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Laboratory Investigation of Haematological and Biochemical Parameters Throughout the Periparturient Phase in Fat-Tailed Ewes

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Abstract

The periparturient period is defined as the period immediately preceding and following parturition. Dairy animals encounter obstacles during this period. The goal of the study was to identify the various physiological stressors that ewes encounter during this time and the kind of special care that should be offered to them in order to deal with this problem. Thirty fat-tailed ewes were used in this research. All animals were examined clinically and ultrasonography. Blood samples were taken firstly during the diestrus stage (group 1), then during late gestation (group 2), and finally during postpartum (Group 3). Haematological and biochemical parameters were assessed. The results revealed a significant decrease in RBCs count, haemoglobin concentrations, and hematocrite % during the periparturient phase compared to the diestrus phase. WBCs count exhibited a significant increase during the periparturient period. The metabolic profile involves total protein and albumin, which decreased significantly during the periparturient period compared to the diestrus phase, while urea concentration showed the opposite trend. Total cholesterol and triglycerides showed a significant increase over the periparturient phase. Mineral investigations indicate that ferritin and calcium concentrations decreased significantly throughout the periparturient period compared to the diestrus phase, while phosphorus and magnesium concentrations showed non-significant changes. The study revealed a wide variety of alterations in several clinical, haematological, and biochemical parameters of ewe's blood throughout the periparturient phase. This study will provide an opportunity to identify management changes for the prophylaxis of ovine diseases, improving overall herd health and animal welfare.

Keywords: late gestation; post-partum; laboratory diagnosis; ferritin; sheep.

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Introduction

The transitional period, or the periparturient period, represents one of the most crucial phases in the life of ewes and many other animal species. It is considered as to begin three weeks before and end three weeks after parturition (Mee., 2013). During the periparturient phase, the dam undergoes profound physiological stressors, which can strongly influence its metabolism (Elischer et al., 2015, Zamuner et al., 2020). Furthermore, metabolic stress may also be caused by the mother's inability to adjust physiologically productive lactation. Chronic inflammation and unregulated adipose tissue lipolysis have complex impacts on these metabolic stresses (Zamuner et al., 2020, Sordillo and Mavangira, 2014). The majority of postpartum health issues are caused by high metabolic stress, which could greatly decrease animal well-being and raise economic losses (Stoldt et al., 2015). Additionally, the transitional period has a number of risk factors that contribute to metabolic stress, which in turn raises the prevalence of certain medical conditions such as mastitis, abomasum displacement, and ketosis (Sordillo and Mavangira, 2014).

Periparturient metabolic disorders usually originate from a lack of nutritional and/or non-nutritional management that may lead to the susceptible occurrence of infectious diseases, which reduce subsequent fertility and cause negative impacts on subsequent reproduction (Drackley and Cardoso, 2014). It was reported that checking the post-partum period for confirming the presence of energy balance, the absence of metabolic imbalances, and the use of preventative measures are crucial and can lower the occurrence of such disorders (Amin et al., 2021).

Clinical examination, haematological and biochemical analyses, when combined with other genetic, environmental, and managerial factors, offer trustworthy indicators of an animal's health, which can be highly helpful for disease detection and diagnosis (Oikonomidis et al., 2018). Published data on the hematological and biochemical parameters of indigenous sheep throughout the periparturient period is still limited.

Total protein, triglycerides, free fatty acids, and urea are some of the biochemical characteristics of the blood that are significant indicators of the metabolic processes in lactating animals. Peripartum diseases have been predicted by investigating lipid profiles, as circulating blood triglycerides contribute to milk fat synthesis (Skotnicka et al., 2011). Triglyceride (TG) and cholesterol concentrations are two frequently used markers of the energy profile and nutritional condition in early-lactating animals (Puppel and Kuczynska, 2016).

The blood serum lipid profile of sheep during late pregnancy is associated with high levels of total cholesterol, triglycerides, and lipoproteins as a result of the target tissues' decreased responsiveness to insulin and the increased mobilization of fatty acids from adipose tissue. These changes open up new sources of energy for the development of the fetus (Puppel and Kuczynska., 2016).

Total protein (TP), albumin, globulin, and urea are the blood indicators used to assess the protein level (Puppel and Kuczynska., 2016). Throughout the final stages of pregnancy, blood protein concentration was found to decrease in sheep, indicating that amino acids are used in protein synthesis in the fetal muscles (Antunovic et al., 202). Additionally, around week 10 of pregnancy, urea levels in the blood increased and peaked at parturition (Raboisson et al., 2017). This might be explained by the fact that cortisol stimulates the breakdown of proteins in the bodies of domestic ruminants (Silanikove, 2000).

It is well-known that successful treatment of pregnancy toxaemia in susceptible animals requires early detection of the disease. It was demonstrated that pregnancy toxaemia in sheep and goats might lead to impairment of renal function, proteinuria, and increased blood urea concentration (Vasava et al., 2016). Regular investigation of certain blood parameters in ewes during pregnancy and during the post-partum phase provides a prediction of the incidence of the disease. Therefore, this research presents a comprehensive picture of the dynamics of the stressors of the transition period through investigation of the hematological and biochemical blood parameters related to these stressors and subsequently, aims to avoid the incidence of such stressors. This study is considered a guideline for the management strategies of ewes for better ovine health care and prevention. These guidelines would guarantee the metabolic requirements for ewes throughout the periparturient phase and minimize the economic loss.

Materials and Methods

Ethical Approval

Experimental procedures were approved by the ethical research committee of the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt (No. 73/01.10.2022).

Animals and Experimental Design

The study was performed on 30 fat-tailed ewes (3.0±0.6 years old, with a mean body weight of 50.0±5.0 kg) during the diestrus period, late gestation, and postpartum period which were examined by ultrasound. Ewes were free from internal and external parasites and belonged to a farm at South Valley University, Qena, Egypt. All animals were housed in a barn and fed twice a day (08:00 and 17:00) with green feed obtained by grazing or offered in the barn and a mix of cereals formulated (fresh matter: 16% crude protein, 2.80% crude fat, 15 % crude fiber, 9 % crude ash, 12% humidity, 32,000 IU Vit. A, 2,000 IU Vit. D, 50 mg Vit. E, and 4 mg copper). The feeding is dependent on maintaining the nutritional requirements of pregnant sheep (NRC, 2007).

