# Original Article

# Comparing Hematological and Biochemical Profiles of Pregnant and Non-pregnant Barb Mares Raised in Tiaret, Algeria

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## ABSTRACT

**Background:** The pure-bred Barb horse is a beloved breed from the Great Maghreb. Despite the breed's prominence in Algeria, no gestational hematological or biochemical research has been done on this breed.

**Objectives:** This study aimed to compare the hematological and biochemical parameters of pregnant and non-pregnant Barb mares in the first, second, and third trimesters of pregnancy.

**Methods:** From 12 pregnant and 6 non-pregnant mares, 102 venous blood samples were taken, and their glucose (Glu), cholesterol (Cho), triglycerides (TG), total protein (TP), urea (Urea), Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), iron (Fer), calcium (Ca), phosphorus (P), and ferric reducing ability of plasma (FRAP) were assessed as biochemical variables. Also, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, white blood cells, and platelets were all measured as hematological variables.

**Results:** The levels of ALP, ALT, GGT, and P decreased significantly throughout gestation, while Ca, TG, Fe, and Glu levels increased. AST concentrations decreased in the second and third trimesters, whereas Cho levels increased in the first and second trimesters. Urea levels increased significantly in the third trimester, and FRAP showed significant differences at different stages of pregnancy. Mean corpuscular hemoglobin concentration was significantly lower in the first and second trimesters, and hemoglobin values were significantly lower in the second trimester. The mean value of white blood cell count was slightly higher in late pregnancy, while platelet values significantly increased throughout all trimesters.

**Conclusion:** The study provides valuable information on the changes in hematological and biochemical parameters during pregnancy in Barb mares. These findings can be used as a reference for future studies on the reproductive physiology of this breed.

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## 1. Introduction

According to archaeological relics, rock carvings, and mosaics depicting horses with similar conformation and type to the present day, Barb horse equids were present in Algeria during the prehistoric era in the fourth millennium (Alimen, 1955). Through archaeological excavations conducted in Algeria, we have successfully identified the skeletal remains of the *Equus algericus* species discovered in the southeastern region of Tiaret (Chaid-Saoudi, 2006). Consequently, the Barb horse is considered a pure-bred horse native to the Greater Maghreb region.

In 1886, the first Algerian studbook of the Barb breed was established. Because of an international desire to rehabilitate the Barb horse, the World Barb Horse Organization (OMCB) was created in Algiers in June 1987 (El-Kohen, 2006).

Approximately 90% of the estimated 250000 horses in Algeria are Barb and Arabian Barb horses. The remaining 10% comprises French Trotters, English Thoroughbreds, and Arabian Horses (Benhamadi et al., 2020).

The breed is also the source of the creation of several breeds, in particular, the English Thoroughbred, the Spanish or Iberian horse, the American quarter horse, the Mustang, the Paso Fino, and the Argentinian Criollo (Benhamadi et al., 2020).

Tiaret houses the national stud farm "Chaou-Chaoua," founded in 1877. It is devoted to preserving and enhancing Algerian horse breeds, focusing on the Arabian Thoroughbred and the Barb.

In equine medicine, hematology and biochemistry are essential diagnostic techniques. Physiological alterations must be distinguished from pathological ones that could endanger the health of the mare or the fetus that necessitate rapid medical attention (Faramarzi et al., 2018).

According to Bazzano et al. (2014b) and Butte (2000), pregnancy is a physiological condition with an elevated need for oxygen. Several metabolic and endocrine alterations characterize this state. Mares may have increased metabolic demands and oxidative stress protection due to the growing nutritional needs of the fetus, especially in the third trimester (Farver, 2008). Significant changes in biochemical values, hematocrit, hemoglobin, white blood cell count, platelet count, prothrombin time, and fibrinogen concentration have been observed associated with foaling (Martuzzi et al., 2019).

Several studies have examined pregnant mares' hematological and biochemical profiles from various breeds. These studies include the Carthusian strain (Satué & Domingo, 2008), Bretonas and Brasilianas (Orozco et al., 2007), Holstein (Milinković-Tur et al., 2005), as well as Thoroughbred, Quarter Horse, Saddlebred, Standardbred, and Morgan breeds (Harvey et al., 2005).

