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Hematological and Biochemical Blood Parameters During Pregnancy in Algerian Rembi Ewes

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PREGNANT animals need high energy to maintain pregnancy and embryo development. Animals supplemented with low energy diets are predisposed to inflammatory processes, immune system dysfunction, and maternal metabolic issues during pregnancy. This research work aimed to declare the changes in hemato-biochemical parameters in pregnant Rembi sheep. Thirty healthy *Rembi* ewes were synchronized in October 2020 for the current study. Beginning in the first month of pregnancy, blood was taken monthly from the jugular vein. Our results showed that plasma glucose (GLU), urea, and globulin(Glob) decreases were highly significant ($P<0.05$) during the last two months of pregnancy. Conversely, the values of triglyceride (TG), Albumin (Alb), Alanine Aminotransferase (ALT), and Aspartate Aminotransferase (AST) recorded a highly significant increase at the end of the pregnancy. Furthermore, serum levels of Calcium (Ca) and Phosphorus (P) recorded a highly significant ($P<0.05$) decrease during the last month of gestation. Total protein (TP) and Gammaglutamyl Transferase (GGT) showed significant ($P<0.05$) decreases during the first gestational month. Regarding the hematological parameters, especially erythrogram, in the last month of gestation, ewes present macrocytic hypochromic anemia with a highly significant ($P<0.05$) decrease in the values of red blood cells (RBC) and hemoglobin (HBC). White blood cells (WBC) were found to be significantly higher ($P< 0.05$) in the final month of pregnancy compared to the first. Blood parameters may change during pregnancy, and that can reflect the health of the ewes.

Keywords: Ewe, Biochemical Parameters, Hematological Parameters, Pregnancy, *Rembi.*

Introduction

In Algeria, sheep represent the highest domestic animal population. They are important to the country's economy, particularly in meeting population needs such as meat and wool [1]. In 2017, the Algerian steppes had an important growth in livestock numbers, which increased up to 28 million ovine heads [2]. The classification of the Algerian sheep population based on mapped breeding zones in the study of Djaout et al. [3] showed that 12 ovine breeds are phenotypically characterized.

Rembi sheep is an important breed in livestock farming in Algeria; it is well adapted to extreme cold and mountain conditions and is known by its cold and drought resistance [4]. This breed is predominant in the regions of Ouarsenis and the highlands of Tiaret, west Algeria [3].

Several studies have shown great variation in the hematological parameters during the different pregnancy stages in small ruminants [5, 6]. According to Plaza Cuadrado et al. [7], blood biochemical parameters are important indicators of metabolic activity in ewes. As per the stages of life,

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mineral components play an essential role in cellular metabolism, homeostasis, reproduction and growth.

In small ruminants, pregnancy is physiologically stressful and considered one of the most critical periods in the reproductive cycle of females, during which the endocrine milieu impacts the nutritional and metabolic profile [8]. According to Yaqub et al. [9] ewe's gestation period is characterized by important adaptive changes, principally in hematological parameters, in order to provide a favorable environment for fetal development. These adaptive mechanisms can disturb homeostasis, thereby compromising the health of the ewes and their fetuses.

In veterinary medicine, blood parameter analysis is important in that it complements clinical findings and contributes to an accurate diagnosis. Hematological and biochemical parameters are often used for assessing the health status of individual animals and homeostatic impairments [10].

In order to ensure an accurate interpretation of analysis results, reference blood values should be available for each animal species taking into account the environment [11], the management of herds, detailed characterization of breeds, breeding systems and critical phases. However, these parameters are often misinterpreted as being obtained from other breeds without taking these factors into consideration.

Therefore, the present study aimed to determine the reference values for hematological and biochemical profiles in *Rembi* ewes in Algeria during different stages of pregnancy. This knowledge is necessary to help in better management practices, nutritional practices, and the diagnosis of health conditions, particularly because of the large heterogeneity of values within this species due to differences in breed, gender, age, nutrition, management systems [11], and physiological status [12].

The data obtained in this study will contribute to the diagnosis of various physiological and pathological cases encountered during gestation, the monitoring of the course of diseases, as well as future research on *Rembi* ewes.

Material and Methods

Ethical approval

All procedures used in this study were approved by Tiaret University's Animal Ethics Committee (Algeria).

