**Factors Influencing the Hematological Parameters in Ewes of the Rembi Breed during Late Pregnancy in Tiaret Region, West of Algeria**

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**Abstract**

Late pregnancy is a critical period in prolific ewe, due to the metabolic and hormonal changes necessary for fetal development. These variations in physiological status affect the normal hematological values of animals. In addition, factors such as age, parity, and aliment also influence these hematological values. This paper details a study carried out on 74 ewes, of *Rembi*breed, aged 2 to 7 years old, and situated in semi intensive system coming from 10 farms in the region of Tiaret. The choice of ewes reaching the third trimester of pregnancy was made randomly in order to assess the changes in hematological profile of the ewes during this period, and the influence of certain factors on the latter. An increase in white blood cell WBC (18.3 × 103 / mm3), characterized mainly by anincrease of neutrophils and monocytes, and decrease in haematocrit(Ht), haemoglobin (HB)and red blood cell(RBC) values compared to normal values, were recorded. Statistical analyzes showed an influence of farm, type of aliment, and parity on the values of WBC, RBC, HB and Ht. The end of gestation in prolific sheep requires improvement in terms of type of aliment and hygiene in order to avoid anemia, metabolic diseases and infectious diseases.

**Keywords:** *Ewes, Hematological Parameters, Late Pregnancy, Rembi.*

**Introduction**

In Algeria, sheep farming consists of 28.39 million heads, and holds a strategic importance in the agricultural economy (FAOSTAT, 2017). *Rembi* breed occupies almost the entire East to West steppes and highlands of the country, due to their great rusticity. This type of farming is particularly suitable for the regions of Ouarsenis and the highlands of Tiaret (Djaout, *et al.* 2017).

Diseases, reduced production performance and animal reproduction, can cause significant economic losses. Several hematological parameters can be used for the prognosis and diagnosis of such diseases (AL-Hadithy, *et al.,* 2014). This is because hematological parameters are reliable tools for detecting certain changes in animal's health and their physiological status, which cannot be detected during clinical examination (Badawi, *et al.,*  2014).

Recent studies showed that a number of factors can influence blood parameters in small ruminants. These factors include season, breed (Rathwa, *et al.,* 2017), environmental factors (Titaouine, *et al.,* 2017), stress (Oramari, *et al.,* 2014), the production/breeding system (Antunović, *et al.,*2017), and the physiological stage of the ruminants (Stevanović, *et al.,*2015).

From a health, production and nutrition perspectives, peripartum, especially late pregnancy and initial lactation, is the most critical period in the life of prolific ewes (Antunovic, *et al.,*2011) because of the high nutritional requirements for fetal development and milk production (Piccione, *et al.,* 2009). During this period, the hematological and biochemical parameters are effective assessors of the nutritional aspects, homeostasis, health and adaptation of the various sheep breeds (Ali, *et al.,* 2010).

To this end, the aim of our study is to evaluate the variations of hematological profile for *Rembi* ewes in their late pregnancy, in different farms in the region of Tiaret. The influence of factors such as aliment, age, parity, farm, season, body score (BS), was evaluated.

**Materials and methods**

**Study area**

The city of Tiaret is located in the west of Algeria, occupying 20 050 05 km². This region is considered as a semi-arid area (ANDI, 2013).

**Animals**

This study was conducted on 74 *Rembi* breed ewes during late pregnancy, clinically healthy, primiparous and multiparous the mean of here parity estimated (5.12 ± 2.65), aged 2 to 7 years with an average of (4.53 ± 1.72) and distributed over 10 different farms in Tiaret region. All the farms visits were almost in the same conditions of livestock management.Each farms used a different type of aliment, the ewes of the first farm were fed barley with lentil bran, the second farm uses barley and straw, the third farm aliment here ewes by the ground barley and the straw, farm number four uses barley and wheat bran, the sheep of the fifth farm were pastured and supplemented with barley, the sheep of the sixth farm were pastured and supplemented with wheat bran, ewes from the seventh and eighth farms were only pastured, the ninth farm uses the same aliment as the sixth one supplemented with wheat bran and pasture, the tenth farm uses a ration consisting of corn and barley and pasture. Note that the amount ingested by pregnant ewes rarely exceeded 1 kg per day. Our study runs from December 2018 to June 2019, we give ourselves the name of winter season for all samples taken in the month of December, January and February, the name of spring season for samples taken in the month March, April, May and June. The body score (BS) of the ewes was assessed by two operators using a scoring grid ranging from 1 to 5 (1 = emaciated to 5 = obese) (Russel, *et al.,* 1969). The recorded BS in our sampling had a mean of (2.3±0.44).