To accurately interpret the tests used for screening, diagnosis, and monitoring during pregnancy and assess the severity and systemic consequences of diseases, it is necessary to understand the direction and degree of these changes in maternal hematology, biochemistry, and oxidative stress consequences throughout pregnancy. Importantly, using proper reference ranges in high-risk pregnancies aids in adapting the treatment to the individual animals (Farver, 2008).

The Barb horse is a particularly rustic horse of great sobriety because of its resistance to dryness and climatic variations. In particular, they require a lower food intake than Thoroughbred horses. Previous research (Chikhaoui et al., 2018) has demonstrated that neither age nor gender affects hematological values in Barb horses. The current study hypothesized that the pregnancy status does not impact hematological values, and pregnant Barb mares can maintain satisfactory biochemical parameters without supplementation during gestation.

To the best of our knowledge, there is currently no available publication of reference values for hematological and biochemical parameters specific to pregnant Barb mares. Therefore, this study aimed to investigate the changes in hematological and biochemical values between non-pregnant Barb mares and pregnant Barb mares during pregnancy's first, second, and third trimesters. These values could be useful in clinical practices to assess the health status of pregnant Barb mares.

## 2. Materials and Methods

### Study animals

Venous peripheral blood samples were collected from 12 pregnant Barb mares for hematological and biochemical analysis. Six non-pregnant Barb mares were utilized as control subjects for comparison. All of the mares involved in the study were owned by the Chaou-Chaoua National Stud located in Tiaret (35° 22′ 0″ N, 1° 19′ 0″ E). They were all kept and fed under identical conditions, receiving approximately 5 kg of hay and 4 kg of barley on a daily basis, along with unlimited access to water. Throughout the third trimester of gestation, no additional supplementation was provided. Additionally, the mares were given daily access to pasture from 10 AM to 3 PM.

The included mares were aged 4 to 17 (Mean±SD 7.82±3.95 years in both groups) with body condition scores ranging from 3 to 5, as determined by the Body Condition Score Chart provided by Kentucky Equine Research, USA.

Before the study, all the mares underwent a general clinical evaluation performed by the same veterinarian. Only mares with no signs of trauma or disease were selected for study inclusion. Furthermore, subjects with blood parasites detected before or during the research were excluded based on blood smear examination.

The day of pregnancy was calculated based on the conception date. Pregnancy confirmation was achieved through transrectal ultrasonography (SonoScape A6, Italy) conducted between days 14 and 30 after natural insemination performed by the farm veterinarian. The study was initiated following the confirmation of pregnancy through ultrasonography and concluded during the week preceding delivery.

## Study samples

In total, 102 blood samples were collected, with 8 samples obtained from each mare. Among these samples, 96 were collected from pregnant mares. Control samples (n=6) were obtained from the same mares before conception. The mares in the control group were sampled once. Sampling of the mares took place every 4 weeks, starting from April 2019 until February 2020. During each sampling event, the pregnant mares were at different stages due to being inseminated at various time points during the preceding breeding season. Pregnancy was divided into three stages: I (from conception to 110 days), II (111-220 days), and III (over 220 days). An 18-gauge needle attached to a 10-mL sterile syringe (KD-JECT® III, KDM®, Germany) was used to draw venous blood from the jugular vein. The blood was then stored in K3EDTA×4-mL test tubes (FL Medical, Italy) for hematological tests and in heparinized vacutainer tubes for biochemical analysis. Blood samples were examined right away in the laboratory of the veterinary institute. For 15 minutes, the tubes lacking an anticoagulant were centrifuged at 3000 g. After collection, the aspirated plasma was promptly frozen and stored at -20°C. The biochemical levels were examined two weeks after the samples were obtained to ensure accurate analysis. This precaution was taken into account since freezing can alter the biochemical values.

## Hematological examination

On blood samples, the following parameters were analyzed: red blood cell count (RBC), hematocrit (Hct), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), and platelet count (PLT). A hematology analyzer designed for horses, Orphee Mythic 18 Hematology Analyzer® (China), was utilized for the measurements.

## Biochemical analysis

In blood plasma, the following parameters were determined: Glucose (Glu), cholesterol (Cho), triglycerides (TG), total protein (TP), urea (Urea), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Gamma-glutamyl transferase (GGT), iron (Fer), calcium (Ca), and phosphorus (P). These biochemical parameters were determined using a split beam UV/Vis spectrophotometer (OPTIZEN 2120UV Plus Korea).