Study area

Tiaret lies at the southern end of the Ouarsenis Massif (in the Tell Atlas Mountains) on the slopes of Mount Guezoul (1375 meters above sea level) on the High Plateau. Tiaret is a major agricultural center on the Sersou Plateau, dealing in wheat and livestock.

According to the Köppen classification, the climate in the Tiaret region is CWa, characterized as a hot, summer Mediterranean climate. The average rainfall is 472 mm per year, and the mean temperature is 15.5 °C.

Animals

The current study included 30 ewes of the *Rembi* breed, aged 3 to 7 years and apparently healthy, with an estimated weight of 45.61 ± 5.45 kg. They were housed in the facilities of the Technical Institute of Breeding of Qsar Challala in Tiaret. This farm›s breeding system was intensive, and the ewes were fed 500 g of hay per animal per day, as well as Alfalfa hay ad libitum. In October 2020, these ewes were synchronized with the male effect, and their pregnancy was confirmed definitively by ultrasound examination for 3 months afterwards.

Blood Sampling

Blood was collected via jugular vein puncture in Ethylen Diamine Tetra Acetic (EDTA) tubes for hematological analysis and heparin tubes for biochemical analysis plasma in the morning before feeding the animals. They were then transported to the medical hematology-biochemistry laboratory of the Tiaret Institute of Veterinary Sciences, within approximately 2 hours. The samples in heparin tubes were centrifuged at 3000 turns per minute for 15 minutes. Plasma was aspirated, immediately frozen, and stored at -20°C until been analyzed. *Laboratory Analyzes*

Blood metabolites were determined by spectrophotometer (Optizen®) using Spinreact® kits for the following parameters: Glucose (GLU); Cholesterol (CHO); Triglyceride (TG); (TP); Albumine (Alb) ; Globuline (Glob); Urea(Urea); Alanine Aminotransferase(ALT); Aspartate Aminotransferase(AST); Gammaglutamyl Transferase (GGT); Calcium (Ca); Phosphorus (P); Iron (Fe). Whereas red blood cells (RBC), haemoglobin (HGB), hematocrit (HTC), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and white blood cell (WBC) were analyzed using an automated (Orphée ®) mythic 18.

Statistical Analysis

Statistical analysis of the collected data was performed using STATISTICA software (version 7, Statsoft, Tulsa. OK) to evaluate the variation of the means with the standard deviation of each of the studied parameters. The one-factor ANOVA was used to compare the means, followed by Duncan's post-hoc test. Differences were considered statistically significant at a *p*-value of less than 0.05 across all statistical analyses.

Results

The results illustrated in Table 1 show that the mean of plasma glucose decreases gradually with gestational advancement, and a highly significant ($P < 0.05$) value is registered in the 1st month. On the other hand, we found that the plasma triglyceride concentration in pregnant *Rembi* ewes registered a highly significant value ($P < 0.05$) in the ٤th month of pregnancy compared to the other months.

In the current study, cholesterol plasma concentration had a significant ($P < 0.05$) influence, and the higher value was signaled in the 4th month. Furthermore, we find a significant ($P< 0.05$) difference in total protein (TP) plasma levels during pregnancy, with the highest value occurring in the first month. In addition, the plasmatic concentration of albumin gradually increases with gestation, reaching the highest significant ($P< 0.05$) values in the last month. The value of globulin recorded its lowest value in the fourth and fifth months of pregnancy. Regarding urea plasma concentrations, the lowest significant value in these ewes was observed in the last month of gestation.

Analyses of ALT and AST activities showed a highly significant ($P <0.05$) increase in the last two months of pregnancy compared to other months. Likewise, GGT activity in this study recorded a highly significant ($P <0.05$) increase in the second month of gestation compared to the other months. A highly significant ($P <0.05$) increase was registered in serum levels of phosphorus in the 4th month. Conversely, the calcium plasma concentrations of these ewes decreased significantly ($P <0.05$) in the 5th month of gestation as compared to the other months. The first month of pregnancy had a higher significant ($P< 0.05$) value in plasmatic iron than the other months of pregnancy.