**Blood samples**

Blood samples were collected in EthylenDiacid Tetra Acetic (EDTA) tubes **(FLmedical®),** from pregnant ewes in the morning before feeding, by puncturing the jugular vein, using disposable needles. They were then transported to the medical hematology-biochemistry laboratory of Tiaret Institute of Veterinary Sciences, within approximately 2 hours.

**Laboratory analyzes**

Hematological analysis was performed by means of a mythic 18 automat **(Orphée ®).** The studied hematological parameters were: white blood cells (WBC), lymphocytes (Lym), monocytes (Mono), neutrophils (Neu), eosinophils (Eos), basophils (Baso), red blood cell (RBC), hemoglobin (HB), haematocrit (Ht), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and platelets (PLT). Blood smears for each studied subject were prepared on slides previously cleaned, then stained with RAL 555, and observed under the immersion microscope (x100) **(OPTIKA ®)** for the determination of the leukocyte formula.

**Statistical analysis**

The global mean value, standard deviation, maximum and minimum value of each hematology parameter of the studied ewes were calculated. These calculated global means were compared with the reference standards of Kramer, et al. (2006). Moreover, the variation of the means with the standard deviation of each of the studied parameter were evaluated according to the type of aliment, farms, (BS) body score, parity, season and age.

Statistical analysis of collected data was performed using R Studio open source software (3.6.1). The level of significance was set to P < 0.05.

**Results**

The results in Table (1) show an increase in the values of WBC, by an increase of neutrophils and monocytescompared to the reference values determined by Kramer, *et al.*(2006). While we note a decrease in the means of RBC, HB, and Ht, the mean values of Lym, Eos, Baso, MCHC, and MCV remained similar to the reference values.

The means of WBC, Mono, Neu, Eos, MCHCin our study varied according to the farm and type of aliment, with a highly significant difference (P <0.0001). The values of red blood count (RBC, HB, Ht, and MCHC) of*Rembi*ewes during late pregnancy in our study was influenced by type of aliment, farm, and parity (P <0.05). In addition, age showed no significant difference in the hematological parameters in the ewes at the end of gestation (Table 2).

**Discussion**

The knowledge of blood reference values during pregnancy and lactation helps the veterinarian in the diagnosis, prognosis and the treatments used to improve the production and reproduction of ewes (Roubies, *et al.,* 2006).

The results presented in Table (1) show that the mean of WBC (18.3 × 103 / mm3) is higher than the reported reference value (Kramer, *et al.,* 2006). These results were also found high as compared to those obtained in ewes during late pregnancy in the *Santa Inês* and *Morada Nova* breed in Brazil (Bezerra, *et al.,* 2017), in the *Pelo Criollos* breed in Colombia (Alberto, *et al.,*2019), in Turkey (Cihan, *et al.,* 2016) and in Nigeria (Adeyeye, *et al.,* 2016). This increase in the value of WBC in small ruminants during the late stage of pregnancy could be due to the increase in bone marrow activities (Waziri, *et al.,* 2010). The stress animals go through during pregnancy stimulates the secretion of certain hormones which can increase hematopoietic activities and movements of blood cells in the circulatory system, thereby increasing the number of WBC (Dellmann, *et al.,* 1987).

Neutrophils means were the highest in *Rembi*ewes during late pregnancy, followed by those of the monocytes which were moderately increased. These results are similar to those reported by Adeyeye, *et al.*(2016), and Bezerra, *et al.*(2017) who indicated that neutrophils are highest in pregnant ewes compared to other WBC values. This increase in neutrophils in our study may be due to the increase in hormonal secretion that occurs under stress, and the negative energy balance that occurs in ewes during late pregnancy. Stress can lead to leukocytosis due to the increase and release of bone marrow neutrophil reserves, induced by glucocorticoids (Sugito, *et al.,* 2009). Găvan, *et al.*(2010) pointed out that the negative energy balance activates the function of the adrenal glands, which can lead to an increase in the levels of catecholamines, cortisol and endorphins.

