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Hematological Parameters as Indicators for Litter size and Pregnancy Stage in Awassi Ewes

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A B S T R A C T

Physiological status and litter size can indeed have a significant impact on ewes' hematological parameters, which are essential indicators of their health. Therefore, this study examined the hematological profiles of ewes during pregnancy with single and twins in the Awassi ewes. The present study involved 232 ewes in good health and at sexual maturity. Among them, 123 ewes had single pregnancies, while 109 ewes had twin pregnancies. The age range of the ewes included in the study was between 3.5 and 4.5 years. Hematological tests were conducted on the sheep's blood samples promptly following collection. The findings demonstrated variations in hematological parameters among pregnant ewes, with differences based on litter size. Ewes carrying twin pregnancies exhibited significantly higher levels of red blood corpuscular, hemoglobin, packed cell volume, and mean corpuscular hemoglobin concentration during pregnancy. In comparison to single-pregnant ewes, Awassi ewes with twin pregnancies displayed elevated counts of white blood cells, lymphocytes, granulocytes, and granulocyte percentage compared to ewes with single pregnancies. Awassi ewes with twin pregnancies also exhibited a strong positive correlation with the leukocytes and erythrocytes constituents. In conclusion, these findings indicate that litter size significantly influences hematological parameters, highlighting the importance of considering the physiological status and litter size as indicators of ewes' health. The findings have practical implications in sheep breeding and reproduction, as they can be utilized to enhance the diagnosis, prognosis, and treatment of related conditions.

Keywords: birth type, blood profile, pregnancy, sheep

INTRODUCTION

Sheep production plays a vital role in the agricultural industry, providing a valuable source of meat, wool, and other by-products. The economic success of sheep farming is influenced by various factors, with litter size emerging as a crucial determinant. It serves as a key indicator of an ewe's productivity and substantially impacts the overall income derived from sheep production. The economic aspect of sheep production is largely influenced by litter size, making it a crucial factor (1, 2). Higher litter sizes increase production levels, leading to higher marketable output. This aspect is particularly important in commercial sheep farming, where maximizing productivity is essential for profitability (3, 4). In addition to the direct economic implications, litter size also influences other aspects of sheep production. It affects the overall reproductive efficiency of the flock, as ewes with larger litter sizes contribute to a faster rate of flock expansion (2). In previous studies, Bezerra et al. (5) and Khalif et al. (6) demonstrated that litter size and reproductive stages affected non-pregnant ewes in semi-arid climates,

specifically in Awassi, Santa Inês, and Morada Nova. These findings underscore the relationship between litter size and hematological characteristics, further emphasizing their relevance in assessing sheep and others animal health and physiological status (7, 8).

Moreover, Sheep farming requires continuous monitoring and assessment of the physiological condition of individual animals to ensure their overall well-being and productivity. Hematological parameters have proven to be valuable indicators in evaluating the physiological status of sheep. By examining various hematological parameters, valuable insights can be obtained regarding the health and well-being of sheep (9, 10).

Hematological parameters in sheep and goats exhibit significant variability influenced by litter size, age, sex, physiological state, and genotype of the animals (11-14). Specifically, twin pregnancy exerts an effect on the blood and biochemical characteristics of ewes (15). Habibu et al. (16) demonstrate increased erythrocyte production in twin-pregnant goats to meet the oxygen demand of the fetuses. Furthermore, Khalif et al. (6) observe variations in the constituents of erythrocytes and leukocytes based on litter size in non-pregnant ewes. These findings collectively emphasize the significance of considering hematological parameters in assessing animals' physiological status and health, particularly in the context of pregnancy and litter size. Santarosa et al. (17) showed the impact of pregnancy-associated physiological changes on maternal hematopoiesis, highlighting that alterations during late pregnancy can affect fetal birth weight and neonatal viability. Notably, the fourth and fifth months of pregnancy are critical periods when the growth of lambs is most affected. Kenyon et al. (18) emphasize the rapid fetal growth during the final 50 days of pregnancy, accompanied by distinct variations in feed demand across different pregnancy stages.

