded when non-significant. The season and the experimental station were included in the model as a covariate.

RESULTS

There were significant differences ($P \leq 0.05$) between the ewes carrying singleton and twins in live body weight and BCS. Ewes carrying twins gained more weight and BCS during pregnancy compared to singleton (Table 1). Based on the litter size and physiological status, hematological changes were investigated in this study. The least-square means of erythrocyte constituents as affected by the litter size and physiological condition are presented in Table 2. The amount of RBC, PCV%, MCH, and MCHC were significantly higher ($P \leq 0.05$) during pregnancy months in Awassi ewes with twin birth than in ewes with a single pregnancy, however, none of the other parameters were significantly different ($P \geq 0.05$).

Table 1 Live body weight and body condition scores (BCS) at pregnancy and postpartum in single and twin pregnancies of Awassi ewes

Indices	Months	Litter size (LSM ± SE)		*P*-value
		Single (123)	Twin (109)	
Live body weight (kg)	4th Month	48.52 ± 2.66[b]	52.00 ± 2.41[a]	**0.04**
	5th Month	52.60 ± 3.76 [b]	55.09 ± 2.64 [a]	**0.03**
	Postpartum	55.00 ± 2.33 [b]	56.74 ± 2.73 [a]	0.12
BCS	4th Month	2.58 ± 0.20[b]	2.65 ± 0.18[a]	**0.04**
	5th Month	2.63 ± 0.17[b]	2.74 ± 0.31[a]	**0.05**
	Postpartum	2.69 ± 0.29[b]	2.72 ± 0.21[a]	0.09

[a,b] Significant differences in means represent differences in the same raw within each classification.

Table 2 The effect of litter size and physiological status on erythrocyte constituents in Awassi ewes

Indices	Months	Litter size (LSM ± SE)		*P*-value
		Single (123)	Twin (109)	
RBC ($\times 10^6/\mu l$)	4th Month	7.12 ± 0.47[b]	8.66 ± 0.41[a]	**0.04**
	5th Month	8.41 ± 0.67[b]	8.89 ± 0.67[a]	**0.03**
	Post-parturition	7.41 ± 0.09[a]	7.52 ± 0.09[a]	0.41
Hb (g/dl)	4th Month	7.61 ± 0.80[a]	8.03 ± 0.38[a]	0.07
	5th Month	7.75 ± 0.67[a]	7.98 ± 0.71[a]	0.16
	Post-parturition	8.18 ± 0.09[a]	8.21 ± 0.91[a]	0.31
PCV (%)	4th Month	27.56 ± 1.36[b]	33.01 ± 1.26[a]	**0.04**
	5th Month	32.00 ± 2.14[a]	36.06 ± 2.01[a]	**0.02**
	Post-parturition	28.73 ± 0.33[a]	28.95 ± 0.91[a]	0.65
MCV (fl)	4th Month	29.83 ± 1.62[a]	30.20 ± 1.11[a]	0.13
	5th Month	29.60 ± 0.41[a]	29.26 ± 0.16[a]	0.65
	Post-parturition	29.26 ± 0.33[a]	29.13 ± 0.33[a]	0.78
MCH (pg)	4th Month	10.30 ± 0.81[b]	11.13 ± 0.73[a]	**0.01**
	5th Month	9.10 ± 0.37[b]	10.83 ± 0.34[a]	**0.05**
	Post-parturition	11.15 ± 0.16[a]	11.00 ± 0.26[a]	0.54
MCHC (g/dl)	4th Month	30.96 ± 0.19[b]	35.51 ± 0.18[a]	**0.01**
	5th Month	30.93 ± 0.75[b]	34.55 ± 0.71[a]	**0.04**
	Post-parturition	34.40 ± 0.93[a]	34.05 ± 0.53[a]	0.79

LSM ± SE, Least square means ± Standard error. [a,b] Significant differences in means represent differences in the same raw within each classification. RBC, red blood corpuscular; Hb, hemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

According to the leukocyte constituents, the Awassi ewes with twin birth showed higher WBC, lymphocyte, granulocytes, lymphocyte%, and granulocytes% during pregnancy months than ewes with single birth, while no statistically significant difference was observed for the other leukocyte profile ($P \geq 0.05$) (Table 3). Univariate regression analysis further explored the association of the litter size with hematological parameters (Table 4).

