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DYNAMICS OF HEMATOBIOCHEMICAL AND CLINICAL PATTERNS IN EWES IN DIFFERENT REPRODUCTIVE STAGES UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

Asmaa Rabea[1], Yassin Mahmoud Abdelraof[1], Heba Mohamed El-khaiat[1],
Mohammed Abdelhameed Mohammed Kamal[2]* and Mahmoud Atef Youssef Helal[1]*

[1]Department of Animal Medicine, Faculty of Veterinary Medicine, Banha University, Egypt 13511
[2]Department of Veterinary Hygiene and Management, Faculty of Veterinary Medicine, Cairo University, Egypt 12211

***Corresponding author:** mohamed.a.kamal@cu.edu.eg (MAMK); mahmoud.halal@fvtm.bu.edu.eg (MAYH)

ABSTRACT

The study aimed to investigate the clinical patterns and blood profile dynamics in ewes across different reproductive stages. The research involved analyzing clinical patterns, hematological parameters, and various serum parameters from nonpregnancy to postpartum ewes, as well as mid-pregnancy ewes categorized by age (young vs. old) and litter size (single vs. twin). Analysis of serum minerals demonstrated notable changes in the levels of calcium, phosphorus, magnesium, copper, zinc, and selenium across the reproductive cycle. Calcium decreased significantly during late pregnancy (7.11±0.39mg/dL) and postpartum (9.69±0.06mg/dL), phosphorus remained consistent except for a decrease in late pregnancy (4.15±0.36mg/dL), and magnesium declined gradually from nonpregnancy to postpartum. Similarly, the examination of serum leptin, vitamin D, glucose, and triglycerides provided valuable insights into metabolic status and energy homeostasis. Leptin showed a significant increase during pregnancy (5.40±0.31, 5.80±0.06, 6.33±0.09ng/dL). The evaluation of hematological parameters revealed dynamic variations in RBC, Hb, HCT, WBC, lymphocyte, neutrophil, and MID levels throughout the reproductive stages. Additionally, the analysis of clinical patterns including body temperature, pulse rate, respiratory rate, and ruminal movement showed the adaptive changes occurring in response to pregnancy. Furthermore, comparisons between young vs. old age groups and single vs. twin lamb pregnancies indicated limited effects of age and litter size on the measured parameters. In conclusion, this research provides comprehensive insights that contribute to a better understanding of ewe health, reproductive performance, and metabolic adaptations, offering valuable information for effective management strategies, mineral supplementation, and overall care during different reproductive stages.

***Keywords*:** Blood parameters, Clinical pattern, Ewes, Hematology, Serum biochemistry

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1. INTRODUCTION

The transition period in ewes under controlled environmental conditions, occurring approximately 4 weeks before and after lambing, is a physiologically demanding critical phase in ovine life. This period is marked by noteworthy metabolic and immunological hurdles as the ewe's body strives to meet the increased demands placed upon it. During the last 4-6 weeks of pregnancy, approximately 75-80% of fetal growth takes place, which can lead to notable complications in productivity and reproduction (El-Sayed et al. 2020).

Improving lamb numbers can be achieved through nutritional interventions in ewes, offering the potential for a sustainable meat supply. Ensuring that the reproductive process and overall health of the ewe are maintained during this critical period is vital for the long-term success of sheep production. Inadequate nutrition often leads to reproductive failures in ewes, resulting in lower-than-expected quantities and quality of offspring (Nurlatifah et al. 2022). Micronutrients, specifically minerals, play a crucial role in preserving ewe health by supporting antioxidant defenses against tissue damage caused by free radicals. Selenium, copper, and zinc are essential elements for the body's antioxidative mechanisms (Razavi et al. 2023). When comparing mineral levels in ewes' blood from nonpregnancy to postpartum, it is essential to consider the specific minerals of interest. Calcium and phosphorus are essential for proper bone development, milk production, and muscle function. During the transition from nonpregnancy

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to postpartum, blood calcium levels may decrease due to the high demand for calcium during fetal skeletal development and milk production. Conversely, phosphorus levels might increase to support the increased metabolic needs of the ewe (Antunović et al. 2021). Magnesium is involved in various enzymatic reactions and plays a crucial role in muscle function and energy metabolism. Blood magnesium levels may fluctuate during the transition period, potentially influenced by dietary intake and hormonal changes. Monitoring mineral levels through blood tests and adjusting dietary supplementation accordingly can help maintain proper mineral balance and support ewe health and productivity during the transition period (Singh et al. 2022).

The well-being of the pregnant ewe is greatly influenced by her nutritional status. Evaluating blood hematology provides valuable insights into the ewe's health status (Nurlatifah et al. 2022). Pregnancy induces changes in hematological parameters, which can be attributed to factors such as increased energy requirements for fetal development and lactogenesis, as well as hormonal and metabolic alterations associated with preparing the body for parturition and lactation (Greguła-Kania et al. 2020).

In addition, hematological parameters serve as valuable indicators for assessing the nutritional status, overall health, and adaptability of animals. During the initial week following parturition, these parameters can reflect the organism's return to a state of homeostasis (Greguła-Kania et al. 2020). In sheep, the levels of hemoglobin and packed cell volume (PCV) typically show an increase during late gestation, then drop before lambing and decrease further in the initial stages of milk production (Karaşahin et al. 2023). This pattern can be attributed to the hormonal stress response mediated by ACTH. Stress and neuroendocrine changes directly influence the counts of neutrophils and lymphocytes during the prepartum period (Nurlatifah et al. 2022).

Leptin, which is known as the hormone responsible for energy expenditure, interacts with specific brain regions that play a role in regulating energy metabolism. During the transition period, leptin is involved in coordinating metabolism by controlling feed intake and energy storage. When leptin levels are low or absent in the bloodstream during this period, it serves as a signal to the central nervous system that there is an energy deficiency in the body. Consequently, various adaptations occur, such as a decline in reproductive and immune capabilities and an augmentation in metabolic augmentation (El-Sayed et al. 2020).

Except for newborns, domestic animals typically have upper critical temperatures ranging from 25–30°C, while summer temperatures often surpass 40°C for extended durations. The thermoneutral zone is influenced by both internal and external factors, with internal factors such as genotype, species, and physiological status (e.g., pregnancy) and external factors such as relative humidity, ambient air velocity, and solar radiation level playing a role (Goma and Phillips 2022). In experimental trials, the ideal controlled climatic conditions for sheep have been established as an ambient temperature ranging from 13 to 20°C, wind speed between 5 to 18km/hr, relative humidity in the range of 55 to 65%, and a moderate level of illumination (Chauhan et al. 2021). Sheep performance and reproduction are adversely affected by any alterations in climatic conditions (Godde et al. 2021).