Three groups of animals were established: group 1 (G1 or control ewes in the diestrus phase); group 2 (G2 ewes during late gestation); and group 3 (G3 ewes during postpartum). All ewes were examined clinically (Constable et al., 2017) including general inspection, mucous membranes, body temperature, heart rate, respiration and rumen motility (Jackson and Cockcroft, 2002). All ewes were examined by ultrasound. The schematic cartoon of the experimental design is shown in Figure 1.

The sheep involved in this study were chosen from a herd of approximately 200 dairy sheep under the same management conditions. The herd was free from infectious diseases. The selected sheep were housed individually in straw-bedded box stalls until 3 weeks after calving, and their health status was monitored every day.

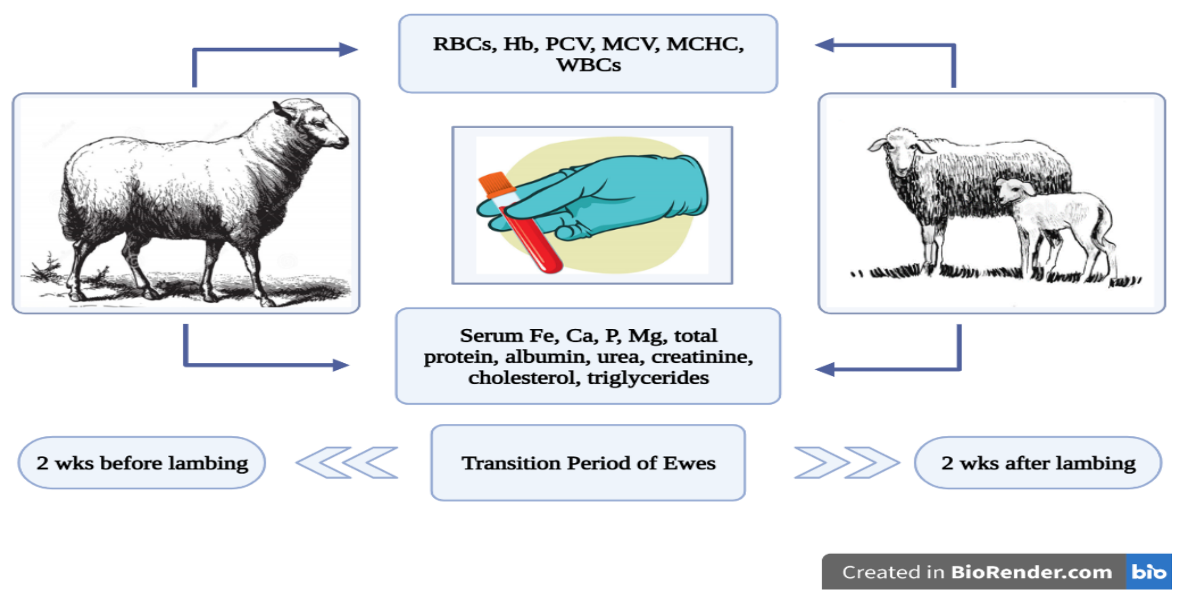

Figure 1. The schematic cartoon of the experimental design — It was created with BioRender.com.

Blood Sampling

Blood samples were taken from all ewes early in the morning prior to feeding.

First samples were planned when the ewes were not pregnant during the diestrus phase. Then they were collected after the occurrence of pregnancy (at late gestation), 2 weeks before the anticipated lambing date. Thereafter, they were collected 2 weeks after parturition (post-partum).

Approximately 5 mL of blood samples were withdrawn from the jugular vein of each ewe early in the morning prior to feeding, and from there, 2 mL of the blood sample was transferred to a sterile vial containing ethylene diamine tetra-acetic acid (1 mg/mL of blood) for measuring the hematological parameters using the Veterinary Hematology Analyzer (Exigo H400). The measured hematological parameters include red blood cells count (RBCs), hemoglobin (Hb), packed cell volume percentage (PCV %), mean erythrocyte volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count. In addition, total white blood cell count (WBC) and differential leukocytic count were estimated. The remaining 3 mL of the blood samples were used for the production of serum by their transfer to a plain vacutainer tube and centrifugation at 3000 rpm for 15 minutes, then kept at -20 °C for later use in biochemical analysis. Serum samples were analyzed using a biochemical analyzer (Humalyzer-3000, USA) to estimate ferritin (Fe), calcium (Ca), phosphorus (P), and magnesium (Mg). Total protein, albumin, urea, creatinine, cholesterol, and triglycerides were measured by using commercial kits (Spectrum-Diagnostics Company) and by using the UV Spectrophotometer (SEAC, Slim, Florence, Italy).

Statistical Analysis

All results were presented as means and standard deviation (SD). The analysis of variance (one-way ANOVA) was used to test the overall significance of differences among the means using IBM-SPSS software 22.0. Bonferroni's Multiple Comparison Test was applied for post hoc comparison .The significant difference was considered when $P \leq 0.05$

Results

Clinical Examination of Ewes Throughout the Periparturient Phase

The results of clinical findings through diestrus, late gestation, and post-partum periods are presented in (Table 1). Ewes during late pregnancy and post-partum were suffered from anemia this is evidenced by pale mucous membranes, increase in capillary refill time (CRT ≥ 2 sec) and decreased appetite. On the other hand, the clinical findings of the examined ewes in the diestrus period showed bright red mucosa, normal capillary refill time (CRT < 2 sec) and a normal appetite.

The results showed no change in body temperature during late gestation, post-partum and the diestrus period, while there was a non-significant increase in heart rate and a significant increase in respiratory rate (P<0.001) in late gestation and post-partum periods when compared with those values in the diestrus period.