Concerning the hematological profile of pregnant *Rembi* ewes (Table 2), we recorded lower significant ($P <0.05$) values of RBC and Hb in the 1st month of gestation compared to the other months. A highly significant difference ($P <0.05$) is recorded in the MCHC and MCH values in the 2nd month of gestation. However, no significant difference was recorded in the values of HTC. The leuckograms significantly ($P <0.05$) higher for the WBC, monocytes and neutrophils in the 5th month.

TABLE 1. Variation of biochemical parameters in *Rembi* ewes during the months of pregnancy.

Parameters	1st (N=30)	2nd (N=30)	3rd (N=30)	4th (N=30)	5th (N=30)
GLU (g/l)	0.4910 ± 0.13 *	0.4459 ± 0.20	0.2583 ± 0.07	0.1697 ± 0.24	0.2434 ± 0.09
CHO (g/l)	1.1903± 0.67	1.2417 ± 1.22	0.9155 ± 0.47	1.1697 ± 1.21	1.2141 ± 0.65
TG (g/l)	0.3286± 0.24	0.4483 ± 0.29	0.1569 ± 0.09	0.5434 ± 0.46*	0.4603 ± 0.21
TP (g/l)	6.9276± 1.31 *	5.6528 ± 1.67	5.9552 ± 1.35	5.6648 ± 5.97	5.9793 ± 0.94
Alb (g/l)	1.4959± 0.29	3.4769 ± 0.69	4.1245 ± 1.44	4.1162 ± 4.95	4.9593 ± 1.13 *
Glob (g/l)	5.4314± 1.38*	2.1752 ± 1.47	1.8307 ± 1.28	1.5497 ± 1.02	1.0241 ± 0.59
Urea (g/l)	0.4931± 0.20 *	0.4683 ± 0.12	0.4186 ± 0.18	0.3362 ± 0.29	0.2900 ± 0.11
ALT (U/L)	21.9648 ± 19.70	29.1469 ± 19.38	25.9490 ± 19.43	48.2345 ± 36.01 *	33.5914 ± 22.73
AST (U/L)	73.8221 ± 47.29	83.8197 ± 45.04	100.5348 ± 31.09	145.5317 ±122.56	172.0824 ± 111.45*
GGT (U/L)	30.8162 ± 15.99	57.7059 ± 35.62 *	22.2338 ± 11.18	26.2328 ± 8.56	32.0600 ± 15.81
Ca (mg/l)	8.1521 ± 1.79	8.3417 ± 2.28 *	7.5362 ± 2.59	8.0752 ± 1.71	5.0593 ± 1.11
P (mg/l)	6.4907 ± 1.74	6.4093 ± 1.94	7.6214 ± 2.37	8.3228 ± 2.36*	5.0141 ± 2.68
Fe (mg/l)	0.1414 ± 0.06 *	0.1200 ± 0.04	0.1045 ± 0.04	0.1231 ± 0.03	0.1176 ± 0.03

*Refers to a significant difference in the same line ($P < 0.05$). GLU = glucose; CHO = cholesterol; TG = Triglyceride; TP = total protein; Alb = Albumine; Glob = Globuline; Urea= (Urea); ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gammaglutamyltransferase; CA = calcium; P = phosphorus; Fe = iron.

TABLE 2. Variation of hematological parameters in *Rembi* ewes during the months of pregnancy.

Parameters	1st (N=30)	2nd (N=30)	3rd (N=30)	4th (N=30)	5th (N=30)
RBC ($x10^6$ /mm^3)	6.3179± 0.84	8.4217± 0.81	8.7345± 0.65 *	8.5683± 0.77	7.5741± 0.83
HGB (g/dL)	7.338± 1.23	11.217± 1.25	11.117± 0.82	9.662± 0.82	8.452± 0.85 *
HTC (%)	28.062± 37.58	28.479± 2.94	28.879± 2.12	28.859± 1.92	26.303± 2.19
MCV (fL)	33.641± 2.53	33.824± 1.63	33.090± 1.53	33.917± 1.90	34.876± 2.10 *
MCH (pg)	12.000± 4.56^A	13.310± 0.75 *^{B}	12.738± 0.59^A	11.290± 0.58^B	11.179± 0.60^B
MCHC(g/dL)	35.369± 10.27	39.366± 0.86 *	38.493± 0.72	33.328± 1.24	32.103± 1.13
WBC(/mm^3)	7855.17± 4208.96	10596.55± 2220.12	11272.41± 2482.64	12427.59± 1781.63	14837.93± 1755.08 *
LYM(/mm^3)	2122.10± 1471.75*	1464.55± 490.76	1708.55± 718.42	1493.21± 883.54	897.10± 578.12
MON(/mm^3)	1948.76± 1254.17	4013.34± 1473.52	4331.00± 1372.39	3884.03± 1323.19	4690.93± 1442.290*
NEUT(/mm^3)	3431.97± 2060.58	4843.07± 1571.42	4094.14± 1369.01	5987.24± 1337.55	8747.00± 1821.33 *
EOS(/mm^3)	179.21± 222.18	292.28± 328.61	403.14± 318.49	436.59± 382.24 *	222.24± 234.54
BAS (/mm^3)	125.97± 159.80	142.41± 191.66	700.21± 543.24 *	631.59± 744.37	290.14± 420.37