When comparing different changes in clinical patterns and blood parameters in ewes from nonpregnancy to postpartum, it is essential to consider that these changes are influenced by factors such as fetal growth, lactation and hormonal fluctuations. The current study was conducted to understand these changes and implement appropriate management strategies, mineral supplementation, and overall care to address any imbalances or deficiencies that can contribute to the overall health and reproductive success of ewes during this critical period.

2. MATERIALS AND METHODS

2.1. Ethical Approval

All examinations were performed after approval of the Ethics Committee of Benha University with approval number BUFVTM09052023 and Cairo University with approval number Vet CU 09092023799.

2.2. Study Design

This research employed a randomized prospective observational and followed cohort design for a duration of six months (from November to April) to compare blood parameters (hematological and biochemical) and clinical patterns in different groups of ewes. The study was conducted at a commercial farm in Kalubia, Egypt, which housed a total flock of 100 healthy meat Baladi ewes (mature and maiden). Sheep were kept indoors but had access to the outdoors, exposing them to natural seasonal variations in photoperiod, temperature, and relative humidity. Extreme climatic conditions were controlled indoors through shading, windows, fans, and heaters.

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2.3. Sample Size Calculation

The size of the sample was determined according to the available population of ewes on the farm. A total of 39 healthy Baladi meat ewes were included in the study, isolated in separate pens under controlled environmental conditions, and distributed across four groups. Each enclosure was furnished with straw bedding, temperature regulation, distinct ventilation setups, and hand-operated feed dispensers.

2.4. Sample Population

The parameters observed and employed for the classification and comparison of sheep were as follows: age: young multiparous (1-2 years) vs. old multiparous (7-10 years) (Chen et al. 2008), litter size: single vs. twin (confirmed via ultrasonography and validated postlambing). The study included four groups of ewes. Group (1): 21 single young ewes, Group (2): 6 twin young ewes, Group (3): 6 single old ewes, Group (4): 6 nonpregnant ewes.

2.5. Animal Management and Farm Environment

All ewes in the study were managed under similar conditions at the facility with environmental manipulation to avoid both cold and heat stress. The intensity of heat stress primarily relies on dry bulb temperature and RH% content, which can be amalgamated into a THI, or temperature–humidity index (Goma and Phillips 2022). Marai et al. adapted the THI equation (in °C) for sheep and goats as follows: $THI = db°C - \{(0.31 - 0.31\ RH)\ (db°C - 14.4)\}$, where db°C represents the dry bulb temperature (°C), and RH signifies the relative humidity (RH%)/100. According to this revised formula, the optimal THI for sheep and goats in Egyptian conditions was determined as follows: <22.2 = no heat stress, 22.2 to <23.3 = mild heat stress, 23.3 to <25.6 = significant heat stress, and above 25.6 indicates extremely severe heat stress (Table 1) (Marai et al. 2001). Ewes were housed in shaded, semiopen quarters and fed concentrate (cottonseed cake, corn, wheat bran, soya bean meal) in addition to green fodder (green herbage, grass, berseem, and darawa), a well-balanced ration according to NRC. Each sheep was given 250gm of concentrate twice daily at two months of pregnancy and 375gm twice daily during the final four weeks (NRC 1985, El-Sayed et al. 2020), while water was always available *ad libitum*. Ewes were provided with veterinary care according to standard management practices to ensure their welfare and health.

Table 1: Descriptive statistics (Maximum-Minimum) of temperature, Relative humidity (RH%), air velocity (AV m/s), and temperature–humidity index (THI) during the study 6 months in the farm outdoor and indoor climates

	November		December		January	
	Outdoor	Indoor	Outdoor	Indoor	Outdoor	Indoor
Temp (°C)	24.6-16.9	24.9-18.5	22.3-12.9	23.9-14.6	20.3-11.7	23.9-15.5
RH%	78.3-35.2	80.9-36.1	79.5-37.9	76.5-35.0	78.4-34.7	72.8-30.5
AV (m/s)	18.9-0.9	6.1-0.1	19.0-1.0	5.9-0.2	20.0-1.5	6.0-0.3
THI	22.6-16.8	22.8-18.3	20.7-13.1	21.9-14.6	19.1-11.8	21.8-15.3
	February		March		April	
Temp (°C)	18.5-10.8	23.6-15.9	24.8-14.9	25.7-19.5	27.9-16.8	24.7-17.8
RH%	76.0-38.5	66.8-28.9	73.7-25.6	69.4-20.9	71.2-22.8	84.2-36.0
AV (m/s)	22.3-2.1	6.4-0.6	28.9-1.9	9.0-0.3	25.6-4.9	8.7-1.7
THI	17.7-11.0	21.5-15.7	22.3-14.8	22.9-19.0	24.6-16.6	22.6-17.6

2.6. Blood Parameters

Blood samples were collected via jugular venipuncture (5mL was transferred into standard 10mL EDTA vacuum tubes (Vacutainer® System Europe; Becton Dickinson, France) and another 5mL into a standard 10mL plain tube) from each ewe using aseptic techniques. Samples were collected at a specific time point determined based on the stage of pregnancy or nonpregnancy for each group (Pesántez-Pacheco et al. 2019). Samples were collected every 45 days of pregnancy (first samples on the 45th day of early pregnancy, then on the 90th day of mid-pregnancy, 135th day of late pregnancy, and then on the 30th day postpartum).

2.7. Biochemical Parameters

A blood sample was obtained in a plain tube and then subjected to centrifugation at 3000rpm for 15min to separate the serum, which was subsequently stored at -20°C for future biochemical analysis. The serum biochemical parameters, including cholesterol and triglycerides (provided by Chronolab Chemicals, Barcelona, Spain) and magnesium (supplied by BioMed, Egypt), were analyzed spectrophotometrically using commercial test kits following

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the standard protocols provided by the suppliers. Leptin analysis was conducted utilizing a solid phase double antibody sheep leptin ELISA test kit provided by SinoGene- clon (Hangzhou, China) (Krawczyńska et al. 2022). Glucose levels in mg/dL were determined spectrophotometrically using specialized kits, following the method described by Barham and Trinder (1972). Calcium levels in mg/dL were measured spectrophotometrically using specialized kits following the method outlined by Rifai (2017). Phosphorus levels in mg/dL were determined spectrophotometrically using specialized kits, following the manufacturer's instructions as outlined by Young et al. (1975). Serum 25-hydroxyvitamin D3 (25(OH)D3) levels were examined following extraction with cyclohexane/ethyl acetate and subsequent separation on Sep-pak silica cartridges (Waters Associates, Northwich, Cheshire). This analysis was conducted using the radioimmunoassay method described by Clemens et al. (1979). To ascertain the levels of trace minerals in the serum, serum samples were subjected to digestion using a mixture of perchloric and nitric acid (in a 3:7 ratio). Copper, selenium, and zinc concentrations were quantified utilizing an atomic absorption spectrophotometer (Shimadzu Asc- 6100, Japan) (Bouman et al. 1986). The results for serum trace mineral levels were reported in µmol/L of serum.