Pesántez-Pacheco and his colleagues (19) also show that litter size influences dam physiological conditions during late pregnancy. To the best of our knowledge, there is a lack of comprehensive research examining the hematological profiles of Awassi ewes during pregnancy, specifically concerning their litter size and physiological status. Given the significance of the Awassi breed within the sheep industry, this study aimed to address this gap by comparing the hematological parameters of Awassi ewes during gestation, specifically focusing on the differences between single and twin pregnancies.

MATERIALS AND METHODS

Animals, Sampling, Hematological Examination

Animal experiments were conducted between July 2021 and April 2022 with the approval of the Institutional Animal Care and Use Committee, Al-Qasim Green University, Babylon, Iraq (Agri, No. 015, 7, 20). A total of 232 sexually mature, multiparous Awassi ewes, aged between 3.5 and 4.5 years, were included in the study. These ewes were determined to be clinically healthy by experienced veterinarians. The selection of ewes was carried out randomly at the Barakat Abu Al-Fadhl Al-Abbas sheep station, a subsidiary of Al Kafeel Company for general investment, located in the Holy Karbala Province. Among the participants, there were 123 ewes with single pregnancies and 109 ewes with twin pregnancies. The weights of the pregnant ewes ranged from 40-60 kg. The study focused on ewes in the fourth and fifth months of pregnancy. Pregnancy status, whether single or twin, was confirmed through an ultrasound on day 40 of gestation. Ultrasonography was performed on all ewes between days 30-100 after mating. Several different ultrasound examinations were performed (transrectal and transabdominal). Brightness mode a real-time linear array, B-mod scanner equipped with a 5 MHz and 7.5 MHz linear array prostate probe (ALOKA Co Ltd, USA). An ultrasound machine with a probe (3.5 MHz) and a laptop connected to the light wave record were used as the second machine. A gel was applied to the probe before the examination for ultrasound transmission. The diameter of the placenta was measured using ultrasonography in single and twin pregnancies, and big-size placentomes were chosen for this study.

All selected ewes were raised under similar management conditions at the sheep-raising station. They were provided seasonal green grass during spring and autumn and housed indoors during winter. Additionally, the ewes received concentrated feed twice daily, amounting to 2.5% of their body weight (20). The feed consisted of 59% barley, 40% bran, and 1% salt. Each animal was given 3 kg of green alfalfa and 1 kg of straw. Access to drinking water was provided at all times.

Blood samples were obtained from the external jugular vein of each sheep using a sterile, disposable 18-gauge needle and vacutainer tubes containing EDTA as an anticoagulant (21-26). The blood collection done at a single time point, specifically at 120 and 150 days of gestation, before administering the morning meal. To preserve sample integrity, the collected blood samples were immediately cooled at 4 °C and promptly subjected to a comprehensive hematology evaluation (27-29). The laboratory analysis involved using a hematology analyzer (Vet.18, Mythic Company) to assess various hematological parameters, including erythrocytes and leukocytes.

Statistical Analysis

The data analysis was performed using statistical software (SPSS for Windows, version 23.0, SPSS Inc., Chicago, IL). ANOVA-repeated measures were employed to compare the measurements across different time points. The following model was utilized for the analysis; $Yijk = \mu + Li + Pj + pk(j) + eijk$, where μ is the overall mean, Li is the main effect of litter size (single, twin) (fixed w/Σi Li=0), Pj

is the main effect of the physiological stage (fixed w/Σj Pj =0), pk(j) is the main effect of subjects N (0, σ^2 p), and eijk is random error assumed eijk ~ N (0; σ^2). Tukey-Kramer tests were employed to compare the means of significant main factors. A significance level of 0.05 was used to determine the presence of statistical significance. The potential impact of factor interactions, season (autumn, winter, and spring), and the station was assessed and disregarded if found to be non-significant. The correlation was analyzed using the Pearson correlation coefficient, and significance was set at $P \leq 0.05$.

RESULTS

This study detected hematological changes concerning litter size and physiological status. Table 1 displays the least-square means of erythrocyte constituents, taking into account the influence of litter size and physiological condition. The measured values for all parameters fall within the expected range for sheep. The analysis revealed that the levels of red blood corpuscular (RBC), hemoglobin (Hb), packed cell volume (PCV %), and mean corpuscular hemoglobin concentration (MCHC) were significantly higher ($P<0.05$) during the pregnancy months in Awassi ewes with twin births compared to those with single pregnancies. However, no significant differences ($P>0.05$) were observed in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH).