Rumen motility exhibits a low rate during the periparturient period, especially during late gestation, in comparison with that of ewes during the diestrus period, however, all parameters were within the normal reference ranges of body temperature, heart rate, respiration, and rumen motility in sheep.

Table 1. Temperature, heart rate, respiration, and ruminal movement in diestrus period, late gestation, and postpartum period in ewes.

Signs of health	Non-preg. ewe (diestrus) Control	Late gestation	Post-partum	Sig. (P-value)
Temperature (ċ)	39.03±0.80	39.53±0.14	39.33±0.24	^aP =0.105 ^bP =0.156
Heart rate (beat/ min)	76.33±2.30	81.67±2.40	82.67±1.45	^aP =0.091 ^bP =0.081
Respiration (breaths/ min)	18.76±0.88	26.50±0.50[a]	24.67±1.45[b]	^aP =0.000 ^bP =0.000
Rumen motility (movements/ min)	3.00±0.57	2.33±0.33	2.66±0.66	^aP =0.183 ^bP =0.201

Data is represented as (mean ± SD). SD = standard deviation.
[a]P<0.001 in comparison with the control group.
[b]P<0.001 in comparison with the control group.

Complete Blood Picture in Ewes Throughout the Periparturient Phase

As shown in Table 2, the RBC count and Hb concentration decreased significantly (P=<0.001) in the late gestation period and during the post-partum period in ewes when compared to values throughout the diestrus period. In addition, the PCV decreased significantly (P=<0.001) in late gestation (26.87 ±1.02 %)and during the postpartum period (26.57±0.56%) when compared to values in the diestrus period (32.71±0.26%). While, MCH, MCHC, MCV, and platelet count do not have significant differences throughout late gestation and the postpartum phase compared with the diestrus phase.

Table 2. Hematological parameters in the diestrus period, late gestation, and postpartum period in ewes.

Parameters	Non-preg. ewe (diestrus) Control	Late gestation	Post-partum	Sig. (P-value)
RBCs (count×10^6/μL)	9.57±0.32	8.3±0.37^a	8.05±0.37^b	^aP =0.000 ^bP =0.000
Hb conc (g/dl)	10.53±0.33	8.62±0.55^a	8.62±0.45^b	^aP =0.000 ^bP =0.000
PCV %	32.71 ±0.26	26.87 ±1.02^a	26.57±0.56^b	^aP =0.000 ^bP =0.000
MCH (pg)	11.02±0.49	10.48±0.91	10.79±0.69	^aP =0.105 ^bP =0.481
MCHC (g/dL)	32.17± 0.35	33.07± 0.99	32.43± 1.19	^aP =0.829 ^bP =0.575
MCV (fL)	34.23±1.25	32.60±1.91	33.28±1.51	^aP =0.031 ^bP =0.195
Platelets (count×10^3/μL)	319.50±32.35	289.50±56.59	285.00±55.50	^aP =0.186 ^bP =0.130

Data is represented as (mean ± SD). SD = standard deviation.
^aP<0.001 in comparison with the control group.
^bP<0.001 in comparison with the control group.

As shown in Table 3, the leukocytic count throughout the late gestation phase (10.05±0.45×10^3/μL) and the post-partum phase (9.88±0.49×10^3/μL) increased significantly when compared with the results throughout the diestrus phase (6.85±0.23×10^3/μL). Whereas, either the increase of neutrophil and eosinophil percentages during late gestation phase or their decrease during the post-partum phase was not statistically significant compared with the results during the diestrus phase. Differences in the percentages of lymphocytes, monocytes, and basophils were not significant during all stages of the study.

Table 3. Differential leukocytic count in ewes during the diestrus period, late gestation, and post-partum period.

Leukocytic count	Non-preg. ewe (diestrus) Control	Late gestation	Post-partum	Sig. (P-value)
WBCs (count×10^3/μL)	6.85±0.23	10.05±0.45^a	9.88±0.49^b	^aP= 0.000 ^bP=0.000
Neutrophils (count×10^3/μL)	2.33±0.83	3.52±0.93	3.16±0.53	^aP= 0.112 ^bP=0.101
Lymphocytes (count×10^3/μL)	3.70±0.53	5.23±0.42	5.43±0.41	^aP= 0.081 ^bP=0.094

Monocytes (count×10^3/μL)	0.41± 0.23	0.50± 0.21	0.59± 0.23	^aP= 0.105 ^bP=0.091
Eosinophils (count×10^3/μL)	0.64± 0.11	0.80± 0.13	0.69 ± 0.12	^aP=0.071 ^bP=0.104
Basophil (count×10^3/μL)	0.06 ± 0.03	0.04 ± 0.01	0.02 ± 0.03	^aP= 0.082 ^bP=0.061

Data is represented as (mean ± SD). SD = standard deviation.
^aP<0.001 in comparison with the control group.
^bP<0.001 in comparison with the control group.

Serum Biochemical Analysis in Ewes Throughout the Periparturient Period

As shown in Table 4, the biochemical investigation showed that there was a significant drop in the total serum protein and serum albumin values throughout the late gestation and the postpartum phase compared with the values throughout the diestrus phase. In contrast, the urea concentration was significantly higher throughout the late gestation phase and postpartum phase (38.70±2.11 mg/dl) (42.60±2.7 mg/dl) when compared to the diestrus phase (28.10±3.92 mg/dl). Furthermore, creatinine values were found to increase during late gestation phase (0.98±0.08 mg/dl) and during the postpartum phase (1.12±0.13 mg/dl), but this increase was not significant during late gestation.

The result of this study revealed that the levels of cholesterol and triglycerides during late gestation were significantly higher (P<0.001) than other periods.

Table 4. Comparison of biochemical profile in ewes during diestrus period, late gestation and post-partum period.