2.8. Hematological Parameters

Blood samples were collected in vacutainer tubes containing EDTA to evaluate the complete blood picture. This analysis was conducted using an automated blood cell counter (Exigo– Veterinary Hematology system, Boule Medical AB, Sweden). The blood picture encompasses various parameters, including hemoglobin concentration (Hb), total erythrocyte count (RBCs), hematocrit test (Hct), mean corpuscular volume (MCV), and total and differential leukocyte count (WBC). These measurements were conducted using a Hematology Analyzer (Jain 1993).

2.9. Clinical Patterns

The examined ewes had complete clinical examinations in accordance with the standard protocols (Constable et al. 2016). Clinical observations were recorded for each ewe, including body condition score, appetite, rectal temperature (°C), respiratory rate (respiratory cycle/min), pulse rate (beats/min), ruminal movement /2min, and any signs of illness or abnormal behavior. We conducted these observations at regular intervals throughout the study period.

2.10. Statistical Analysis

Data and results were collected and computed using Microsoft Excel 2016. To analyze the data, we utilized the Statistical Package for Social Sciences software, specifically version 25.0 (SPSS Inc., Chicago, IL). We computed descriptive statistics, including means and standard errors, for each group and parameter. To compare blood parameters and clinical patterns among the various groups, we employed a one-way analysis of variance (ANOVA). Post hoc tests, such as Tukey's or Dunn's test, were performed for pairwise comparisons if significant differences were detected.

3. RESULTS

The results present the findings of the comparative analysis of serum levels of various biochemical parameters, hematological parameters, and clinical patterns from nonpregnancy to postpartum ewes, then, categorized by age (young and old) and litter size (single and twin). This section provides a comprehensive overview of the observed differences and variations among the different stages of pregnancy and postpartum, as well as the influence of age and litter size on the measured parameters.

3.1. Serum Mineral Levels

The investigation of serum mineral levels (Table 2) focused on key elements such as calcium, phosphorus, magnesium, copper, zinc, and selenium. The comparative analysis provides insights into the fluctuations in serum mineral concentrations during the different stages of pregnancy and postpartum, highlighting potential variations in mineral metabolism and requirements. Table 2 shows a significant ($P<0.05$) difference in the serum mineral levels observed in the different stages.

3.2. Serum Leptin, Vitamin D, Glucose, and Triglycerides

The assessment of serum levels of leptin, vitamin D, glucose, and triglycerides offers valuable information regarding metabolic status and energy homeostasis in ewes during the reproductive cycle. These parameters are closely linked to nutrient utilization, energy balance, and overall metabolic health. The comparative analysis

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provides insights into the variations in these parameters, indicating potential adaptations to meet the energy demands associated with pregnancy, lactation, and postpartum recovery. Table 2 shows a significant (P<0.05) difference in their serum levels observed in the different stages.

Table 2: Assessment of serum biochemical parameters from nonpregnancy to postpartum with their significant difference

Parameters	Units	Control	45th day EP	90th day MP	135th day LP	30th day PP	Sig.
Calcium	mg/dL	9.70±0.15a	8.08±0.04b	7.79±0.27c	7.11±0.39d	9.69±0.06a	0.000
Phosphorus	mg/dL	5.51±0.14a	5.00±0.16a	5.01±0.18a	4.15±0.36b	5.47±0.12a	0.007
Magnesium	ppm	2.84±0.16a	2.48±0.13b	2.21±0.06c	2.05±0.04d	2.34±0.06e	0.003
Leptin	ng/dL	2.78±0.11a	5.40±0.31b	5.80±0.06c	6.33±0.09d	2.30±0.12a	0.000
Vitamin D	nmol/L	12.12±0.19a	9.50±0.17b	7.74±0.29c	6.10±0.23d	10.20±0.12e	0.000
Glucose	mg/dL	86.33±1.86a	77.10±1.03b	70.32±0.55c	66.03±3.07d	86.78±0.12a	0.000
Triglycerides	mg/dL	46.15±2.31a	79.33±4.98b	75.18±1.09c	83.74±2.90d	72.00±0.06e	0.000
Copper	ppm	107.22±1.12a	138.27±7.82b	130.40±1.45c	127.72±6.17d	107.00±0.06a	0.002
Zinc	ppm	137.94±8.56a	130.89±0.59a	141.50±0.29a	150.99±0.30b	130.11±0.06a	0.018
Selenium	ppb	62.67±1.48	34.50±14.65	43.57±0.38	40.97±0.63	33.00±0.06	0.060

Mean±SE values within the rows bearing different letters are significantly different (P<0.05). Control (Non-Pregnant), EP=Early Pregnancy; MP=Mid Pregnancy; LP=Late Pregnancy; PP=Postpartum; Sig=Significance.

3.3. Hematological Parameters

The analysis of hematological parameters (Table 3) sheds light on the alterations in blood composition during the different reproductive stages. We examined parameters such as total erythrocyte count (RBCs), hemoglobin concentration (Hb), hematocrit test (Hct), mean corpuscular volume (MCV), total leukocyte count (WBC), and differential leukocyte count (neutrophils, lymphocytes). The results elucidate the hematological adaptations occurring in response to the energy demands, hormonal changes, and immune system dynamics characteristic of each reproductive stage. Table 3 shows a significant (P<0.05) difference in the hematological parameters observed in the different stages.