## Ferric reducing ability power measurement

Plasma antioxidant status was assessed using the ferric reducing ability power (FRAP) assay. The FRAP assay uses antioxidants as reductants in a colorimetric method linked to redox reactions. In this assay, a ferric-tripyridyl triazine ($Fe^{III}$-TPTZ) complex is reduced to its ferrous form, which appears blue, under low pH conditions. The reduction process is monitored by measuring the change in absorption at 593 nm using a split-beam UV/Vis spectrophotometer (OPTIZEN 2120UV Plus, Korea). The extent of absorbance change is directly proportional to the electron-donating antioxidants' reducing power present in the plasma. To quantify the FRAP value (in µmol/L), the difference in absorbance at 593 nm of the test sample is compared to that of a standard solution with a known FRAP value (Benzie & Strain, 1996).

## Statistical analysis

A Shapiro-Wilk test ($P<0.05$) was employed to assess the normality of the data, while Levene's test ($P<0.05$) was used to examine the equality of variances. The data from 4 groups, NP, T1, T2, and T3, were compared, with $P<0.05$ considered statistically significant. For normally distributed data, the one-way analysis of variance (ANOVA) was conducted, and in case of significance, Tukey tests were performed to determine the differences between the categories. In the case of non-normally distributed data, the Kruskal-Wallis test was used. If the data showed statistical significance, the Mann-Whitney test was conducted to determine the differences between the categories. All statistical analyses were carried out using SPSS software, version 18; IBM, Armonk, New York, USA).

## 3. Results

### Hematological profile

Mean±SD, minimum, and maximum values for blood erythrocyte parameters (RBC, Hb, Hct, MCV, and MCHC), WBC, and PLT counts in the three trimesters of pregnancy and the control group (non-pregnant) are presented in Table 1. Pregnant mares had significantly lower MCHC values in the first and second trimesters than non-pregnant mares. The Hb levels were significantly lower in the second trimester than in non-pregnant mares. The WBC count was significantly higher in the third trimester. The PLT count considerably increased across all three trimesters compared to non-pregnant mares. No significant changes were observed in RBC count, Hct, and MCV.

### Biochemical profile

We compared different stages of pregnancy with non-pregnant mares by investigating various blood biochemistry parameters. The data were presented as Mean±SD, minimum, and maximum values in Table 2. Among the measured and calculated parameters in this breed, only the TP value did not show a significant change throughout pregnancy compared to non-pregnant mares. However, most biochemical markers exhibited changes with the stages of pregnancy compared to non-pregnant mares.

Compared to non-pregnant mares, the mean values of ALP, ALT, GGT, and P significantly decreased during pregnancy in the first, second, and third trimesters. On the other hand, the levels of Ca, TG, Fe, and Glu significantly increased when comparing non-pregnant mares with the first, second, and third trimesters of gestation, respectively.

In contrast, AST concentrations significantly decreased as the pregnancy progressed to the second and third trimesters. When comparing pregnant mares to non-pregnant mares, Cho levels increased in the first and second trimesters of pregnancy. Additionally, Urea levels were significantly higher in the third trimester of pregnancy than in non-pregnant mares.

Statistically significant differences in FRAP were observed between non-pregnant and pregnant Barb mares. The levels of FRAP decreased during the various stages of pregnancy compared to non-pregnant mares.

## 4. Discussion

Veterinarians require appropriate reference values for their patients based on factors such as species, breed, sex, age, and reproductive status. These reference values are essential for making accurate treatment decisions, as blood analysis plays a crucial role in the clinical evaluation of animals. Pregnancy is a physiologically dynamic state, so that blood chemistry markers may change during this period (Barbosa et al., 2018). Considering these physiological changes when interpreting blood analysis results in pregnant mares is important to ensure appropriate diagnosis and treatment.

Our study compared potential variations in hematological and biochemical values between pregnant Barb mares and non-pregnant mares. Our investigation revealed no significant differences in the RBC, Hct, and MCV values between pregnant and non-pregnant Barb mares and between different gestational trimesters ($P>0.05$). These findings are consistent with the study conducted by Nagel et al. (2016), where they also reported no changes in erythrocyte, hemoglobin, and hematocrit levels during late gestation.

However, our results contradict the findings of Faramarzi et al. (2018), who reported significantly lower RBC, Hct, and Hb levels in pregnant mares compared to non-pregnant mares.