Item	Non-preg. ewe (diestrus) Control	Late gestation	Post-partum	Sig. (P-value)
Total Protein (g/dl)	6.23±0.18	3.41±0.09^a	3.28±0.07^b	^aP= 0.000 ^bP=0.001
Albumin (g/dl)	3.37±0.20	1.65±0.14^a	1.47±0.09^b	^aP= 0.001 ^bP=0.001
Cholesterol (mg/dl)	65.90±3.07	141.30±3.26^a	118.80±6.28^b	^aP= 0.001 ^bP=0.001
Triglyceride (mg/dl)	51.50±3.62	80.90±4.45^a	67.70±5.87^b	^aP= 0.001 ^bP=0.001
Urea (mg/dl)	28.10±3.92	38.70±2.11^a	42.60±2.7^b	^aP= 0.001 ^bP=0.001
Creatinine (mg/dl)	0.91±0.15	0.98±0.08	1.12±0.13^c	^cP=0.003

The data is represented as (mean ± SD). SD = standard deviation.
$^aP \leq 0.001$ in comparison late gestation group with the control group.
$^bP \leq 0.001$ in comparison post-partum group with the control group.
^cP < 0.01 in comparison post-partum group with the control group.

Selected Serum Macro Elements in Ewes Throughout the Peripartурient Phase

As shown in Table 5, serum ferritin and calcium concentrations decreased significantly ($P \leq 0.001$) in ewes throughout late gestation and the postpartum phase in comparison to the levels in the diestrus period. The decrease in phosphorus and magnesium content in ewes throughout the late gestation and the post-partum phase was not significant in comparison to the levels in the diestrus phase.

Tables 5. Comparison of selected serum macro elements in ewes during diestrus period, late gestation and post-partum period.

Item	Unit	Control	Late gestation	Post-partum	Sig. (*P*-value)
Ferritin	*ng/ml*	84.1±13.8	54.70±15.7^{a}	21.854±17.6^{b}	^{a}P= 0.000 ^{b}P=0.000
Ca^{++}(total Calcium)	*mmol/L*	9.22±0.27	8.27±0.21^{a}	8.43±0.19^{b}	^{a}P= 0.000 ^{b}P=0.000
Ph (phosphorus)	*mmol/L*	4.3±0.18	4.13±0.26	4.33±0.17	^{a}P= 0.092 ^{b}P=0.092
Magnesium	*mmol/L*	2.6±0.19	2.09±0.29^{a}	2.26±0.08	^{a}P= 0.000 ^{b}P=0.082

The data is represented as (mean ± SD). SD = standard deviation.
$^{a}P \leq 0.001$ in comparison late gestation group with the control group.
$^{b}P \leq 0.001$ in comparison post-partum group with the control group.
$^{c}P < 0.01$ in comparison post-partum group with the control group.

Discussion

Clinical Findings of Ewes Throughout the Periparturient Period

Clinical examination in ewes during the periparturient period showed decreased appetite, pale mucous membranes, increased CRT (≥ 2), this attributed to the impact of physiological status in late pregnancy and early lactation. It was found that during late pregnancy, the energy intake is lower due to increased intra-abdominal pressure due to a gravid uterus, in addition to other physiological changes in this period (Al-Bayati and Luaibi, 2020). Lactation is also a particularly stressful time for ewes as their nutritional requirements increase due to milk production (David et al., 2020, Soliman, 2014). On the other hand, the examined ewes in the diestrus period showed normal appetite, and normal mucous membranes and the capillary refill time (CRT) was < 2 S.

In this study, there was an alteration in respiratory rate and heart rate in ewes during the periparturient period in comparison to those in the diestrus period. The reason for these changes is attributed to the increased demands of fetal growth and the development of extrauterine tissues in late gestation. During the early lactation period in ewes, there was an increase in the respiratory rate and heart rate with the increased metabolic needs during pregnancy and the early postpartum (Al-Bayati and Luaibi, 2020, Singh et al.,2022).

Increased intra-abdominal pressure from the gravid uterus on the rumen during late gestation may be the cause of decreased ruminal movements during the periparturient period in ewes and a somewhat reduced calcium level during the postpartum period; these results agreed with the previous results (Al-Bayati and Luaibi, 2020).

Hematological Findings in Ewes Throughout the Periparturient Period

Hematological parameters changed easily and quickly during the transition period. Our findings revealed a significant decrease in RBCs count, Hb conc, and PCV% throughout the late gestation phase and postpartum phase compared to the prenatal period. Our findings are in agreement with the prior study carried out on sheep throughout the peri-partum phase, which showed that the hematological picture exhibits significant variations throughout the gestational period (David et al., 2020). The authors also reported that the lowest values of hemoglobin concentration (Hb) were observed around the date of birth. Additionally, another study investigated the impact of physiological condition on the hematological picture in Ossimi sheep and observed a significant decrease in PCV% (P< 0.05) throughout late-gestation and early-lactation in ewes compared to non-pregnant ewes (Soliman, 2014). Pregnancy-related physiological hemodilution causes PCV to decrease during this period (Brito et al., 2006). The RBCs count and PCV% may have decreased due to the hemodilution impact brought on by an increase in plasma volume and the enhanced water mobilization to the mammary gland via the bloodstream during the periparturient phase (Raboisson et al., 2017, Bamerny, 2013).

The findings of our study demonstrated that MCH levels drop in the late gestation period, followed by an increase during the postpartum period, but either the decrease or the increase was not significant. These findings are almost identical to those mentioned in the previous report (David et al., 2020), who found that MCH concentration decreased around the time of delivery. In the present study, MCV exhibits a gradual decrease from diestrus to late gestation and postpartum. Similarly, it was reported that MCV exhibited a gradual decrease over the course of the study (pregnancy, birth, and lactation) (David et al., 2020). Other authors reported that MCHC fell below the range of normal values, which is a sign of hypochromia. (Sharma et al., 2015, Pereira et al., 2015, Bezerra et al., 2017).