Table 3: Comparative hematological parameters analysis in ewes at different pregnancy stages compared to nonpregnancy and postpartum with their significant difference

Parameters	Units	Control	45th day EP	90th day MP	135th day LP	30th day PP	Sig.
RBCs	10^{12}/L	6.96±0.48a	6.45±0.14a	5.44±0.32bc	4.75±0.11c	6.23±0.17ab	0.002
Hb	g/dL	12.10±0.68ab	12.37±0.94b	10.53±0.19ac	9.23±0.32c	8.77±0.38c	0.003
HCT	%	25.93±1.51a	26.40±0.85a	21.40±0.64b	19.63±0.27b	19.30±0.38b	0.000
MCV	fL	41.30±0.21a	41.80±0.32a	42.53±0.35a	47.83±2.42b	43.10±0.78a	0.017
WBCs	10^9/L	21.53±1.27a	22.10±0.56a	23.73±1.23a	36.63±2.38b	22.10±0.81a	0.000
Lymphocyte count	10^9/L	21.13±3.53a	18.97±1.23a	17.23±0.50a	11.03±1.59b	17.93±0.20a	0.029
Lymphocyte	%	69.33±1.13a	77.03±0.61b	73.27±1.60ab	59.43±1.99c	71.80±1.91a	0.000
Neutrophil count	10^9/L	4.50±0.35a	3.87±0.26a	4.20±0.15a	10.93±0.52b	5.70±0.38c	0.000
Neutrophil	%	17.10±0.21a	15.50±1.08a	15.97±1.01a	24.30±1.18b	16.23±0.52a	0.000
MID	%	4.97±0.26	4.73±0.41	4.77±0.48	5.90±0.38	4.57±0.23	0.154
MID count	10^9/L	1.90±0.44	1.90±0.06	1.90±0.06	2.80±0.25	1.83±0.12	0.068

Mean±SE values within the rows bearing different letters are significantly different (P<0.05). Control (Non-Pregnant), EP=Early Pregnancy; MP=Mid Pregnancy; LP=Late Pregnancy; PP=Postpartum; Sig=Significance.

3.4. Clinical Patterns

Comparative analysis of clinical patterns (Table 4) revealed distinct variations throughout the reproductive cycle of ewes. These patterns encompassed aspects such as body temperature (°C), pulse rate (beats/min), respiratory rate (respiratory cycle/min), and ruminal movement /2min. The results provide insights into the physiological changes and potential health-related implications associated with each stage of pregnancy and postpartum, allowing for a better understanding of the overall well-being of ewes during these critical periods. Table 4 shows a significant (P<0.05) difference in the comparative clinical patterns observed in the different stages.

3.5. Age and Litter Size Comparisons

To further explore the influence of age and litter size in mid-pregnancy on the measured parameters, additional analyses were performed. By comparing the results between young and old ewes and between single and twin ewes within each reproductive stage, it becomes possible to discern potential age-related or litter size-related significant differences in blood parameter levels (Table 5), hematological parameters (Table 6), and clinical patterns at mid-

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pregnancy (Table 7). These comparisons contribute to a more comprehensive understanding of the impact of these factors on the reproductive physiology and overall health of ewes.

Table 4: Comparative clinical patterns analysis in ewes at different pregnancy stages compared to nonpregnancy and postpartum with their significant difference.

Parameters	Units	Control	45th day EP	90th day MP	135th day LP	30th day PP	Sig.
Body temperature	°C	38.6±0.04a	38.7±0.04a	38.6±0.04a	38.55±0.03a	38.525±0.03a	0.033
Pulse rate	Beats/min	81.75±0.25a	82.5±0.29a	83.75±0.25b	86.25±0.48c	82±0.41a	0.000
Respiratory rate	Cycle/min	12.5±0.29a	12.75±0.25a	14±0.41b	17.25±0.48c	12.75±0.25a	0.000
Ruminal movement	/2min	4±0.41a	3.75±0.25ab	3±0.00bc	1.75±0.25d	3.75±0.25ac	0.000

Mean±SE values within the rows bearing different letters are significantly different (P<0.05). Control (Non-Pregnant), EP=Early Pregnancy; MP=Mid Pregnancy; LP=Late Pregnancy; PP=Postpartum; Sig=Significance

Table 5: Mid-pregnancy ewes' age and litter size comparisons in serum mineral levels and levels of leptin, vitamin D, glucose, and triglycerides with their significant difference.

Parameters	Units	Age-Wise Difference			Single/Twin Lamb Difference		
		Young age	Old age	Sig.	Single lamb	Twin lamb	Sig.
Calcium	mg/dL	8.00±0.06	7.69±0.25	0.203	7.79±0.19	6.67±0.23	0.822
Phosphorus	mg/dL	4.70±0.12	5.17±0.09	0.770	5.01±0.13	2.89±0.24	0.220
Magnesium	ppm	2.15±0.06	2.24±0.05	0.963	2.21±0.04	1.55±0.19	0.127
Leptin	ng/dL	5.70±0.12	5.85±0.03	0.219	5.80±0.04	5.64±0.07	0.241
Vitamin D	nmol/L	7.20±0.12	8.01±0.11	0.982	7.74±0.21	5.66±0.22	0.697
Glucose	mg/dL	70.86±0.12	70.06±0.48	0.211	70.32±0.39	58.07±2.67	0.135
Triglycerides	mg/dL	76.10±0.06	74.72±0.99	0.132	75.18±0.77	71.97±5.47	0.057
Copper	ppm	130.00±0.06	130.61±1.44	0.127	130.40±1.03	141.88±0.43	0.347
Zinc	ppm	140.00±0.58	141.75±0.14	0.219	141.50±0.20	137.00±0.71	0.134
Selenium	ppb	43.60±0.06	43.55±0.38	0.170	43.57±0.27	41.98±0.42	0.333

The values are presented as the mean±SE.

4. DISCUSSION

The stages of pregnancy are regarded as critical due to the numerous metabolic changes and adjustments associated with the animal's altered physiological condition (Araujo et al. 2014). The present study suggests that the variations in serum levels of various parameters, hematological parameters, and clinical patterns imposed by meat ewes' pregnancy depend on ewes' reproductive stage, age, and litter size. Comparable findings have been documented in ewes raised for meat production (Pesántez-Pacheco et al. 2019, El-Sayed et al. 2020).

4.1. Serum Mineral Levels

The investigation of serum mineral levels provides a deep understanding of mineral metabolism and homeostasis during different reproductive stages. Table 2 shows significant (P<0.05) changes in calcium, phosphorus, magnesium, copper, and zinc levels across the reproductive cycle. Calcium levels decrease significantly during late pregnancy (7.11±0.39mg/dL) and postpartum (9.69±0.06mg/dL), reflecting the mobilization of calcium for fetal development and milk production. Adequate calcium supplementation is essential during these stages to prevent hypocalcemia. Phosphorus levels remained consistent except for a decrease in late pregnancy (4.15±0.36mg/dL), indicating an increased demand for fetal skeletal development.