Table 3 White blood cell composition of Awassi ewes concerning litter size and physiological status

Indices	Months	Litter size (LSM ± SE)		*P-value*
		Single (123)	Twin (109)	
WBCs (×10³/µl)	4th Month	8.81 ± 3.86[b]	12.05 ± 3.26[a]	0.04
	5th Month	7.83 ± 2.82[b]	13.40 ± 3.31[a]	0.002
	Post-parturition	10.37 ± 2.27[a]	11.48 ± 3.90[a]	0.53
Lymphocytes (×10³/µl)	4th Month	6.18 ± 2.70[b]	8.88 ± 2.70[a]	0.02
	5th Month	6.55 ± 1.99[b]	10.26 ± 2.99[a]	0.001
	Post-parturition	6.40 ± 2.89[a]	7.25 ± 1.89[a]	0.61
Monocytes (×10³/µl)	4th Month	0.22 ± 0.001[a]	0.21 ± 0.001[a]	0.13
	5th Month	0.46 ± 0.006[a]	0.41 ± 0.006[a]	0.26
	Post-parturition	0.53 ± 0.008[a]	0.58 ± 0.008[a]	0.55
Granulocytes (×10³/µl)	4th Month	3.38 ± 0.14[b]	4.85 ± 0.26[a]	0.05
	5th Month	3.01 ± 0.49[b]	4.91 ± 0.49[a]	0.05
	Post-parturition	5.85 ± 0.71[a]	5.55 ± 0.71[a]	0.25
Lymphocytes (%)	4th Month	54.81 ± 3.60[b]	73.41 ± 3.29[a]	0.03
	5th Month	59.91 ± 4.14[b]	77.60 ± 3.78[a]	0.04
	Post-parturition	59.60 ± 4.61[a]	63.08 ± 4.20[a]	0.08
Monocytes (%)	4th Month	2.88 ± 0.01[a]	2.91 ± 0.01[a]	0.09
	5th Month	3.31 ± 0.09[a]	2.98 ± 0.09[a]	0.63
	Post-parturition	3.26 ± 0.02[a]	4.01 ± 0.02[a]	0.07
Granulocytes (%)	4th Month	24.28 ± 3.14[b]	35.66 ± 2.13[a]	0.03
	5th Month	25.76 ± 2.56[b]	38.41 ± 2.46[a]	0.005
	Post-parturition	34.25 ± 2.04[a]	37.90 ± 4.04[a]	0.09

LSM ± SE, Least square means ± Standard error. [a,b] Significant differences in means represent differences in the same raw within each classification. WBC, white blood cell.

Table 4 Logistic regression analysis of litter size with hematological parameters in Awassi ewes

Characteristic	Univariate logistic regression		
	Estimate	Odds ratio (95% CI)	*P*-value
RBC (×10⁶/µl)	1.22	3.38 (1.11-6.28)	**0.04**
Hb (g/dl)	1.09	2.97 (0.01-4.96)	**0.01**
PCV (%)	0.42	1.52 (0.07-3.94)	0.07
MCV (fl)	0.29	1.33 (0.27-3.03)	0.56
MCH (pg)	0.18	1.19 (0.11-3.83)	0.33
MCHC (g/dl)	0.47	1.59 (0.21-3.84)	0.39
WBCs (×10³/µl)	1.04	2.82 (1.32-6.05)	**0.007**
Lymphocytes (×10³/µl)	1.05	2.85 (0.14-4.83)	**0.01**
Monocytes (×10³/µl)	0.30	1.34 (0.01-3.34)	0.12
Granulocytes (×10³/µl)	1.05	2.85 (0.11-4.09)	**0.05**
Lymphocytes (%)	1.22	3.38 (0.61-5.62)	**0.01**
Monocytes (%)	0.55	1.73 (0.31-3.69)	0.23
Granulocytes (%)	1.35	3.85 (1.17-5.01)	**0.01**

The *P*-value with statistical significance is indicated in bold numbers; CI: confidence interval. RBC, red blood corpuscular; Hb, hemoglobin; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration, WBC, white blood cell.

DISCUSSION

Body condition score (BCS) is a precise and practical indicator of the nutritional status of a flock, with a strong correlation between ewe body condition and conception rate (Guyoti et al., 2015). Furthermore, body weight and BCS are used widely by farmers and researchers for determining the physiological state of animals (Semakula et al., 2020; Al-Thuwaini and Al-Hadi, 2022). Pesántez-Pacheco et al. (2019) indicate that body weight and body condition score are used widely during mid-pregnancy and late pregnancy; these variables are affected by the number of fetuses and correlate with changes in circulating factors that manage energy metabolism. By evaluating the metabolic status of ewes during these demanding periods, it may be possible to identify more accurate methods for assessing ewe nutrition and thereby optimize performance (Semakula et al., 2020).