In the current study, evaluation of platelet count occurs in parallel with the hematological picture, which indicates the presence of a lower platelet count in late gestation and postpartum than diestrus periods. A drop in platelet count throughout the postpartum phase was found in Gaddi sheep (Sharma et al., 2015), which is similar to our findings, while other researchers illustrated that they found opposite findings in the incidence of an increase in the platelet count (Santarosa, 2021).

The results of this study provide evidence for the theory that haematological patterns can significantly alter throughout pregnancy and the postpartum phase. It underlines the necessity for additional, more detailed research on these parameters during physiological conditions related to periods of pregnancy and postpartum to avoid misinterpretation of results.

WBCs count exhibited the same tendency (Santarosa, 2018). The neutrophilia identified at late gestation can be attributed to the elevated cortisol levels. Thus, the release of mature neutrophils into the bloodstream may have been caused by the release of stress hormones (Meyer and Harvey, 2004). Moreover, during postpartum, a mild leucocytosis associated with neutrophilia was observed along with a little left shift brought on by the rise in cortisol, the hormone that initiates delivery. Corticosteroids are also secreted from the adrenal glands of lambs and temporarily enhance neutrophil numbers by causing neutrophil endothelial demargination (Byers and Kramer, 2010, Fonteque et al., 2013). Thus, it can be concluded that the physiological stress-related alterations that took place during late gestation (near to lambing) led to a significant rise in the total number of WBCs count due to neutrophilia and the presence of band cells, which were both distinguished by a slight shift to the left in both groups (late gestation and postpartum) (sharma et al.,2015). Also, these findings support the idea that the increased cortisol concentration might affect the bone marrow and the bloodstream's neutrophil population (Fonteque et al., 2013).

In the current study, neutrophilia was observed in samples collected at late gestation and slightly decreased at postpartum. As the initial line of defense against infection, segmented neutrophils work. The activity of these leukocytes just before birth has been studied by numerous researchers (Fonteque et al., 2013). The same authors noted a decline in segmented neutrophils and total leukocytes during postpartum. Nonetheless, the later authors' observation after delivery also revealed the increase in segmented neutrophils as well as total leukocytes, indicating the potential requirement to replenish the quantity of circulatory cells as a result of the flow to the uterus.

In the present study, eosinophilic counts gradually increased from diestrus to late gestation, and decreased slightly in the postpartum. These results are similar to the previous reports who recorded increased eosinophilic counts gradually until the day of birth before sharply declining on that day (David et al., 2020, Fonteque et al., 2013).

Biochemical Findings in Ewes Throughout the Periparturient Period

It is crucial to understand the metabolic profile during the periparturient period in order to determine nutritional status and prevent illnesses that can disrupt reproduction and production. Total protein (TP), albumin, globulin, and urea are the blood markers used to assess the protein level (Puppel and Kuczynska, 2016). The background information on a total serum protein gives details on protein synthesis, consumption, and excretion, as well as kidney function, hepatic function, and nutritional status. In our study, total protein concentration decreased significantly throughout late gestation and the postpartum phase in comparison with the diestrus phase; this was also evidenced by the lower level of albumin concentration during the periparturient period than it was during the diestrus period. This drop-in serum total protein throughout late pregnancy could be attributed to the usage of the fetus to the amino acids gained from his mother in the synthesis of all its proteins. Synthesizes of proteins are required for the growth of the fetus which is particularly accelerated to the peak in the last third of gestation (Antunovic et al., 2002).

Serum albumin and protein levels dropped during the periparturient period, especially the day after delivery (David et al., 2020). This drop can be anticipated to occur during the third trimester of pregnancy due to three main reasons. First, during late pregnancy and early lactation, less dry matter and crude protein are consumed. Second, as 70 % of the fetus's growth occurs in the final 50 days of pregnancy, there is a greater needing form the fetus for albumin and protein at this time. Finally, the nutritional requirements still increase until the peak of milk production (Houdijk, 2008).

Throughout the experiment, cholesterol levels and triglycerides were significantly higher (*P*< 0.001), throughout the periparturient period when compared with the control group. The highest levels of cholesterol and triglycerides were during the late gestation period compared with the other periods. These results agreed with those authors noticed that pregnancy significantly affected the serum lipid and cholesterol concentrations of Iranian fat-tailed sheep (Nazifi et al., 2002). There was a rise in blood cholesterol and triglyceride levels as the pregnancy progressed.

It was found that the concentrations of cholesterol, triglycerides, HDL-cholesterol, and VLDL-cholesterol were higher (*P*< 0.05) in the week prior to parturition than at other times (Nazifi et al., 2002). Increased lipolysis around parturition is hormonally controlled rather than a sign of low energy. In pregnant ewes, insulin directly affects the metabolism of adipose tissue.

The rise in total cholesterol, triglycerides, and lipoprotein concentrations was attributed to the diminished response of the target tissues to insulin (Iriadam, 2007). This, along with an increase in the mobilization of fatty acids from adipose tissue, provides new sources for fetal growth. As circulating blood triglycerides largely contribute to milk fat production, lipid profiles have been used to predict periparturient diseases (Nazifi et al., 2002).

In contrast to this point of view, there is no differences in cholesterol concentration between pregnant and non-pregnant ewes (Ribeiro et al., 2004).

Urea is produced by the liver's urea cycle and is considered a by-product of protein metabolism. Increased ammonia detoxification may be indicated by a high serum urea level, which is also a risk factor for lipomobilization (Park et al., 2010). In sheep and goats, plasma urea content is regarded as a key predictor of dietary protein supply (Nazifi et al., 2003). Our study revealed that the mean urea concentration increased significantly throughout the periparturient period compared to the gestational one. The same was recorded by the authors who discovered that gestation and lactation characterized by high uremia (Antunovic et al., 2002, Deghnouche et al., 2011). The urea levels start to increase around the ninth week of pregnancy and peak at delivery (Raboisson et al., 2017). The decrease in glomerular filtration rate and lower urea clearance during late pregnancy and lactation are to blame for this increase in blood urea levels (Raoofi et al., 2013).