Table 6: Mid-pregnancy ewes' age and litter size comparisons in hematological parameters with their significant difference.

Parameter	Units	Age-Wise Difference			Single/Twin Lamb Difference		
		Young age	Old age	Sig.	Single lamb	Twin lamb	Sig.
RBCs	10^{12}/L	5.44±0.32	5.44±0.32	0.984	5.44±0.32	5.37±0.30	0.880
Hb	g/dL	10.54±0.18	10.43±0.19	0.964	10.53±0.19	10.43±0.19	1.000
HCT	%	21.40±0.64	21.33±0.67	0.963	21.40±0.64	21.03±0.58	0.812
MCV	fL	42.53±0.35	42.53±0.32	0.791	42.53±0.35	42.30±0.36	0.923
WBCs	10^9/L	23.73±1.23	23.70±1.19	0.953	23.73±1.23	23.57±1.27	0.956
Lymphocyte count	10^9/L	17.23±0.50	17.23±0.56	0.849	17.23±0.50	16.97±0.55	0.903
Lymphocyte	%	73.27±1.60	73.17±1.60	1.000	73.27±1.60	73.07±1.55	0.937
Neutrophil count	10^9/L	4.20±0.15	4.17±0.12	0.587	4.20±0.15	4.07±0.07	0.147
Neutrophil	%	15.97±1.01	15.90±1.00	0.973	15.97±1.01	15.70±1.03	0.944
MID	%	4.77±0.48	4.77±0.44	0.823	4.77±0.48	4.57±0.47	1.000
MID count	10^9/L	1.90±0.06	1.87±0.07	0.609	1.90±0.06	1.77±0.12	0.205

The values are presented as the mean±SE.

Table 7: Mid-pregnancy ewes' age and litter size comparisons in clinical patterns with their significant difference.

Parameters	Age-Wise Difference			Single/Twin Lamb Difference		
	Young age	Old age	Sig.	Single lamb	Twin lamb	Sig.
Body temperature (°C)	38.60±0.04	38.63±0.05	0.506	38.60±0.06	38.53±0.03	0.561
Pulse rate (beats/min)	83.75±0.25a	79.00±0.91b	0.012	83.67±0.33	83.00±0.58	0.561
Respiratory rate (respiratory cycle/min)	14.00±0.41	12.00±0.41	1.000	12.67±0.33	13.67±0.67	0.148
Ruminal movement /2min	3.00±0.00	3.00±0.00	1.000	3.00±0.00	3.00±0.00	1.000

Mean±SE values within the rows bearing different letters are significantly different (P<0.05).

Magnesium levels decline gradually from nonpregnancy to postpartum (2.84±0.16, 2.48±0.13, 2.21±0.06, 2.05±0.04, and 2.34±0.06ppm, respectively), possibly due to increased utilization. Copper levels increased in early (107.22±1.12ppm) and mid-pregnancy (138.27±7.82ppm), while zinc levels remained consistent. The present outcomes are concordant with those of Fırat and Özpınar (2002) who indicated that ewes experience elevated nutrient demands in late pregnancy because of accelerated fetal growth. Increased fetal growth and mammary gland development can lead to an elevated demand for nutrients, which, if not met, poses a higher risk of losses due to nutrient imbalance (Akraeim 2021). However, selenium levels showed no significant differences. Maintaining optimal mineral levels is crucial for ewe health and productivity, impacting reproduction, immune function, and overall metabolic well-being. Understanding these variations is important for managing mineral supplementation to support optimal reproductive performance and overall health in ewes.

4.2. Serum Leptin, Vitamin D, Glucose, and Triglycerides

The analysis of serum levels of leptin, vitamin D, glucose, and triglycerides provides valuable insights into metabolic status, energy homeostasis, and nutrient utilization throughout the reproductive cycle of ewes. These parameters play critical roles in regulating energy balance, reproductive function, and overall metabolic health. Leptin levels show a significant increase during pregnancy (5.40±0.31, 5.80±0.06, 6.33±0.09ng/dL), indicating its involvement in energy balance and metabolic adaptations. Leptin levels show a significant drop in the postpartum period. The findings substantiate the notion that ewes commence lactation with a diminished intake capacity, resulting in a negative energy balance, a phenomenon widely observed in dairy ruminants (Ghaffari et al. 2022, Iqbal et al. 2022). Glucose levels decrease throughout pregnancy (77.10±1.03, 70.32±0.55, 66.03±3.07mg/dL), meeting the energy demands of fetal development and lactation, and return to early pregnancy levels in the postpartum stage (86.78±0.12mg/dL). The rise in postpartum blood glucose levels could also be attributed to the increased cortisol release observed following lambing in meat crossbred ewes (Schmitt et al. 2018). Nonetheless, it is noteworthy that glucose levels in all the animals within our study fell within the physiological range for sheep, as reported in previous studies (Pesántez-Pacheco et al. 2019, Iqbal et al. 2022). This observation suggests that the nutritional management practices employed on the study farm were adequate.

Vitamin D levels decline progressively during pregnancy (9.50±0.17, 7.74±0.29, 6.10±0.23nmol/L), reflecting increased demand and utilization of this fat-soluble vitamin. Triglyceride levels significantly increased during pregnancy (79.33±4.98, 75.18±1.09, 83.74±2.90mg/dL), serving as an energy store for the upcoming lactation period. These findings align with the observations made by (Ghaffari et al. 2022), who noted that the process of lipid mobilization could commence several weeks prior to lambing, particularly in ewes carrying multiple fetuses. Conversely, (Pesántez-Pacheco et al. 2019) reported that the sheep in their investigation maintained consistent levels of cholesterol and triglycerides throughout pregnancy. Following lambing, both mature and maiden sheep exhibited a reduction in triglyceride levels. These variations demonstrate the dynamic adjustments in energy metabolism and nutrient partitioning to support reproductive processes and postpartum recovery. Understanding these metabolic adaptations enhances our knowledge of energy regulatory mechanisms in ewes during the reproductive cycle.