The analysis of leukocyte constituents revealed that Awassi ewes with twin births exhibited higher levels of white blood cells, lymphocytes, granulocytes, and granulocyte percentage during the pregnancy months compared to single births. However, no statistically significant difference was observed for the remaining leukocyte constituents ($P>0.05$) (Table 2).

Table 1. The effect of litter size and physiological status on erythrocyte constituents in pregnant Awassi ewes

Indices	Months	Litter size		*P*-value	Normal range of sheep*
		Single (123)	Twin (109)		
RBC (×10⁶/µL)	4th	9.63 ± 0.61 ^{bB}	10.4 ± 0.90 ^{aB}	0.03	9 - 15.8
	5th	10.6 ± 0.65 ^{bA}	11.8 ± 1.07 ^{aA}	0.03	
Hb (g/dL)	4th	9.43 ± 0.82 ^{bB}	10.1 ± 0.57 ^{aB}	0.05	9 - 15
	5th	10.6 ± 0.53 ^{bA}	11.2 ± 0.94 ^{aA}	0.04	
PCV (%)	4th	30.7 ± 2.25 ^{bB}	33.2 ± 2.16 ^{aB}	0.01	27 - 45
	5th	33.2 ± 1.41 ^{bA}	36.3 ± 1.35 ^{aA}	0.02	
MCV (fl)	4th	29.7 ± 1.52	30.0 ± 1.54	0.11	28 - 40
	5th	29.6 ± 1.63	29.4 ± 1.19	0.43	
MCH (pg)	4th	10.1 ± 1.05	10.3 ± 0.94	0.29	8 - 12
	5th	9.43 ± 0.62	9.81 ± 0.84	0.34	
MCHC (g/dL)	4th	31.3 ± 2.39 ^{b}	32.1 ± 1.18 ^{a}	0.04	31 - 34
	5th	31.6 ± 2.19 ^{b}	34.1 ± 2.26 ^{a}	0.03	

The values represented the LSM ± SE, Least square means ± Standard error. ^{a,b} Significant differences in means represent differences in the same row within each classification. $^{A, B}$ Different capital letters indicate a significant difference in the same column 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, *Adapted from: Research Animal Resources [RAR] (28)

Table 2. White blood cell composition of Awassi ewes concerning litter size and physiological status

Indices	Months	Litter size		*P*-value	Normal range of sheep*
		Single (123)	Twin (109)		
WBCs (×10³/µL)	4th	9.27 ± 1.44 ^{bB}	11.8 ± 1.67 ^{aB}	0.02	4 - 12
	5th	10.7 ± 1.64 ^{bA}	12.7 ± 1.26 ^{aA}	0.03	
Lymphocytes (×10³/µL)	4th	6.39 ± 0.44 ^{bB}	7.96 ± 0.74 ^{aB}	0.02	2 - 9
	5th	7.51 ± 0.73 ^{bA}	10.4 ± 0.63 ^{aA}	0.02	
Monocytes (×10³/µL)	4th	0.24 ± 0.04	0.25 ± 0.04	0.42	0 - 0.8
	5th	0.42 ± 0.03	0.41 ± 0.05	0.31	
Granulocytes (×10³/µL)	4th	3.45 ± 0.84 ^{b}	4.81 ± 1.11 ^{a}	0.05	0.7 – 6.0
	5th	3.14 ± 0.55 ^{b}	4.90 ± 1.09 ^{a}	0.02	
Lymphocytes (%)	4th	72.5 ± 2.68	73.5 ± 3.11	0.18	40 - 70
	5th	76.7 ± 3.77	77.7 ± 4.06	0.29	
Monocytes (%)	4th	3.84 ± 0.26	3.71 ± 0.30	0.30	2 - 9
	5th	3.11 ± 0.31	3.01 ± 0.21	0.41	
Granulocytes (%)	4th	25.2 ± 3.98 ^{bB}	34.5 ± 3.01 ^{aB}	0.01	10 - 63
	5th	28.4 ± 3.51 ^{bA}	37.4 ± 2.54 ^{aA}	0.001	

The values represented the LSM ± SE, Least square means ± Standard error. ^{a,b} Significant differences in means represent differences in the same row within each classification. $^{A, B}$ different capital letters indicate a significant difference in the same column within each classification. WBC, white blood cell, *Adapted from: Research Animal Resources [RAR] (28)

The correlation coefficient between birth types and physiological status with phenotypic traits of the Awassi ewes is shown in Tables 3 and 4. A strongly positive correlation ($P<0.01$) was recorded among ewe with twin pregnancies during pregnancy months with phenotypic traits of the Awassi ewes.