*Refers to a significant difference in the same line ($P < 0.05$)
RBC = red blood cell; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; WBC = white blood cell; LYM = Lymphocytes; MON = Monocytes; NEUT = Neutrophils; EOS = Eosinophils; BAS= Basophils.

Discussion

Although it is a physiological state, pregnancy is associated with a disturbance in homeostasis. The present study showed a gradual decrease in plasma glucose concentration during gestation. Subsequently, a highly significant ($P <0.05$) value was observed in the first month. Similarly, in the study of Ismaeel *et al.* [13] the concentration of plasma glucose decreased progressively with gestation. This could be linked to the gradual decrease in maternal glucose levels and the increase in fetal needs. On the other hand, research has shown that blood glucose concentration increases with advancing gestation or reaches a maximum value in the last third. This increase is linked to the decrease in insulin levels, which could be due to the negative energy balance [8].

The pregnancy period in the current study had no significant influence on cholesterol plasma concentration. In contrast, significant increase in cholesterol level was recorded during late pregnancy in *Ouled Djallal* ewes as a result of the decreased sensitivity of adipose tissue to insulin [14].

Plasma triglyceride concentration in pregnant *Rembi* ewes registered significant increase ($P<0.05$) in the 4th month of pregnancy compared to the other months. Previous findings on serum triglyceride in pregnant sheep related hypertriglyceridemia during late pregnancy to increased lipid mobilization in response to high energy demands [15,16], while others reported no significant changes in plasma concentration of triglyceride during gestation [17].

Changes in total proteins, albumin, and globulin levels in plasma may reflect the animal's liver function, energy metabolism, and immune function status. All of the total protein plasma samples showed significant ($P< 0.05$) differences between the months of pregnancy. The decrease in total protein values observed in ewes during the month of gestation is probably due to the active placental transport of amino acids to meet the needs of fetal development during this period.

Our results are in agreement with those reported in pregnant ewes [18, 13, 19] and are consistent with the finding of a significantly lower TP concentration during the end of pregnancy. In contrast, no significant difference was recorded in the serum concentration of the total proteins throughout gestation and post-partum. In the present study, the plasmatic concentration of albumin increases gradually with gestation, reaching the highest values in the last two months. Conversely, the levels of globulin record the lowest value in the 4th and 5th months of pregnancy.

According to Soliman [21] , the increase in albumin at the end of ewes› gestation coincides with the increase in energy requirements for fetal growth. Our findings regarding the decline in plasma globulin during late pregnancy are in line with a previous study [22].These researchers attributed hypoproteinemia and hypoglobulinemia during the peripartum period to the extensive extraction of globulin for colostrum production in the mammary glands.

Moreover, the lowest serum concentrations of urea in the ewes of the current study were observed during the 5th month of gestation with a significant difference (P <0.05), while others reported a significant increase in uremia at the end of sheep pregnancy [23,17]. This could be due to decreased glomerular filtration rate and reduced urea clearance.

The significant (P <0.05) elevated levels of ALT and AST activities were observed in the last two months compared to other months in our results. These findings were similar to those of previous studies carried out on pregnant ewes and reflect the increase in ALT and AST activities during late pregnancy, which has been linked to the accumulation of triglycerides in the liver that contributes to its dysfunction [15, 17, 24]. According to Roy *et al.* [25], the increase in ALT activity during the prepartum period can be due to the release of this enzyme from the placenta and uterus.