It was recorded that plasma urea levels began rising in the tenth week of pregnancy and reached their peak at parturition (El-sherif and Assad, 2001, Durak and Altinek, 2006). They hypothesized that the cause of the highest values might be the body's higher cortisol levels, which have an impact on protein catabolism.

The present study showed a significant increase in creatinine level during the postpartum period compared to the late gestation period, but the concentration of creatinine during the late gestation period showed a non-significant increase when compared with the diestrus period. The total body creatinine content, which in turn depends on dietary intake, the rate of creatinine synthesis, and muscle mass, determines how much creatinine is formed (Piccione et l.,2009).

Macro Elements Concentrations in Ewes Throughout the Periparturient Phase

Comparing late pregnancy and postpartum to diestrus, there were obvious variations in the serum levels of ferritin, calcium, and phosphorus (*P*< 0.001).

Ferritin is a major iron storage protein, is crucial for blood synthesis and physiological conditions; serum ferritin is affected by many pathological cases. A ferritin test helps to understand how much iron is stored in the body (Badawi, 2014). The results revealed the presence of significant decrease in ferritin levels in blood of sheep during late gestation (54.70±4.97 *u*g/ dl) and during post-partum (21.85±5.57 ug/dl) changes when compared with diestrus period (84.1±4.35 *u*g/ dl). These findings agreed with the authors who detected alterations in a number of minerals, including iron, in sheep (David et al., 2020).

Calcium levels decreased significantly in late pregnancy and during the postpartum period. These findings corroborated those who noticed that calcium values decreased during the peak of milk production, which might be related to milk production requirements (David et al., 2020). The authors stated that calcium and magnesium are essential for healthy skeletal growth and that a significant amount is secreted in both milk and blood. This shows that the decrease in this mineral's level during the peaks of milk production and during the periparturient period may be related to the demand for milk production. These results are in contrast to the previous report (Boudebza et al., 2016), who conceded that calcium plasma levels greatly rise in late pregnancy and drop significantly in early lactation when compared to the dry period.

Large amounts of phosphorus (P) are also necessary for the mineralization of the skeleton. In the current study, phosphorus levels decreased in the late gestation period compared to their levels in the diestrus period. Some researchers hypothesized that this drop in serum P levels during late pregnancy was caused by a higher rate of P mobilization from the mother's bloodstream into the fetus, which is supplied by rising P absorption from the gut or P resorption from the mother's bones (Gurgoze et al., 2009). The slight fluctuation in the phosphorus profile of the serum was unrelated to the body's physiological status. Nonetheless, values lower than the normal range for other sheep breeds were recorded (5.1 to 7.3 mg/ dL) in some stages of pregnancy, peri-partum, and lactation. This mineral can be transformed into adenosine triphosphate in the postpartum period, which results in a drop in its content. It acts as an indirect measure of energy metabolism (De Oliveira et al., 2014).

Magnesium (Mg) is one of the most frequent enzyme activators, which is necessary for healthy skeletal development. In our study, the lowest Mg levels were found in late pregnancy, while the postpartum period suffered from a slight, non-significant decrease compared to the gestational period. The fact that sheep are usually in a negative energy balance during this time may help to explain why magnesium levels fall throughout the postpartum period, in addition to the stress of lactation and parturition, which results in lipomobilization and magnesium consumption (Martens and Rayssiguier, 1980).

Conclusions

It is concluded that the stressors of the transition period reflected on marked changes in certain clinical, hematological and biochemical parameters of sheep. This alterations during the transition period not only identify which animals are sick but also which animals are prone to get sick. Current findings improve the understanding of the diagnosis and prognosis of diseases in ewes throughout this period, aiming to prevent a drop-in production and, subsequently, an economic loss. Therefore, it is strongly advised that animals consume diets rich in iron and Calcium combinations throughout late gestation, lambing, and the postpartum period to prevent conditions like anemia and osteomalacia.

Institutional Review Board Statement Experimental procedures were approved by the Faculty of Veterinary Medicine committee of South Valley University, Qena, Egypt. All methods were carried out in accordance with relevant guidelines and regulations. All methods are reported in accordance with ARRIVE guidelines. Animals are cared for and used in research and education in accordance with Egyptian laws and OIE animal welfare guidelines. The owners signed an informed consent form to use the animals in the current study.

Conflict of Interest: The authors declare that there is no conflict of interest.

Authors' Contributions: RMI and SYN are mutually contributed to the hypothesis and the design of the scientific manuscript. YAA, FZ, and ZF provided the chemicals and the materials used in this work. RMI and SYN, YAA, FZ, and ZF performed the experimental procedures and analysis. All authors participated in this research work. They also participated in writing the manuscript's draft and revision. All authors have read and approved the final manuscript. The authors agreed to publish the findings generated from this work.

Data Availability Statement: All the data analysed during this study are included in this published article.

References

Al-Bayati OA, Luaibi OK. **2020**. Clinical and biochemical profile of Iraqi local breed cows during pregnancy and early lactation. *Iraqi J Vet Med*. 44(E0): 51-56. doi: 10.30539/ijvm. v44i(E0).1021

Amin YA, Ali RA, Fouad S, Ibrahim RM. **2021**. The deleterious effect of postpartum pyometra on the reproductive indices, the metabolic profile, and oxidant/antioxidant parameters of dairy cows. *Vet World*. 14(2):329. doi: 10.14202/vetworld.2021.329-338

Antunović Z, Senčić Đ, Šperanda M, Liker B. **2002**. Influence of the season and the reproductive status of ewes on blood parameters. *Small Rum Res*. 45(1):39-44. doi: 10.1016/S0921-4488(02)00109-8

Badawi NM**. 2014**. Evaluation of some hematological values and ferritin concentration in normal and emaciated Iraqi sheep. *Kufa J Vet Med Sci*. 5(2). https://journal.uokufa.edu.iq/index. php/kjvs/article/view/4176

Bamerny A. **2013**. Changes in some haemato-biochemical and electrolytes parameters in female Meriz goats during pregnancy and after parturition. *J Anim Sci*. 2(1):11-4.