The RBC count was higher in late-pregnant mares. Yazlik et al. (2021) and Satué and Domingo, (2008) found an analogous trend. In contrast, Satué et al. (2012) reported mild anemia near the end of pregnancy.

In the present study, Hb showed a significant difference between non-pregnant and pregnant mares in the second trimester ($P<0.005$). This value returned to normal in the third trimester.

These differences could be attributed to physiological changes associated with late pregnancy. These changes may include increased blood volume, necessary to support the increased blood supply demands to the kidneys and uterus. These blood volume and circulation modifications have been documented to occur during late pregnancy and are believed to be essential for maintaining a healthy pregnancy (Bazzano et al., 2016).

No significant difference was observed in the Hct concentration in the present study. Additionally, a mild difference was found in Hb levels during the second trimester, suggesting no evidence of iron deficiency or pregnancy-related anemia in Barb mares.

MCV did not significantly change in the current study, which is consistent with earlier investigations on mares, which found no significant changes in MCV (Bazzano et al., 2014b; Faramarzi et al., 2018).

In contrast to the studies conducted by Bazzano et al. (2014b) and Faramarzi et al. (2018), our findings revealed a significant difference in MCHC between non-pregnant mares and the first and second trimesters of pregnancy.

The Barb breed is classified as a cold-blooded horse, not of Arabian ancestry, and is known for its docile temperament and lower metabolic rate. As a result, these horses typically exhibit lower levels of red blood cells (RBC), hematocrit, and hemoglobin compared to horses with a hot temperament, as observed by Kramer (2000). This finding is further supported by Chikhaoui et al. (2018) study, which demonstrates that neither age nor sex impacts hematological values in Barb horses. This discrepancy could also be due to the low-quality feeding of the animals during the breeding period (Karaşahin et al., 2023).

In our study, a significant difference was observed in platelet count (PLT) between non-pregnant mares and throughout the first ($P<0.008$), second ($P<0.0001$), and third ($P<0.003$) trimesters of pregnancy. This finding contradicts the results reported by Bazzano et al. (2014b), Chaid-Saoudi (2006), and Faramarzi et al. (2018), who found no significant differences in PLT count. However, our findings are consistent with a study by Mariella et al. (2014) in Standardbred mares and Aoki et al. (2012) in heavy draft mares.

The peak in PLT levels observed around foaling in our study may reflect a hypercoagulable state that physiologically occurs in mares around the time of parturition, as described by Bazzano et al. (2014b).

Consistent with previous studies by Yazlik et al. (2021) and Vaz et al. (2000) in Arabian pregnant mares, our findings indicated a slight increase in leukocyte count during the late stages of pregnancy. This increase in leukocyte count can be attributed to the rising release of endogenous adrenaline as pregnancy progresses. Elevated adrenaline levels contribute to the mobilization of cells and increase leukocyte count (Orozco et al., 2007). However, parturition disrupts the physiological rhythm of an animal and exposes it to physical stress, which may change the neutrophil and lymphocyte balance (Danyer & Bilal. 2021; Kaveh Baghbadorani et al., 2022).

Biochemical parameters are valuable tools for assessing the physiological changes during pregnancy in mares. The analysis of biochemical markers plays a crucial role in diagnosing, prognosing, and monitoring the progression of diseases, as well as understanding the physiological alterations induced by pregnancy (Harvey et al., 2005; Orozco et al., 2007).

In our study, significant increases in glucose levels were observed during the first, second, and third trimesters of pregnancy ($P<0.0001$). This finding is consistent with the research conducted by Vincze et al. (2015), who reported higher plasma glucose levels during the last three months of gestation. However, other studies, such as Harvey et al. (2005) and Fowden et al. (2000), have shown that glucose is significantly absorbed by the gravid uterus, fetus, and uteroplacental tissues during the middle of gestation. On the other hand, Aoki et al. (2012) and Mariella et al. (2014) argued that stress, which elevates blood cortisol levels, would lead to increased glucose levels near parturition. Furthermore, it has been demonstrated that the maternal cells develop mild insulin resistance during pregnancy to facilitate the transfer of glucose through the placenta to the growing fetus (Hoffman et al., 2003; Tavanaeimanesh et al., 2022). Similar to Vincze et al. (2015), we believe that the observed glucose increase in our study was likely due to the maturation of maternal cell insulin resistance.