Based on the hematology analysis, blood parameters can help determine the return to physiological status (Bezerra et al., 2017). In addition, the hematological analysis provides reliable indicators of animal health and physiological state (Oikonomidis et al., 2018). According to this study, RBC, PCV %, MCH, and MCHC were significantly higher during pregnancy months in Awassi ewes with twin birth than in those with a single pregnancy but remained in the normal range for sheep. The elevated metabolic rate and high oxygen consumption during pregnancy could account for this increase. In addition, these increases are likely the result of the increased volume of red blood cells throughout pregnancy (Bamerny et al., 2022). This variation can also result from the need for oxygen, which triggers an adaptive response that increases hemoglobin concentrations and nutrients in physiological status (Gravena et al. 2010). The physiological condition of the sheep (sex and lactation) affected blood parameters such as RBC, PCV, WBC, Hb, and lymphocyte count, according to Njidda et al. (2014) and Owusu et al. (2016).

Bezerra et al. (2017) showed that the physiological status affected the total red cell count and lymphocyte count. Additionally, twin pregnancy affects hematological and biochemical parameters, which are health indicators among sheep (Yenilmez et al., 2021). According to Khalif et al. (2020), the erythrocyte, hemoglobin, PCV (%), WBC, lymphocyte, monocyte, and granulocyte counts were higher in twin ewes than in single ewes. Compared to pregnancy, the RBCs and PCV decreased during the postpartum period, which is caused by the hemodilution effect of a significant increase in plasma volume and the increasing mobilization of water to the mammary gland via the vascular system (Sharma et al., 2015; Simsek et al., 2015).

In terms of leukocyte composition, twin-birthed Awassi ewes had higher WBC, lymphocytes, granulocytes, lymphocyte %, and granulocyte % during pregnancy than single-birthed ewes. Pregnancy stress might be responsible for this result since it stimulates the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH), which activates the adrenal cortex to release glucocorticoids that induce neutrophil mobilization from the body pool into the peripheral circulation (Soliman, 2014). Total WBC levels in pregnant sheep and goats may rise because of an ACTH-related hormonal stress reaction (Ahmed et al., 2018). These glucocorticoids release bone marrow neutrophil reserves, causing stress-induced leukocytosis (Bamerny et al., 2022). The neutrophil/ lymphocyte ratio also increases according to physiological status and can be used to evaluate stress and physiological conditions in animals (Yaqub et al., 2013; David et al., 2020). Likewise, stress is demonstrated to stimulate the release of colony-stimulating factors and leukocyte-inducing factors. These factors have been demonstrated to enhance the production of blood cells (Bamerny et al., 2022). In addition, the number of lambs exhibits a direct relationship to the level of stress experienced by the dam and its effects on blood hematology. The stress on the ewe is increased when more lambs are born per ewe (Nurlatifah et al., 2022). Comparatively, ewes' postpartum lymphocyte values are significantly lower than their prepartum values. These results may be due to lymphocyte migration into different tissues with neutrophils (Awad et al., 2021). The result of the present study is consistent with the study of Santarosa et al. (2021), who revealed that the Dorper ewes with twin pregnancies exhibited higher erythrogram values and neutrophil: lymphocyte ratio than did ewes with single pregnancies.

CONCLUSIONS

The litter size affected hematological parameters in Awassi ewes during pregnancy months. The number of erythrocyte and leukocyte components in pregnant Awassi ewes with twins increased significantly than those with a single. Determining the effect of litter size during pregnancy on hematological parameters could improve maternally and their kid's health management and use of these tools to improve sheep breeding and reproduction.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the staff of two sheep stations (Babylon and Karbala) for their facilities that supported the Awassi ewe population.

AUTHOR CONTRIBUTIONS

All authors contributed equally.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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How to cite this article;

Ahmed Fadel Kadhem and Tahreer Mohammed Al-Thuwaini. Influence of litter size on the hematologic profile of Awassi ewes during gestation and lactation. Veterinary Integrative Sciences. 2022; 20(3): 625- 633.