Table 3. Correlation between birth type and blood variables in pregnant Awassi ewes

	Birth type			
	Single pregnancy		Twin pregnancy	
Variables	***r***	***P*-value**	***r***	***P*-value**
RBC (×10⁶/µL)	0.40	0.03	0.84	0.04
Hb (g/dL)	0.30	0.001	0.73	0.02
PCV (%)	0.48	0.002	0.81	0.04
MCV (fl)	-0.14	0.19	0.26	0.12
MCH (pg)	0.50	0.001	0.70	0.001
MCHC (g/dL)	0.51	0.001	0.88	0.002
WBCs (×10³/µL)	0.71	0.001	0.87	0.001
Lymphocytes(×10³/µL)	0.93	0.001	0.98	0.001
Monocytes (×10³/µL)	0.55	0.001	0.88	0.001
Granulocytes (×10³/µL)	0.32	0.001	0.74	0.01
Lymphocytes (%)	0.40	0.003	0.45	0.01
Monocytes (%)	0.12	0.43	-0.07	0.67
Granulocytes (%)	0.42	0.002	0.47	0.01

$P \leq 0.05$: Significant, $P \geq 0.05$: Not significant, r: Pearson correlation coefficients

Table 4. Correlation between physiological status and blood variables in pregnant Awassi ewes

	Physiological status			
	4th month		5th month	
Variables	***r***	***P*-value**	***r***	***P*-value**
RBC (×10⁶/µL)	0.62	0.02	0.70	0.001
Hb (g/dL)	0.96	0.001	0.71	0.001
PCV (%)	0.84	0.04	0.72	0.001
MCV (fl)	0.43	0.18	0.37	0.080
MCH (pg)	0.61	0.004	0.52	0.002
MCHC (g/dL)	0.78	0.003	0.66	0.030
WBCs (×10³/µL)	0.91	0.001	0.89	0.001
Lymphocytes(×10³/µL)	0.98	0.001	0.96	0.001
Monocytes (×10³/µL)	0.88	0.001	0.81	0.001
Granulocytes (×10³/µL)	0.76	0.001	0.65	0.001
Lymphocytes (%)	0.49	0.003	0.34	0.060
Monocytes (%)	0.11	0.50	0.39	0.030
Granulocytes (%)	0.51	0.002	0.37	0.040

$P \leq 0.05$: Significant, $P \geq 0.05$: Not significant, r: Pearson correlation coefficients

DISCUSSION

Hematological parameters are important indicators of overall health and physiological well-being in animals (31). In this study, comparing blood parameters between ewes with single and twin births sheds light on the influence of litter size on pregnancy-related hematological parameters. The significant differences observed in erythrocyte constituents, such as RBC, Hb, PCV%, and MCHC, between the two groups indicate that litter size can impact the red blood cell parameters during the pregnancy months, despite remaining within acceptable limits. Awassi ewes with twin births displayed higher levels of these parameters than those with single births, suggesting an increased demand for oxygen-carrying capacity and potentially higher metabolic activity in twin-pregnant ewes. Furthermore, the higher volume of red blood cells throughout pregnancy may contribute to these observed variations (32). These changes can also be linked to the adaptive response triggered by the need for oxygen, resulting in increased hemoglobin concentrations and nutrient levels during the physiological state (33).

Furthermore, the leukocyte constituents also demonstrated interesting trends concerning litter size. Awassi ewes with twin births exhibited higher levels of white blood cells, lymphocytes, granulocytes, and granulocyte percentage during the pregnancy months than those with single births. This could indicate boosting immune response and potential physiological adjustments for carrying multiple fetuses. Bezerra et al. (5) demonstrated that physiological status impacts the total red cell and lymphocyte counts. Furthermore, twin pregnancy has been found to affect hematological and biochemical parameters, which serve as indicators of health in sheep (15). According to Khalif et al. (6), twin-bearing ewes exhibited higher counts of erythrocytes, hemoglobin, packed cell volume (PCV%), white blood cells (WBC), lymphocytes, monocytes, and granulocytes compared to single-bearing ewes.