The findings of this study demonstrate a significant increase in cholesterol concentration during the first ($P<0.0001$) and second ($P<0.0001$) trimesters of pregnancy compared to non-pregnant mares. However, no difference was observed between non-pregnant mares and late pregnancy. Similar cholesterol concentrations were also observed in late pregnant and non-pregnant mares in a study by Yazlic et al. (2021).

Danyer and Bilal. (2021) suggested that the rise in cholesterol concentration during pregnancy may be necessary to support an increased rate of hormone synthesis and that reproductive hormones, which regulate reproductive cyclicity, are closely associated with pregnancy. Additionally, the elevated cholesterol levels observed during pregnancy may be secondary to the hormonal changes associated with gestation (Silva et al., 2019).

**Table 1.** Hematological values in non-pregnant and pregnant mares, during the first, second, and third trimesters of pregnancy (SI units)

| | Non-pregnant (NP) | | 1st Trimester (T1) | | 2nd Trimester (T2) | | 3rd Trimester (T3) | |
|---|---|---|---|---|---|---|---|---|
| Parameter | Mean±SD | Min-Max | Mean±SD | Min-Max | Mean±SD | Min-Max | Mean±SD | Min-Max |
| RBC ($10^{12}$/L) | 6.61±1.01 | 5.4-8.2 | 6.49±0.5 | 5.7-7.3 | 6.65±0.58 | 5.4-7.8 | 5.93±0.93 | 4.6-7 |
| Hb (g/L) | 117.5±18.59 | 92.0-144 | 100.50±10.32 | 84.5-124 | 93.91±07.12** | 78.0-104 | 105.63±08.13 | 93.0-116.5 |
| Hct (L/L) | 0.33±0.51 | 0.26-0.4 | 0.32±0.25 | 0.28 -0.36 | 0.32±0.23 | 0.27-0.35 | 0.29±0.4 | 0.23-0.36 |
| MCV (fL) | 50.5±1.38 | 49-52 | 50.42±1.48 | 47.3-52.5 | 48.71±2.01 | 44.7-51.6 | 50.2±2.52 | 46.2-54.6 |
| MCHC (g/L) | 355±05.48 | 350-360 | 312.77±18.55** | 286-339 | 290.18±05.33** | 282-298 | 364.08±39.16 | 316.0-426.5 |
| PLT (x/mm$^3$) | 107.5±36.9 | 57-158 | 240.00±101.21** | 122-412 | 295.18±65.59*** | 237-445 | 336.27±150.93** | 136-532 |
| WBC ($10^9$/L) | 8.28±0.76 | 7.6-9.7 | 8.33±1.47 | 6.6-10.8 | 8.16±1.24 | 7.0-10.6 | 8.78±1.19** | 7.4-9.8 |

Abbreviations: RBC: Red blood cell; Hb: Hemoglobin; Hct: Hematocrit; MCV: Mean corpuscular volume; MCHC: Mean corpuscular hemoglobin concentration; PLT: Platelet; WBC: White blood cell.

*P<0.05, **P<0.001, ***P<0.0001.

**Table 2.** Biochemical values in non-pregnant and pregnant mares during the first, second, and third trimesters of pregnancy (SI units)