Our results show that the effect of farms and the type of aliment on the WBC values were highly significant with P = 2.35e-08 \*\*\* and 4.4 e-04 \*\*\*, respectively. This can be due to the hygiene of the farms, or some infectious pathology such as parasites diseases. Similarly, the values of neutrophils, monocytes, and basophils in our study varied with the farms and the type of aliment with a highly significant difference in the latter, as depicted in Table 2. The decrease in the lymphocyte’s mean in ewes at the end of pregnancy may be due to the negative energy balance. A study conducted by Hefnawy, *et al.*(2011) in Egypt in toxemic goats showed a significant decrease in lymphocytes due to the increase in ketone bodies (βHB), which have a significant impact on the immune system. In addition, the lymphocyte values were found to be influenced by the farm effect (P = 0.0191 \*), and the seasonal effect (P = 0.0142 \*), with higher significance in the winter season as compared to those obtained in springseason.

The RBC recorded a mean of 7.81 × 106 / mm3, which is much less than the mean found in Egypt by El-Malky, *et al.*(2019) in ewes during late pregnancy of the *Barki* and the *Ossimi* breeds, and also by Bezerra, *et al.*(2017) andAlberto, *et al.*(2019). Moreover, the study of Soliman (2014) in *Ossimi* ewes breed, showed that the physiological state can lead to significant modifications (P <0.05) on the hematological parameters, such as HB and RBC with an increase in late pregnancy as compared to the early lactation. However, Brito, *et al.*(2006) found no variation in the hematological parameters between pregnant and lactating ewes when they are well fed.

Our results concerning the value of HB (8.12 g / dl) are similar to those of El-Malky, *et al.*(2019) in pregnant *Ossimi* and *Barki*breeds, and Alberto, *et al.*(2019), but different from those reported by Bezerra, *et al.*(2017) and Adeyeye, *et al.*(2016), who recorded higher values of HB in ewes at the end of pregnancy.

The percentage of Ht was found to be reduced as compared to the results obtained by El-Malky, *et al.*(2019) in Egypt and Cihan, *et al.*(2016) in Turkey in ewes in their late pregnancy. The study of Sharma, *et al.*(2015) in India, however, recorded a significant increase (P<0.05) in the HBand Ht concentrations in pregnant ewes. This could be due to an increased demand for oxygen and nutrients. Other authors have observed no difference in the hematological constituents between pregnant and lactating ewes, although Ht was found to decrease with advancing pregnancy as reported by Brito, *et al.*(2006).

According to Iriadam (2007), the decrease in the number of RBC could be due to a factor known as hemodilution. The anemia encountered in our study would probably be due to the hemodilution effect. This phenomenon improves blood circulation in the vessels of placenta, particularly during late pregnancy to increase the diffusion of nutrients and oxygen towards the fetus (Grilli, *et al.,* 2007). This mechanism meets the need for oxygen and nutrients which increases at the late pregnancy period following fetal growth (Adili, *et al.,* 2013).

Weiss, *et al.*(2010)also believe that the anemia found in ewes is due to the increase in plasma volume during late pregnancy and early lactation, while Thrall, *et al.*(2012) suggest that these problems may be related to aliment. According to Okonkwo, *et al.*(2011), HB and Ht levels may be lowered due to increased nutritional requirements in ewes during the peripartum.

In this study, the values of RBC, HB, and Ht varied according to the farm, aliment, and parity (Table 2). The influence of the farm on RBC, HB, and Ht showed more significance than the influence of aliment, and this may be due to parasitic infections. The influence of parity was significant on the values of RBC, HB, and Ht with P = 0.0185 \*, 0.0205 \*, 0.0437, respectively. This significance reduced in multiparous then in primiparous.