4.3. Hematological Parameters

The analysis of hematologic parameters is a reliable approach for assessing the health condition of animals, as affirmed by Cetin et al. (2009). Table 3 displays the results of various hematological parameters at different stages of pregnancy and postpartum in the experimental groups. The findings indicate significant changes in these parameters throughout the reproductive cycle of the ewes. The decline in RBC count and Hb levels observed from the nonpregnant stage to late pregnancy is likely a consequence of hemodilution resulting from increased plasma volume expansion. This physiological phenomenon serves a purpose by reducing blood viscosity, thereby enhancing blood flow in the confined blood vessels of the uterus and udder. This effect is particularly pronounced in ewes carrying twin fetuses, as suggested by (Karaşahin et al. 2023). Similarly, HCT levels followed a similar trend. These results are in accordance with Akraeim (2021) who mentioned that in comparison to nonpregnant and lactating sheep, pregnant sheep exhibited significant reductions in both RBC count and MCV. MCV remained relatively stable, except for a slight increase (47.83±2.42) in late pregnancy. In contrast, Azab and Abdel-Maksoud (1999) discovered that pregnant ewes have greater osmotic tolerance in their red blood cells than dry ewes. The WBC count increased significantly in late pregnancy (36.63±2.38), reflecting an immune response to physiological stress. The lymphocyte count and percentage decrease gradually, while the neutrophil count and percentage remain stable until late pregnancy when they increase significantly, possibly in response to inflammation and preparation for parturition. MID% and MID count exhibit no significant variations. These results provide valuable insights into the dynamic changes in hematological parameters during different reproductive stages in ewes and are similar to the findings acquired by Akraeim (2021), Antunović et al. (2013), and Paape et al. (1992). These findings do not align with the observations made by Azab and Abdel-Maksoud (1999), Mbassa and Poulsen (1991), and Bezerra et al. (2017).

4.4. Clinical Patterns

Table 4 presents the values of several physiological parameters, including body temperature, pulse rate, respiratory rate, and ruminal movement, at different stages of the reproductive cycle in ewes. The body temperature remains relatively stable throughout the reproductive stages, indicating minimal impact from pregnancy and postpartum on ewe body temperature. However, the pulse rate shows a significant increase as pregnancy progresses, with the highest rate observed in late pregnancy (86.25±0.48 beats/min). This elevation is likely due to increased metabolic demands and cardiovascular adaptations. Similarly, the respiratory rate progressively increases from early to late pregnancy (12.75±0.25, 14±0.41, 17.25±0.48 respiratory cycle/min), reflecting the heightened oxygen demand for fetal development. The increase in pulse rate and respiration rate may be related to stress (Kelly 1984). In contrast, ruminal movement decreases significantly from early to late pregnancy, possibly due to limited space caused by fetal growth. This decrease in ruminal movement may be attributed to increased intra-abdominal pressure and low energy rations during late pregnancy, but the decrease on the day of parturition may be regarded as exhaustion and a decrease in serum mineral levels (Goff and Horst 1997). These variations in physiological parameters highlight the adaptive changes occurring during different reproductive stages, such as increased metabolism, cardiovascular adjustments, and gastrointestinal alterations. Monitoring these parameters provides valuable insights into the overall health and well-being of ewes throughout their reproductive journey.

4.5. Age and Litter Size Comparisons

Table 5 presents the comparison of various parameters between young and old age groups of animals, as well as between single and twin lamb pregnancies. The statistical analysis showed no significant differences in calcium, phosphorus, magnesium, leptin, vitamin D, copper, zinc, and selenium levels between the age groups or between singleton and twin lamb pregnancies. Our results contradict those documented by Schlumbohm and Harmeyer (2008), who suggested that, regardless of the cause, the increased nutrient supply with multiple pregnancies likely ensures that the metabolic requirements of all fetuses are satisfied, and the risk of deficiency stress is greater in ewes carrying twins than in those carrying single lambs. Glucose levels exhibited no significant differences between the young and old age groups, while a nonsignificant trend of lower glucose levels was observed in twin lamb pregnancies (58.07±2.67mg/dL). In this context, previous studies have shown that glucose levels do not differ according to ewes' age and litter size (Kappel et al. 1984, Vizcarra et al. 1998, Taylor et al. 2003, Pesántez-Pacheco et al. 2019). In contrast, Abecia and Palacios (2018) reported that the rise in glucose levels from pregnancy to the postpartum period was more significant in sheep with multiple pregnancies than in those with single pregnancies, regardless of whether the sheep were maiden or mature. However, it is worth noting that the glucose levels in all animals in our study fell within the physiological range for sheep, as reported by Kassim and AL-Hellou (2018) and Pesántez-Pacheco et al. (2019), which suggests that nutritional management on the study farm was appropriate. Triglyceride levels also did not significantly differ between the age groups but showed a slight nonsignificant decrease in twin lamb pregnancies (71.97±5.47mg/dL). Nonetheless, Ghaffari et al. (2022) reported that the process of lipid mobilization may initiate several weeks prior to lambing, particularly in ewes carrying multiple pregnancies. These findings suggest that age and lambing status may not have substantial effects on the measured parameters. However, it is important to interpret these results cautiously, considering the limitations of the study and the specific context in which they were obtained. Further research and analysis may be necessary to draw more conclusive findings.

Table 6 presents various hematological parameters comparing mid-pregnancy ewes according to age and litter size. However, the data show no significant differences ($P<0.05$) between young age and old age for any of the parameters. Similarly, there are no significant differences between single lamb and twin lamb conditions for any of the parameters. The results contradict those of Karaşahin et al. (2023), who suggested that pregnancy-induced

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hemodilution, resulting from an expanded plasma volume, has physiological significance. This process reduces blood viscosity, increasing blood flow in the narrow blood vessels of the uterus and udder, and it is particularly prominent in ewes carrying twins. Overall, these results suggest that hematological parameters may not be strongly influenced by age or the number of lambs in the studied population of mid-pregnancy ewes.

Table 7 reveals that there is no significant difference in body temperature between young and old age or between single and twin lambing. However, there was a significant difference in pulse rate between young age and old age, with younger individuals exhibiting a higher pulse rate (83.75±0.25beats/min). On the other hand, Kelly (1984) declared that the increase in pulse rate and respiration rate may be related to stress, heightened oxygen demand for fetal development, increased metabolic demands, and cardiovascular adaptations in twins compared with a single pregnancy. No significant differences were observed in respiratory rate or ruminal movement between age groups or lambing types. However, the current findings contradict those of Goff and Horst (1997), who related decreased ruminal movement to increased intra-abdominal pressure in twin pregnancy. Overall, these findings suggest that age may influence pulse rate, but there are no significant differences in body temperature, respiratory rate, or ruminal movement based on age or lambing type.

In summary, this study elucidates the intricate physiological changes occurring in meat ewes throughout different reproductive stages. It emphasizes the significant impact of factors such as reproductive stage, age, and litter size on serum mineral levels, hematological parameters, and clinical patterns. Notably, these findings stress the importance of tailored nutritional management to ensure optimal ewe health and reproductive success.