| Parameter | Non-pregnant (NP) | | 1st Trimester (T1) | | 2nd Trimester (T2) | | 3rd Trimester (T3) | |
|---|---|---|---|---|---|---|---|---|
| | Mean±SD | Min-Max | Mean±SD | Min-Max | Mean±SD | Min-Max | Mean±SD | Min-Max |
| Glu (g/L) | 0.44±0.1 | 0.3-0.6 | 1.36±0.25*** | 1.0-1.8 | 1.42±0.26*** | 1.0-1.7 | 1.49±0.23*** | 1.2-1.9 |
| Cho (g/L) | 0.57±0.22 | 0.2-0.9 | 1.05±0.19*** | 0.9-1.5 | 0.91±0.25*** | 0.7-1.5 | 0.53±0.1 | 0.4-0.7 |
| TG (g/L) | 0.23±0.1 | 0.1-0.4 | 0.84±0.75** | 0.4-2.6 | 0.72±0.32** | 0.2-1.3 | 0.77±0.32** | 0.3-1.2 |
| TP (g/L) | 80.62±18.79 | 45.5-102.2 | 57.14±5.02 | 48.9-64.7 | 58.51±6.62 | 45.2-72.6 | 57.41±5.74 | 48.9-66.9 |
| Urea (g/L) | 0.21±0.04 | 0.2-0.3 | 0.18±0.02 | 0.2-0.2 | 0.23±0.05 | 0.2-0.3 | 0.31±0.05** | 0.2-0.4 |
| AST (IU/L) | 282.43±51.6 | 236.8-378 | 242.68±107.73 | 95.3-399.4 | 78.41±16.59*** | 56.0-117.3 | 133.64±40.36*** | 81.9-213.1 |
| ALT (IU/L) | 57.17±6.54 | 48.4-66.5 | 20.35±8.61** | 5.1-36.4 | 16.63±7.23** | 8.8-30.9 | 13.29±11.07*** | 0.2-37.3 |
| ALP (IU/L) | 415.80±96.24 | 295.9-586.3 | 263.13±71.75** | 181.1-375.1 | 256.95±79.41** | 138.1-415.8 | 287.55±80.71** | 173.1-427.9 |
| GGT (IU/L) | 15.01±4.28 | 10.7-23.0 | 9.83±1.97** | 8.3-13.9 | 9.92±2.18** | 5.8-12.9 | 9.10±1.46** | 7.0-12.3 |
| Fe (mg/L) | 0.26±0.16 | 0.1-0.5 | 0.93±0.17*** | 0.7-1.3 | 1.60±0.18*** | 1.3-1.8 | 1.39±0.18*** | 1.1-1.7 |
| Ca (mg/L) | 75.26±6.88 | 64.3-84.9 | 84.01±7.52* | 70.3-97.4 | 118.45±14.97*** | 99.4-140.5 | 106.45±10.3*** | 84.8-119.7 |
| P (mg/L) | 53.83±13.05 | 32.7-67.6 | 35.32±6.45* | 25.3-48.2 | 27.29±5.39** | 19.6-34.1 | 23.75±4.94*** | 17.1-30.6 |
| FRAP (µm) | 336.55±39.69 | 292.7-402.7 | 150.02±22.7*** | 114.3-203 | 138.72±16.3*** | 107.9-165.1 | 161.35±25*** | 124.6-224.5 |

Abbreviations: ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CA: Calcium; Cho: Cholesterol; TG: Triglyceride; GGT: Gamma-glutamyl transferase; Glu: Glucose; P: Phosphorus; TP: Total protein; Fe: Iron; FRAP: Ferric reducing ability of plasma.

*P<0.05, **P<0.001, ***P<0.0001.

Bioactive lipids also play a significant role in the function of the corpus luteum responsible for maintaining pregnancy, as indicated by a study conducted by de Oliveira Gobesso et al. (2020).

Moreover, food consumed, food absorbed from the gut, amount of cholesterol generated in the liver, amount used by the body, amount reabsorbed, and amount expelled in feces are all factors that influence blood cholesterol levels (Harvey et al., 2005; Satue & Domingo, 2008).

Our investigation revealed a significant difference in blood triglyceride levels between pregnant mares in the first, second, and third trimesters compared to non-pregnant mares ($P<0.002$, $P<0.006$, and $P<0.002$, respectively). These findings indicate a statistically significant variation in triglyceride levels during pregnancy. Similar results were reported in previous studies conducted by Vincze et al. (2015) and Yazlic et al. (2021). However, Mariella et al. (2014) found no significant differences in triglyceride levels between the last month of pregnancy and the first week after parturition.

An increase in anabolic activity that can impact fat metabolism can be seen when the fetus grows larger in the second half or the last trimester of equine pregnancy. The process of mobilizing fat to make enough nutrients for the developing fetus and the mother may be reflected in the increased maternal plasma TG levels. This phenomenon can be explained by the altered glucose regulation during pregnancy, which leads to the progressive development of insulin resistance and a subsequent rise in plasma TG levels. This process is also thought to be responsible for the previously mentioned fat mobilization in the second half of equine gestation, according to Vincze et al. (2015).

In our study, there was no significant change ($P>0.05$) in the value of TP between pregnant and non-pregnant Barb mares. These results align with previous studies by Butte (2000) and Farver (2008), which also reported no differences in TP and Albumin levels throughout pregnancy. However, these findings contrast with studies conducted on Standardbred mares in Italy by Mariella et al. (2014), Lipizzaner broodmares by Vincze et al. (2015), Thoroughbred mares in Chennai by Naseema et al. (2017), and the Spanish broodmare by Satué et al. (2022) where significant differences in TP levels were observed during pregnancy. These discrepancies in findings across different studies could be attributed to various factors, including differences in breed, sample size, measurement methods, and variations in management and nutrition during pregnancy.