The increase in total white blood cell count during pregnancy in sheep and goats can be attributed to the hormonal stress response mediated by adrenocorticotropic hormone (ACTH) (13). The adrenal cortex then releases ACTH, hormones that have various effects on the body, including the mobilization of neutrophils from their reservoirs within the body to the peripheral circulation. The release of ACTH from the adrenal cortex leads to the depletion of neutrophil reserves in the bone marrow,

resulting in stress-induced leukocytosis (34). Stress also stimulates the release of colony-stimulating and leukocyte-inducing factors, which promote the production of blood cells (32). Moreover, the number of lambs born per ewe directly correlates with the stress level experienced by the dam, influencing blood hematology (35). These findings align with the study by Santarosa et al. (17), which observed higher erythrogram values and a higher neutrophil-to-lymphocyte ratio in Dorper ewes with twin pregnancies compared to those with single pregnancies. Further studies and longitudinal observations are warranted to explore the long-term effects and implications of these hematological changes on the health and productivity of Awassi ewes. By evaluating the hematological profile, veterinarians and breeders can gain insights into ewes' overall health, recovery, and adaptation following parturition. It is imperative to interpret blood parameters alongside other clinical assessments and considerations specific to the breed and environmental factors.

In conclusion, the study emphasizes the importance of considering the physiological status and litter size when evaluating hematological parameters in ewes. Hematological parameters differ by litter size during pregnancy, emphasizing the importance of considering the impact of multiple pregnancies. Such insights can contribute to better management strategies, early detection of health issues, and improve overall care for ewes in the sheep industry. The findings contribute to our understanding of the hematological changes associated with pregnancy and shed light on Awassi ewes' health based on litter size. Further research and monitoring of these parameters are warranted to enhance our knowledge and promote better management practices in the sheep industry.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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التحري عن العلاقة بين المعايير الدموية وعدد المواليد في النعاج العواسية

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الخلاصة

الحالة الفسلجية وعدد المواليد ممكن ان يكون لها تأثير معنوي على معايير الدم للأغنام ،التي هي مؤشرات اساسية لصحتها. لذلك، هذه الدراسة فحصت الصورة الدمّة للنعاج ذات الحمل المفرد والتؤام خلال الحمل في النعاج العواسية. تضمنت الدراسة الحالية ٢٣٢ نعجة بصحة جيدة وناضجة جنسيا. صنفت ١٢٣ نعجة ذات حمل مفرد، بينما ١٠٩ من النعاج كانت حامل بتؤام. يتراوح أعمار النعاج المتضمنة في الدراسة ما بين ٣,٥-٤,٥ سنة. أجريت فحوصات الدم على عينات الدم المجموعة من الأغنام، أظهرت النتائج اختلافات بين النعاج الحوامل، وهذه الاختلافات اعتمدت على عدد المواليد. اظهرت النعاج التي تحمل توائم فرقا معنويا ($P \leq 0.05$) بمستوى اعلى لكريات الدم الحمراء، الهيموغلوبين، حجم مكداس الدم، ومعدل تركيز هيموغلوبين الكرية خلال الحمل. أظهرت النعاج الحامل بتوائم زيادة في عدد خلايا الدم البيض، الخلايا اللمفية، الخلايا الحبيبية والنسبة المئوية للخلايا الحبيبية مقارنة بالنعاج ذات الحمل المفرد. بالإضافة الى ذلك، أظهرت النعاج العواسية ذات الحمل التؤام ارتباط إيجابي قوي ($P \leq 0.05$) مع مكونات كريات الدم الحمراء وخلايا الدم البيضاء. في الاستنتاج، تشير هذه النتائج ان لعدد المواليد تأثير معنوي على معايير الدم، مسلطا الضوء على أهمية اعتبار الحالة الفسلجية وعدد المواليد مؤشرات على صحة النعاج. النتائج لها تطبيقات على تربية وتناسل الاغنام، والتي يمكن الاستفادة منها في التكهن، التشخيص، وعلاج الحالات المقاربة.

الكلمات المفتاحية: عدد المواليد، صورة الدم، الحمل، الاغنام