The MCHC values recorded in this study were higher compared to those obtained by El-Malky, *et al.*(2019) and Alberto, *et al.*(2019) in ewes in the last third of their pregnancy. Cihan, *et al.*(2016) and Bezerra, *et al.*(2017), on the other hand, obtained very high values compared to those recorded in our study.

According to Bezerra, *et al.*(2017) the organism compensates for the decrease in RBC in ewes at the end of gestation by increasing hemoglobin concentration in erythrocytes, while MCHC increases with the advancement of gestation and decreases after lambing. MCHC levels in this study were highly influenced by the aliment (P = 0.000965 \*\*\*), very significant in farms (P = 0.00108 \*\*), and in the BS (P = 0.00743 \*\*) of the pregnant ewes.

The mean of MCV obtained in ewes *Rembi*breed during late pregnancy in our study was more or less high when compared with the mean obtained by Bezerra, *et al.*(2017) and El-Malky, *et al.*(2019). Alberto, *et al.*(2019) recorded higher mean in pregnant *Criollas* ewes than that obtained in this study.

The values of MCV and MCHC are essential transport markers of oxygen, necessary for cell survival. The increased oxygen demand stimulates an adaptive response in which increasing hemoglobin concentrations result in higher levels of oxygen transport (Gravena, *et al.,* 2010).

The mean value of platelets recorded in our study was 897.9. This result agrees with that obtained by Alberto, *et al.*(2019) who found a mean of 873.5 in *Criollas* ewes during late pregnancy. The average obtained by Badawi, *et al.*(2014)in pregnant ewes is, however, much less than that recorded in our study. No significant difference was found, in this study, for platelet values.

**Conclusion**

Hematological analysis provides reliable information on animal health. It is an important means for the assessment, production, reproduction, and adaptability of animals under adverse environmental conditions. It was found that the late stage of pregnancy influenced the hematological profile of *Rembi* ewes. The increase in the mean of WBC in *Rembi* ewes during late pregnancy may be due to the hormonal effect, and thus the hygiene of the farm. The increase in fetal oxygen and nutrient requirements during late pregnancy in suche wes caused a decrease in the means of RBC, Ht, HB. The type of aliment, the amount ingested by the animal, and the parity can reduce the values of RBC, HB, Ht.

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**Conflict**

The authors declare that there is no conflict of interests.

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**Table1.** Values of hematological parameters of *Rembi* ewes during late pregnancy

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Settings (n=74) | Min | Median | Mean | ST | Max | References(Kramer, *et al.*2006) |
| WBC (×103 /mm3)  | 2,9 | 16,9 | **18,3** | 11,97 | 64,2 | 4–12 |
| Lym(×103 /mm3) | 140,3 | 3219,5 | **3,9864** | 3,08 | 1,22 | 2–9 |
| Mono (×103 /mm3) | 280,6 | 4257,5 | **6,1456** | 5,24 | 2,58 | 0–0.75 |
| Neu(×103 /mm3) | 146,4 | 6283,5 | **6,8984** | 5,22 | 3,14 | 0.7–6 |
| Eos (×103 /mm3) | 0 | 527,5 | **0,7476** | 0,732 | 3,61 | 0–1.0 |
| Baso(×103 /mm3) | 0 | 183,5 | **0,307** | 0,42 | 2,38 | 0-0.3 |
| RBC (×106 /mm3)  | 1,540 | 7,705 | **7,810** | 1,76 | 14,14 | 9–15 |
| HB g/dl | 4,900 | 7,600 | **8,120** | 1,90 | 15,8 | 9–15 |
| Ht % | 16 | 24,05 | **25,11** | 5,16 | 47,7 | 27–45 |
| MCHC g/dl | 27,1 | 31,2 | **32,29** | 3,53 | 45,5 | 31–34 |
| MCV fl | 13,2 | 31,7 | **31,7** | 2,80 | 36,1 | 28–40 |
| PLT | 244 | 830,5 | **897,9** | 439,25 | 2465 | 100–800 |