In our study, urea concentrations only showed a slight but significant increase in late pregnancy ($P<0.004$). This finding is consistent with previous studies that reported higher urea concentrations in pregnant mares than in non-pregnant mares, such as those conducted by Benzie and Strain. (1996) and Bazzano et al. (2014a). In the study by Mariella et al. (2014), urea levels were higher in late gestation and at delivery. However, other studies conducted on draft horses, like Aoki et al. (2012), found no significant changes in urea concentrations during pregnancy. Interestingly, in Lipizzaner mares, urea levels declined from the fourth month of pregnancy, as reported by Vincze et al. (2015).

Changes in serum urea might reflect increased energy demand and a higher request for amino acids for the anabolic process. Aoki and Ishii (2012) suggested that these findings might be related to changes in energy metabolism rather than in renal function, and physiological overload of the kidney is not a plausible explanation inlake women (Lockitch, 1997).

In our study, we observed a significant decrease in AST activities during mid gestation ($P<0.0001$) and late-gestation ($P<0.0001$) compared to early gestation in pregnant Barb mares. Furthermore, AST activities during mid-gestation and late gestation were significantly lower compared to non-pregnant Barb mares. These findings indicate a clear trend of decreasing AST activities as pregnancy progresses, suggesting potential physiological changes in liver function during different stages of gestation.

Our findings are consistent with earlier studies conducted by FlisiŃska-Bojanowska et al. (1991) and Mariella et al. (2014), which reported lower AST values in near-term pregnant mares than non-pregnant mares. Similar results were also observed in Spanish pure-bred broodmares by Naseema et al. (2017) and Satué and Montesinos, (2013). However, contrasting results were reported by Castagnetti et al. (2009) and Harvey et al. (2005), who found a slight increase in AST activity near parturition and during early lactation, respectively. The underlying cause for these discrepancies is still unknown, but some researchers, such as Vincze et al. (2015), have suggested that the variations in AST activity may be attributed to the different metabolic processes occurring during late gestation and early lactation, with anabolic processes potentially outweighing catabolic processes.

In the present study, ALP, which is present in several tissues, mostly the liver and bones, significantly decreased in pregnant Barb mares compared with non-pregnant ones (P<0.003) but did not change during pregnancy, which is in agreement with the findings of other studies (Mariella et al., 2014; Satué & Montesinos, 2013) who reported no significant changes in ALP activity throughout gestation. On the contrary, Harvey et al. (2005) found that serum ALP activity was significantly higher during early pregnancy compared to mid and late gestation in pregnant mares of various breeds. These researchers suggested that the liver may experience physiological strain postpartum (Mariella et al., 2014). It is worth noting that the wide range of individual variation among mares (Reese et al., 1984) and the influence of aging (Zinkl et al., 1990) could contribute to the observed variations in ALT activity. According to GAAL (1999), due to the increased activity of osteoclastic cells during this anabolic phase of pregnancy and the placenta's capacity to produce ALP, ALP activity may be higher in late pregnancy in mammals.

In our study, we observed a significant increase in GGT activity in pregnant Barb mares during the first (P<0.006), second (P<0.006), and third trimesters (P<0.002) compared to non-pregnant mares. Similar findings were reported by Mariella et al. (2014) and Naseema et al. (2017). Additionally, higher GGT activity near and during parturition has been reported in other studies, such as Gurgoze and Icen, (2010). Satué and Montesinos, (2013) found that GGT activities were significantly higher during nursing and early pregnancy compared to the last 4-5 months before parturition in Spanish pure-bred mares. These findings collectively suggest that GGT activity can vary throughout pregnancy and may be influenced by different factors, such as the stage of pregnancy and lactation. Animals are frequently diagnosed with hepatic disorders using serum GGT, a useful marker of hepatobiliary system abnormalities. Horses ' high blood GGT activity levels have been linked to several hepatic conditions, including toxic hepatic failure, preclinical hepatopathy, hyperlipemia, and cholestasis (Mariella et al., 2014).