Analyses of GGT activity in this study showed a highly significant (P <0.05) increase in the second month of gestation compared to the other months. A number of studies have reported that the GGT activity in ewes increases significantly at the end of pregnancy following an increased hepatic metabolism and hepatic lesions that occur in ewes during this period [26, 24].

In ruminants, minerals, especially calcium and phosphorus, play an important role in late gestation when the fetal skeleton is mineralized. In our research, serum levels of phosphorus increased gradually to reach their highest value during the 4th month of pregnancy, and subsequently, a highly significant decrease was recorded in the last gestation month. The same findings were reported for pregnant ewes in the study of Boudebza *et al.* [23], which indicated that the plasma phosphorus level was significantly lower at the end of gestation. These authors related the decrease in serum phosphorus levels at the end of pregnancy to an increase in the rate of mobilization of phosphorus out of the maternal circulation towards the fetus. Similarly, in our study, the pregnancy period had a significant influence (P <0.05) on plasma calcium concentration, with the lowest value recorded in the last month of pregnancy.

Other studies also recorded a significant increase in calcium in ewes during late pregnancy and referred their results to increased secretion of the parathyroid hormone during gestation in order to meet calcium needs of the fetus [13, 22].

A higher significant increase (P <0.05) in plasma iron was obtained during the first month of pregnancy compared to the other months in the ewes of our study and this may be related to the consumption of this mineral by the fetus and/or liver damage. In contrast, in *Saidi* ewes in Egypt, serum iron concentration was significantly higher during the last month of gestation compared to that obtained in ewes during the first month postpartum and that obtained in non-pregnant ewes [27]. Nonetheless, the decrease in iron concentration in pregnant ewes in the study of Kozat et al. [28], was related to the infection by gastrointestinal parasites.

The lowest values of RBC and HGB were registered in the first month of pregnancy, and this may be due to the quantity of food ingested by ewes in this period. Concerning RBC and HGB levels, a highly significant decrease (P <0.05) was recorded between the 4th and the 5th month of gestation. These results are in agreement with reports on pregnant *Santa Inês* and *Morada Nova* ewes by Bezerra *et al.* [5] and *Zeta Zuja* ewes by Zvonko *et al.* [29]. Previous findings on ovine erythrocyte indices during pregnancy were contradictory. Several studies have shown that RBC and HTC values in ruminants decrease at the end of gestation due to hemodilution, which is responsible for improved blood supply to the uterus and placenta [30, 9]. However, others have obtained an increase in the indices of the red blood cells [21,31]. In our study, no significant difference was obtained in HTC values throughout gestation.

Additionally, a highly significant difference (P <0.05) was recorded in MCHC values during the different months of gestation in our results, where the lowest values were recorded in the 5th month. Plaza Cuadrado *et al.* [6] attribute the decrease in HGB and MCHC values in ewes at the end of gestation to the deficit of hemoglobin

synthesis in *Poil Créole* ewes, which could be due to the great loss of iron caused by gastrointestinal parasites. On the contrary, MCV values recorded a highly significant ($P < 0.05$) increase in the last month of pregnancy, which agrees with the study of Greguła-Kania et al. [30].The macrocytic hypochromic anemia encountered in ewes during late pregnancy in our study could be a physiological response that preserves oxygen content in the blood for the fetus.

Conclusion

In conclusion, pregnancy is a crucial physiological state in ewes, characterized by important adaptive changes in the metabolism profile, and hematological parameters offer a favorable milieu for fetus growth. Medical analysisof hemato-biochemical parameters can be used as good indicators of these changes in ewes during pregnancy. The end of pregnancy in *Rembi* ewes is characterized by decrease in the plasma concentrations of glucose and mineral profiles. The increase in transaminase (ALT, AST) values occurred around the time of the last weeks of gestation, which could be related to the alteration of hepatic metabolism due to the energy deficit. In *Rembi* ewes, we registered a great change in erythrocyte parameters, resulting in a decrease in RBC and HGB values during the last month of pregnancy due to the hem dilution effect.

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Conflicts of interest

Authors declare no conflict of interest.

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