5. CONCLUSION

This research highlights the necessity of careful calcium supplementation during late pregnancy to prevent hypocalcemia, a crucial consideration for supporting fetal development and lactation. Additionally, the study sheds light on the metabolic adaptations needed to maintain energy balance during pregnancy and lactation, as evidenced by shifts in serum leptin, vitamin D, glucose, and triglyceride levels. Hematological parameters underscore the physiological adjustments essential for fetal development and immune responses, while clinical patterns demonstrate the remarkable adaptability of ewes to the demands of pregnancy. In conclusion, these findings provide valuable insights into the dynamic nature of ewe physiology during reproduction, offering practical implications for improved nutritional management and health monitoring practices. Further research in this area promises to deepen our understanding of the complexities involved and enhance the overall well-being and reproductive outcomes of meat ewes.

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ORCID

Mohammed Abdelhameed Mohammed Kamal https://orcid.org/0000-0002-1708-1826

REFERENCES

Abecia JA and Palacios C, 2018. Ewes giving birth to female lambs produce more milk than ewes giving birth to male lambs. Italian Journal of Animal Science 17: 736–739. Available from: https://doi.org/10.1080/1828051X.2017.1415705.

Akraeim A, 2021. Evaluation the impact of the transition period on some hematobiochemical and hormonal parameters in Native sheep in Algabal Alakhdar governorate in Libya. Benha Veterinary Medical Journal 40: 19–23. Available from: https://doi.org/10.21608/bvmj.2021.71045.1390.

Antunović Z, Novoselec J, Klir Ž and Đidara M, 2013. Hematološki pokazatelji u Alpske koze tijekom laktacije. Poljoprivreda 19: 40–43. Available from: https://hrcak.srce.hr/112548.

Antunović Z, Mioč B, Lončarić Z, Šalavardić ŽK, Širić I, Držaić V and Novoselec J, 2021. Changes of macromineral and trace element concentration in the blood of ewes during lactation period. Czech Journal of Animal Science 66: 129–136. https://doi.org/10.17221/250/2020-CJAS

Araujo CASC, Nikolaus JP, Morgado AA, Monteiro BM, Rodrigues FAML, Vechiato TAF, Soares PC and Sucupira MCA, 2014. Energetic and hormonal profile of Santa Ines ewes in the middle of gestation to postpartum. Pesquisa Veterinária Brasileira 34: 1251–1257. Available from: https://doi.org/10.1590/S0100-736X2014001200019.
Azab ME and Abdel-Maksoud HA, 1999. Changes in some hematological and biochemical parameters during prepartum and postpartum periods in female Baladi goats. Small Ruminant Research 34: 77–85. Available from: https://doi.org/10.1016/S0921-4488(99)00049-8.
Barham D and Trinder P, 1972. An improved colour reagent for the determination of blood glucose by the oxidase system. The Analyst 97: 142–145. https://doi.org/10.1039/an9729700142
Bezerra LR, Oliveira WDC, Silva TPD, Torreão JNC, Marques CAT, Araújo MJ and Oliveira RL, 2017. Comparative hematological analysis of Morada Nova and Santa Inês ewes in all reproductive stages. Pesquisa Veterinária Brasileira 37: 408–414. Available from: https://doi.org/10.1590/S0100-736X2017000400017.
Bouman AA, Platenkamp AJ and Posma FD, 1986. Determination of cobalt in urine by flameless atomic absorption spectroscopy. Comparison of direct analysis using Zeeman background correction and indirect analysis using extraction in organic solution. Annals of Clinical Biochemistry 23: 346–350. Available from: https://doi.org/10.1177/000456328602300317.
Cetin N, Bekyürek T and Cetin E, 2009. Effects of sex, pregnancy and season on some haematological and biochemical blood values in angora rabbits. Scandinavian Journal of Laboratory Animal Sciences 36: 155–162. Available from: https://doi.org/10.23675/sjlas.v36i2.180.
Chauhan SS, Rashamol VP, Bagath M, Sejian V and Dunshea FR, 2021. Impacts of heat stress on immune responses and oxidative stress in farm animals and nutritional strategies for amelioration. International journal of biometeorology 65: 1231–1244. Available from: https://doi.org/10.1007/s00484-021-02083-3.
Chen RL, Kassem NA, Sadeghi M and Preston JE, 2008. Insulin-Like Growth Factor-II Uptake Into Choroid Plexus and Brain of Young and Old Sheep. Available from: https://doi.org/10.1093/gerona/63.2.141.
Clemens TL, Hendy GN, Papapoulos SE, Fraher LJ, Care AD and O'riordan JLH, 1979. Measurement of 1, 25-dihydroxycholecalciferol in man by radioimmunoassay. Clinical Endocrinology 11: 225–234. Available from: https://doi.org/10.1111/j.1365-2265.1979.tb03068.x.
Constable PD, Hinchcliff KW, Done SH and Grünberg W, 2016. Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences. Available from: https://cir.nii.ac.jp/crid/1130000796837212672.
El-Sayed A, El-Ashker M, Ibrahim H, Shoieb S, Ibrahim F, Youssef M and El-Khodery S, 2020. Blood Metabolic Profile in Barki Ewes during Transition Period. Journal of the Hellenic Veterinary Medical Society 71: 2261–2266. Available from: https://doi.org/10.12681/jhvms.25070.
Fırat A and Özpınar A, 2002. Metabolic Profile of Pre-Pregnancy, Pregnancy and Early Lactation in Multiple Lambing Sakız Ewes: 1. Changes in Plasma Gluco se, 3-Hydroxybutyrate and Cortisol Levels. Annals of Nutrition and Metabolism 46: 57–61. Available from: https://doi.org/10.1159/000057641.
Ghaffari MH, Sadri H and Sauerwein H, 2022. Invited review: Assessment of body condition score and body fat reserves in relation to insulin sensitivity and metabolic phenotyping in dairy cows. Journal of Dairy Science. Available from: https://doi.org/10.3168/jds.2022-22549.
Godde CM, Mason-D'Croz D, Mayberry DE, Thornton PK and Herrero M, 2021. Impacts of climate change on the livestock food supply chain; a review of the evidence. Global food security 28: 100488. Available from: https://doi.org/10.1016/j.gfs.2020.100488.
Goff JP and Horst RL, 1997. Physiological Changes at Parturition and Their Relationship to Metabolic Disorders. Journal of Dairy Science 80: 1260–1268. https://doi.org/10.3168/jds.S0022-0302(97)76055-7
Goma AA and Phillips CJC, 2022. 'Can They Take the Heat?'—The Egyptian Climate and Its Effects on Livestock. Animals 12. https://doi.org/10.3390/ani12151937
Greguła-Kania M, Kosior-Korzecka U, Patkowski K, Juszczuk-Kubiak E, Plewik M and Gruszecki TM, 2020. Acute-phase proteins, cortisol and haematological parameters in ewes during the periparturient period. Reproduction in domestic animals 55: 393–400. https://doi.org/10.1111/rda.13628
Iqbal R, Beigh SA, Mir AQ, Shaheen M, Hussain SA, Nisar M and Dar AA, 2022. Evaluation of metabolic and oxidative profile in ovine pregnancy toxemia and to determine their association with diagnosis and prognosis of disease. Tropical Animal Health and Production 54: 338. https://doi.org/10.1007/s11250-022-03339-9
Jain NC, 1993. Essentials of veterinary hematology, comparative hematology of common domestic animals. Lea and Febiger, Philadelphia, PA: 44–46. Available from: http://refhub.elsevier.com/S2405-8440(21)01220-2/sref27.
Kappel LC, Ingraham RH, Morgan EB, Zeringue L, Wilson D and Babcock DK, 1984. Relationship between fertility and blood glucose and cholesterol concentrations in Holstein cows. American Journal of Veterinary Research 45: 2607–2612.
Karaşahīn T, Dursun Ş, Hayat Aksoy N, İpek H and Şentürk G, 2023. Hematological Parameters in Hair Goats During and out of Breeding Season Hair Goats Seasonal Hematological Parameters. Iranian Journal of Veterinary Medicine 17: 113–118. Available from: https://acikerisim.aksaray.edu.tr/dx.doi.org10.32598/ijvm.17.2.1005334.
Kassim WY and AL-Hellou MF, 2018. Effect of geographic location and age on levels of some biochemical parameters of ewes in Southern of Iraq. Journal of Biosciences and Medicines 6: 21–29. Available from: https://doi.org/10.4236/jbm.2018.611003.
Kelly WR, 1984. Veterinary clinical diagnosis. Third. Bailliere Tindall, London, U. K. .