**Table2.**Meanand*P*-value of some factors on hematological parameters of *Rembi* ewes during late pregnancy

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Settings |  Aliments (n=8) | Farms (n=10) | BS (n=4) | Parity (n=2) | Season (n=2) | Age (n=6) |
| **Mean ± ST** | **P value**  | **Mean ± ST** | **P value**  | **Mean ± ST** | **P value**  | **Mean ± ST** | **P value**  |  **Mean ± ST** | **P value**  | **Mean ± ST** | **P value**  |
| WBC (×103 /mm3)  | 18,37 ± 8 | 0,000404 \*\*\* | 16,81 ± 10,18 | 2,35e-08 \*\*\* | 17,33 ± 3,81 | NS | 18,38 ± 0,23 | NS | 16,96 ± 2,8 | NS | 16,84 ± 3,43 | NS |
| Lym (×103 /mm3)  | 3,544 ± 1,478 | NS | 3,120 ± 1,993 | 0,0191 \* | 3,724 ± 0,935 | NS | 3,746 ± 0,661 | NS | 3,190 ± 1,666 | 0,0142 \* | 3,916 ± 2,338 | NS |
| Mono (×103 /mm3)  | 6,424 ± 3,456 | 8,78e-05 \*\*\* | 5,892 ± 4,056 | 2,02e-07 \*\*\* | 5,878 ± 0,946 | NS | 6,579 ± 1,193 | NS | 5,940 ± 0,428 | NS | 5,226 ± 2,121 | NS |
| Neu (×103 /mm3)  | 7,094 ± 3,387 | 0,00022 \*\*\* | 6,613 ± 3,999 | 3,11e-07 \*\*\* | 6,476 ± 1,821 | NS | 6,867± 0,0862 | NS | 6,645± 0,529 | NS | 6,475 ± 1,473 | NS |
| Eos (×103 /mm3)  | 0,388 ± 0,329 | 0,000503 \*\*\* | 0,352 ± 0,309 | 0,000915 \*\*\* | 0,368 ± 0,237 | NS | 0,285 ± 0,0597 | NS | 0,350± 0,090 | NS | 0,258 ± 0,085 | NS |
| Baso (×103 /mm3)  | 0,771 ± 0,357 | NS | 0,688 ± 0,422 | NS | 0,665 ± 0,263 | NS | 0,744 ± 0,0982 | NS | 0,708± 0,082 | NS | 0,855 ± 0,448 | NS |
| RBC (×106 /mm3)  | 7,92 ± 0,79 | 0.0506\* | 7,79 ± 1,05 | 0,0019 \*\* | 7,57 ± 0,72 | NS | 8,1 ± 0,79 | 0,0185 \* | 7,61 ± 0,42 | NS | 7,79 ± 0,63 | NS |
| HB g/dl | 8,05 ± 1,07 | 0,00276 \*\* | 7,95 ± 1,23 | 0,000423 \*\*\* | 7,9 ± 0,39 | NS | 8,42 ± 0,84 | 0,0205 \* | 7,79 ± 0,69 | NS | 7,87 ± 0,81 | NS |
| Ht % | 25,47 ± 2,32 | 0,0494 \* | 25,08 ± 3,12 | 0,00126 \*\* | 24,45 ± 1,84 | NS | 25,83 ± 1,99 | 0,0437 \* | 24,47 ± 1,34 | NS | 24,93 ± 1,81 | NS |
| MCHC % | 31,6 ± 2,25 | 0,000965 \*\*\* | 31,69 ± 2,92 | 0,00108 \*\* | 32,33 ± 2,07 | 0,00743 \*\* | 32,4 ± 0,32 | NS | 31,98 ± 0,64 | NS | 31,47 ± 1,45 | NS |
| M CV fl | 31,53 ± 0,61 | NS | 32,03 ± 0,81 | NS | 32,24 ± 0,85 | NS | 31,63 ± 0,22 | NS | 32,01 ± 0,64 | NS | 31,83 ± 0,23 | NS |
| PLT | 879,61 ± 146,16 | NS | 839,23± 204 | NS | 818,34 ± 162,75 | NS | 939,43 ± 114,41 | NS | 845,52 ± 109,62 | NS | 881,62 ± 140,04 | NS |

**Key:** (P < 0.05)\* significant, (P < 0.01) \*\* very significant, (P < 0.0001) \*\*\* highly significant, (NS) not significant.