Our study found a significant increase in iron values during gestation compared to non-pregnant Barb mares, particularly in the second trimester (P<0.0001). This finding aligns with the results reported by Montesinos and Satue (2013), who observed significantly higher levels of iron and ferritin during the second and third periods than the first. However, Ali et al. (2013) did not find significant differences in serum iron values among mares at various reproductive cycle stages.

These findings suggest that Barb mares possess sufficient iron stores to meet the developing fetus's and feto-placental unit's demands.

The current study found that the calcium levels in mares during the middle and late stages of pregnancy were significantly higher compared to mares in their first period and non-pregnant mares (P<0.0001). These findings oppose the results reported by Harvey et al. (2005) and Naseema et al. (2017), who observed a slight decline in serum calcium levels during pregnancy in mares.

Some authors, including Wooding et al. (2000) and Naseema et al. (2017), have proposed hypotheses to explain the increased calcium concentrations observed in mid- and late-pregnant mares. These hypotheses include faster fetal bone development and mineralization, higher calcium requirements in the third trimester, and the transfer of calcium from the mare to the fetus through the placenta (Esmaeili et al., 2023).

During the peripartum period, mares experience increased mineral demands to meet the needs of both the developing fetus and the growing foal, particularly regarding calcium and phosphorus. The mare's macro mineral profile changes during this time provide valuable insights into mineral interactions and their alterations in specific physiological conditions, such as late pregnancy (Yazlic et al., 2021).

Other studies, like the one by Mariella et al. (2014), have examined the utilization of calcium during foaling and its relationship with uterine contractions.

Serum Ca is also implicated in the detachment of the placenta in the early postpartum, and a study by Sevinga et al. (2002) confirmed that mares with retained placenta had lower calcium concentration within 12 hours after foaling than mares without retained placenta.

Compared to non-pregnant Barb mares, phosphorus levels significantly decreased during the first, second, and third trimesters of pregnancy (P<0.014), (P<0.002) and (P<0.0001), respectively.

Phosphorus and calcium are essential for several body functions, including bone and energy metabolism. Most fetal calcium and phosphorus are deposited in the last 2 months of pregnancy, implying that most skeletal growth occurs at that time (Kavazis et al., 2002). The lower phosphorus levels in periparturient mares compared to non-pregnant mares may be due to fetal mineral consumption and the close relationship between calcium and phosphorus metabolisms (Bazzano et al., 2014a). Furthermore, calcium and phosphorus concentrations normally have inverse relationship. Hence, the higher the calcium levels, the lower the phosphorus levels.

FRAP represents the combined non-enzymatic antioxidant activities of uric acid, ascorbic acid, and proteins. These three components are believed to contribute approximately 60%, 15%, and 10%, respectively, to the total FRAP value in humans. Additional constituents such as tocopherol, bilirubin, and so on comprise around 5% of each total FRAP value in plasma (Benzie et al., 1996).

Our study observed a significant decrease in FRAP levels throughout pregnancy ($P<0.0001$). This decline suggests a general depletion of endogenous antioxidant compounds during pregnancy. These findings align with previous studies conducted in cattle (Marlin & Dunnett 2007), mares (Sgorbini et al., 2015; Martuzzi et al., 2019), and other relevant research studies. Another potential factor contributing to this decrease could be the absence of antioxidant supplementation during the third trimester of pregnancy (Kurhaluk et al., 2022). Therefore, it is strongly recommended to provide antioxidant supplementation during pregnancy to safeguard the well-being of both the mother and the fetus.

## 5. Conclusion

In conclusion, the present study provided the first hematological and biochemical blood analysis data in Barb mares.

Laboratory tests are performed to monitor animal health. The pregnancy period generates changes in different body systems to meet the fetus's and placenta's demands and protect the mare. This research demonstrated that pregnancy significantly influences the biochemical values of Barb mares, and the reference values presented in this study pose a clinically useful tool for the evaluation of blood checks and could be useful in clinical practices to assess the situation of pregnancy in Barb mares.

## Ethical Considerations

### Compliance with ethical guidelines

The European Communities Council Directive (2010/63/EU) for animal experiments was followed in this study. The experimental research took place at Tiaret's Chaou-Chaoua National Stud. The director gave consent for these procedures within a veterinary monitoring system. The authors are members of the Algerian Association of Sciences in Animal Experimentation (AASEA) (Agreement No.: 45/DGLPAG/DVA.SDA.14).

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### Authors' contributions

All authors equally contributed to preparing this article.

### Conflict of interest

The authors declared no conflict of interest.

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