Krawczyńska A, Herman AP, Antushevich H, Bochenek J, Wojtulewicz K and Zieba DA, 2022. The Effect of Leptin on the Blood Hormonal Profile (Cortisol, Insulin, Thyroid Hormones) of the Ewe in Acute Inflammation in Two Different Photoperiodical Conditions. International Journal of Molecular Sciences 23: 8109. Available from: https://doi.org/10.3390/ijms23158109.

Marai IFM, Ayyat MS and Abd El-Monem UM, 2001. Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. Tropical animal health and production 33: 451–462. Available from: https://doi.org/10.1023/A:1012772311177.

Mbassa GK and Poulsen JS, 1991. Influence of pregnancy, lactation and environment on haematological profiles in Danish landrace dairy goats (Capra hircus) of different parity. Comparative biochemistry and physiology. B, Comparative Biochemistry 100: 403–412. Available from: https://doi.org/10.1016/0305-0491(91)90394-s.

NRC, 1985. 5 Nutrient requirements of sheep. National Academies Press. Available from: https://lccn.loc.gov/74000899.

Nurlatifah A, Khotijah L, Arifiantini RI, Maidin MS and Astuti DA, 2022. Change in hematology prepartum and postpartum of garut ewe fed with flushing diet contain lemuru oil. In: IOP Conference Series: Earth and Environmental Science. IOP Publishing, 012005. Available from: 10.1088/1755-1315/1020/1/012005.

Paape MJ, Capuco A V, Lefcourt A, Burvenich C and Miller RH, 1992. Physiological response of dairy cows to milking. Publication-European Association for Animal Production 65: 93. Available from: http://hdl.handle.net/1854/LU-226761.

Pesántez-Pacheco JL, Heras-Molina A, Torres-Rovira L, Sanz-Fernández MV, García-Contreras C, Vázquez-Gómez M, Feyjoo P, Cáceres E, Frías-Mateo M and Hernández F, 2019. Influence of maternal factors (weight, body condition, parity, and pregnancy rank) on plasma metabolites of dairy ewes and their lambs. Animals 9: 122. https://doi.org/10.3390/ani9040122

Razavi SM, Soltan MS, Abbasian K, Karami A and Nazifi S, 2023. Host oxidative stress in piroplasmosis: a review in domestic animals. Veterinary Parasitology: 110011. https://doi.org/10.1016/j.vetpar.2023.110011

Rifai N, 2017. Tietz textbook of clinical chemistry and molecular diagnostics-e-book. Elsevier Health Sciences. Available from: https://lccn.loc.gov/2011030889.

Schlumbohm C and Harmeyer J, 2008. Twin-pregnancy increases susceptibility of ewes to hypoglycaemic stress and pregnancy toxaemia. Research in Veterinary Science 84: 286–299. https://doi.org/10.1016/j.rvsc.2007.05.001

Schmitt E, Maffi AS, Raimondo RFS, Lima ME, Hoffmann DAC, Farofa TS, Montagner P, Rincón JAA, Del Pino FAB and Corrêa MN, 2018. Energetic metabolic profile of ewes presenting low body condition score induced to subclinical hypocalcemia in early postpartum. Austral Journal of Veterinary Sciences 50: 15–20. Available from: http://dx.doi.org/10.4067/S0719-81322018000100104.

Singh R, Singh A, Beigh SA, Sharma N and Singh V, 2022. Effect of physiological status and parity on metabolic and trace element profile of crossbred Rambouillet sheep of Himalayan region. Tropical Animal Health and Production 54. https://doi.org/10.1007/s11250-022-03068-z

Taylor VJ, Beever DE, Bryant MJ and Wathes DC, 2003. Metabolic profiles and progesterone cycles in first lactation dairy cows. Theriogenology 59: 1661–1677. https://doi.org/10.1016/S0093-691X(02)01225-6

Vizcarra JA, Wettemann RP, Spitzer JC and Morrison DG, 1998. Body condition at parturition and postpartum weight gain influence luteal activity and concentrations of glucose, insulin, and nonesterified fatty acids in plasma of primiparous beef cows. Journal of Animal Science 76: 927–936. Available from: https://doi.org/10.2527/1998.764927x.

Young DS, Pestaner LC and Gibberman VAL, 1975. Effects of drugs on clinical laboratory tests. Clinical Chemistry 21: 1D-432D. https://doi.org/10.1177/000456329703400601