Bezerra LR, Oliveira WD, Silva TP, Torreão JN, Marques CA, Araújo MJ, Oliveira RL. **2017**. Comparative hematological analysis of Morada Nova and Santa Inês ewes in all reproductive stages. *Pesquisa Vet Brasil*. 37:408-14. doi:

10.1590/S0100-736X2017000400017

Boudebza A, Arzour-Lakhel N, Abdeldjelil M, Dib A, Lakhdara N, Benazzouz H, Benlatreche C. **2016**. Blood biochemical parameters in Ouled Djellal ewes in the periparturient period. *Der Pharma Chemica*. 8(18):406-10. ISSN 0975-413X

Brito A, González FD, Ribeiro LA, Campos R, Lacerda L, Barbosa PR, Bergmann G. **2006**. Blood and milk composition in dairy ewes from southern Brazil: variations during pregnancy and lactation. *Ciência Rural*. 36: 942-8. doi: 10.1590/S0103-84782006000300033

Byers S, Kramer J. **2010**. Normal hematology of sheep and goats. Schalms Veterinary Heamatology Weiss, DJ and Wardrop, KJ 6th ed *Wiley Blackwell Publishing Ltd Ames*, Iowa, USA. 836-42. ISBN: 978-0-8138-1798-9

Constable PD, Hinchcliff KW, Done SH, Grunberg W. **2017**. Veterinary Medicine, *A textbook of the diseases of cattle, horses, sheep, pigs, and goats*. 11 edition vol.1. ISBN: 9780702052460 (Print), ISBN: 9780702070587 (Online)

David CM, Costa RL, Parren GA, Rua MA, Nordi EC, Paz CC, Quirino CR, Figueiredo RS, Bohland E. **2020**. Hematological, parasitological and biochemical parameters in sheep during the peripartum period. *Revista Colombiana de Ciencias Pecuarias*. 33(2):81-95. ISSN 0120-0690On-line version ISSN 2256-2958

De Oliveira FJ, Perazzoli D, da Silva LGC, Aragão RB, Martins CF, Pereira RA, Ferreira MB, Pino FABD, Rabassa VR, Corrêa MN. **2014**. Avaliação de parâmetros bioquímicos clínicos de ovelhas do grupo genético pantaneiro gestantes e não gestantes. *Brazil J Vet Res Anim Sci*. 51(2):111-7. doi: 10.11606/issn.1678-4456.v51i2p111-117

Deghnouche K, Tlidjane M, Meziane T, Touabti A. **2011**. Effects of the physiological stage on some blood biochemical parameters in Ouled Djellal ewes from arid South East Algeria. *Revue de Méd Vet.* 162(1):3, ISSN: 0035-1555, http://www.revmedvet.com/

Drackley J, Cardoso F. **2014**. Prepartum and postpartum nutritional management to optimize fertility in high-yielding dairy cows in confined TMR systems. *Animals*. 8(s1):5-14. doi: 10.1017/S1751731114000731

Durak MH, Altinek A. **2006**. Effect of energy deficiency during late pregnancy in Chios ewes on free fatty acids, β-hydroxybutyrate and urea metabolites. *Turk J Vet and Anim Sci*. 30(5), 497-502. https://journals.tubitak.gov.tr/veterinary/vol30/iss5/11

El-Sherif M.A, Assad F. 2001. Changes in some blood constituents of Barki ewes during pregnancy and lactation under semi-arid conditions. *Small Rum Res*. 40(3), 269-277. doi: 10.1016/S0921-4488(01)00174-2

Elischer M, Sordillo L, Siegford J, Karcher E. **2015**. Characterizing metabolic and oxidant status of pastured dairy cows postpartum in an automatic milking system. *J Dairy Sci*.

98(10):7083-9. doi: 10.3168/jds.2014-8941

Fonteque JH, Saito ME, Barioni G, Valente ACdS, Takahira RK, Kohayagawa A. **2013**. Leukocyte count and neutrophil oxidative burst in Saanen goats in the pregnancy, parturition and postpartum periods. *Pesquisa Vet Brasil*. 33:63-70. doi: 10.1590/S0100-736X2013001300011

Gürgöze SY, Zonturlu AK, Özyurtlu N, Icen H. **2009**. Investigation of some biochemical parameters and mineral substance during pregnancy and postpartum period in Awasi ewes. *Kafkas Üni Vet Fakültesi Dergisi*. 15(6):957-63. doi: 10.9775/kvfd.2009.858

Houdijk J. **2008**. Influence of periparturient nutritional demand on resistance to parasites in livestock. *Parasit Immunol*. 30(2):113-21. doi: 10.1111/j.1365-3024.2008. 00992.x

Iriadam M. **2007**. Variation in certain haematological and biochemical parameters during the peri-partum period in Kilis does. *Small Rum Res*. 73(1-3), 54-57. doi: 10.1016/j.smallrumres.2006.11.001

Jackson PGG, Cockcroft PD. **2002**. Clinical examination of Farm animals. Blackwell Science Ltd, State, Avenue, Ames, Iowa, USA. 219-247, 301-305. doi: ISBN: 978-0-470-75242-5

Martens H, Rayssiguier Y. **1980**. Magnesium metabolism and hypomagnesaemia. *Digest Physiol Metabol Rum*. 447-466. doi: 10.1007/978-94-011-8067-2_21

Mee JF. **2013**. Why do so many calves die on modern dairy farms and what can we do about calf welfare in the future? *Animals*. 3(4):1036-1057. doi: 10.3390/ani3041036

Meyer DJ, Harvey JW. **2004**. Veterinary laboratory medicine: interpretation & diagnosis. 2a ed PhiladelphiaSaunders. 351. ISBN: 07-216-26548

NRC, National Research Council (US). **2007**. Committee on nutrient requirements of small ruminants, national research council, committee on the nutrient requirements of small ruminants, board on agriculture, Division on Earth, & Life Studies. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. *China Legal Publish House*.

Nazifi S, Saeb M, Ghavami S. **2002**. Serum lipid profile in iranian fat-tailed sheep in late pregnancy, at parturition and during the post-parturition period. *J Vet Med Series A*. 49(1):9-12. doi: 10.1046/j.1439-0442.2002. 00405.x

Nazifi S, Saeb M, Rowghani E, Kaveh K. **2003**. The influences of thermal stress on serum biochemical parameters of Iranian fat-tailed sheep and their correlation with triiodothyronine (T3), thyroxine (T4) and cortisol concentrations. *Comp Clin Path.* 12(3),135-9. doi: 10.1007/s00580-003-0487-x

Oikonomidis IL, Brozos C, Kiossis E, Kritsepi-Konstantinou M. **2018**. Combined and breed specific RIs for hematologic, biochemical, and hormonal analytes in Chios and Florina adult rams. *Vet Clin Path.* 47(1):56-68. doi: 10.1111/vcp.12558

Park A, Shirley J, Titgemeyer E, Cochran R, DeFrain J, Wickersham E, Johnson DE. **2010**. Characterization of plasma metabolites in Holstein dairy cows during the periparturient period. *Inter J Dairy Sci*. 5(4) :253-63. doi: 10.3923/ijds.2010.253.263

Pereira B, Bezerra LR, Marques CAT, Araújo MJd, Torreão JNdC, Machado LP. **2015**. Hematological profile of Santa Inês ewes supplemented on pasture at the last third of pregnancy and postpartum. *Ciência Anim Brasil*. 16:350-7. doi: 10.1590/1089-6891v16i327573

Piccione G, Caola G, Giannetto C, Grasso F, Runzo SC, Zumbo A, Pennisi P. **2009**. Selected biochemical serum parameters in ewes during pregnancy, post-parturition, lactation and dry period. *Anim Sci Papers Reports*. 27(4):321-30. https://www.igbzpan.pl/uploaded/FSiBundleContentBlockBundleEntityTranslatableBlockTranslatableFilesElement/filePath/526/str321-330.pdf

Puppel K, Kuczyńska B. **2016**. Metabolic profiles of cow's blood; a review. *J Sci Food Agri*. 96(13):4321-8. doi: 10.1002/jsfa.7779

Raboisson D, Albaaj A, Nonne G , and Foucras, G. **2017**. High urea and pregnancy or conception in dairy cows: A meta-analysis to define the appropriate urea threshold. *J Dairy Sci*. 100(9):7581-7587. doi: 10.3168/jds.2016-12009.

Raoofi A, Jafarian M; Safi S, Vatankhah M. 2013. Fluctuations in energy-related metabolites during the peri-parturition period in Lori-Bakhtiari ewes. *Small Rum Res*. 109(1):64-8. doi: 10.1016/j.smallrumres.2012.06.012

Ribeiro LAO, Mattos RC, Gonzalez FHD, Wald VB, da Silva MA, La Rosa VL. **2004**. Perfil metabólico de ovelhas Border Leicester x Texel durante a gestação e a lactação Metabolic profile of Border Leicester x Texel ewes during pregnancy and lactation. *Letras*. 7(08):1-7. https://www.semanticscholar.org/paper/Perfil-metab%C3%B3lico-de-ovelhas-Border-Leicester-x-a-e-Ribeiro-Mattos/b6d1a62c8687f603a92a80b84e8765b53bb7c004

Santarosa BP. **2018**. Avaliação clínica, hemogasométrica, do perfil metabólico e do estresse oxidativo de ovelhas prenhes da raça dorper: *Comparação entre Gestações Única e Gemelar*. https://repositorio.unesp.br/handle/11449/153181

Sharma A, Kumar P, Singh M, Vasishta N. **2015**. Haemato-biochemical and endocrine profiling of north western Himalayan Gaddi s heep during various physiological/reproductive phases. *Open Vet J*. 5(2):103-7. ISSN: 2226-4485 (Print), ISSN: 2218-6050 (Online)

Silanikove N. **2000**. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock Prod Sci*. 67(1-2):1-18. doi: 10.1016/S0301-6226(00)00162-7

Singh R, Singh A, Beigh SA, Sharma N; Singh V. **2022**. Effect of physiological status and parity on metabolic and trace element profile of crossbred Rambouillet sheep of Himalayan region. *Trop Anim*

Health Prod. 54(1), 63. doi: 10.1007/s11250-022-03068-7

Skotnicka E, Muszczyński E, Suska ZM. **2011**. Effect of the periparturient period on serum lipid and cholesterol lipoprotein concentrations in goats (Capra hircus). *Acta Vet Hung.* 59(4):445-54. doi: 10.1556/AVet.2011.032Corpus ID: 26094538

Soliman E. **2014**. Effect of physiological status on some hematological and biochemical parameters of Ossimi sheep. *Eg J Sheep Goats Sci*. 9(2):1-10. doi: 10.21608/EJSGS.2014.26736

Sordillo L, Mavangira V. **2014**. The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. *Anim Prod Sci*. 54(9):1204-14. doi: 10.1071/AN14503

Stoldt A-K, Derno M, Nürnberg G, Weitzel JM, Otten W, Starke A, Wolffram S, Metges CC. **2015**. Effects of a 6-wk intraduodenal supplementation with quercetin on energy metabolism and indicators of liver damage in periparturient dairy cows. *J Dairy Sci*. 98(7):4509-20. doi: 10.3168/jds.2014-9053

Vasava PR, Jani R, Goswami H, Rathwa S, Tandel F. **2016**. Studies on clinical signs and biochemical alteration in pregnancy toxemic goats. *Vet World*. 9(8):869. doi: 10.14202/vetworld.2016.869-874

Zamuner F, DiGiacomo K, Cameron A, Leury B. **2020**. Endocrine and metabolic status of commercial dairy goats during the transition period. *J Dairy Sci*. 103(6):5616-28. doi: 10.3168/jds.2019-18040