Evaluation of the hematological profile of pregnant and non-pregnant mares seen at the HVU-BJ/CPCE in Bom Jesus-PI

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ABSTRACT

The blood count is a widely used test in equine medical clinics, being of great importance in the institution of an efficient diagnosis. During pregnancy, physiological adaptations occur such as increased blood volume, uterine expansion, proteins and other nutrients are higher due to fetal development and formation of maternal structure such as placenta, mammary glands and blood. Therefore, it is essential to know the alterations in the hematological constituents in pregnant mares. The objective of this study was to characterize the hematological profile and evaluate possible hematological alterations between pregnant mares and non-pregnant mares. The research was carried out from March 2019 to June 2019, where blood samples were collected from 20 clinically healthy mares that were referred for pregnancy diagnostic examination, these were attended at the large animal outpatient clinic of HVU-BJ/UFPI, at Haras do Hélio Rosa and Haras do Bispo, the last two being private properties, all of them are located in the municipality of Bom Jesus/PI. Of the alterations found, anemia found in high prevalence, leukocytosis, eusinophilia found in low prevalence in both groups, and lymphopenia found in an animal in the non-pregnant group stand out. Therefore, mares that were in the gestation period between 60 and 90 days presented a picture of normocytic normochromic anemia, justified by blood hemodilution, characterizing the stage of gestation that requires more attention.

Keywords: Hematology, Horses, Pregnancy.

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INTRODUCTION

The world equine population has been stable in recent decades and is currently estimated at 113,473,522 head, of which 58,770,171 are horses, 43,496,677 are donkeys and 11,206,674 are mules, as shown in table 1 (FAO, 2008). The world equine population is distributed across the continents as follows: Africa, with 4,519,216 head. (7,7%); America, with 33,594,119 cab. (57,2%); Asia, with 13,870,140 cab. (23,6%); Europe, with 6,374,740 cab. (10,8%); and Oceania, with 411,956 cab. (0.7%), and the concentration of equine production and use in the Americas is evident (FAO, 2008).

Table 1 - World population of horses, donkeys and mules (heads)

Continent	Equine	Asininos	Muares	Total (Equideos)
Africa	4.519.216	18.559.137	1.060.913	24.139.266
America	33.594.119	7.161.527	6.318.150	47.073.079
Asia	13.870.140	17.129.456	3.604.713	34.604.309
Europa	6.374.740	637.557	222.898	7.235.195
Oceania	411.956	9.000	-	420.956
Total	58.770.171	43.496.677	11.206.674	113.473.522

FAO (2008)

The worldwide distribution of horses and also of donkeys and mules between continents or countries reflects productive, sanitary, legal and cultural aspects. However, it should be noted in the last decade the reduction of the equine population in Asia, mainly in China, from 8,916,154 head in 2000 to 6,823,465 head in 2008 (FAO, 2008), associated with the internal migration of the human population. with less use of equidae in transport and agriculture and higher consumption of equine meat. On the other hand, in the United States there was a significant increase in the equine population, from 5,240,000 head in 2000 to 9,500,000 head in 2008 (FAO, 2008), partly due to internal legal restrictions on the slaughter and export of equine meat.

In Brazil, the equine population is currently estimated at 7,986,023 head, of which 5,541,702 are horses, 1,130,795 donkeys and 1,313,526 mules. The national equine population is the fourth largest in the world, with about 5,600,000 animals, which has remained stable in the last decade (IBGE, 2008). In South America, in addition to Brazil, equine production is highlighted in Argentina, with an estimated herd of 3,680,000 animals, and in Colombia, with 2,520,000 animals.

The equine agribusiness complex in Brazil has a turnover of about R$ 7.5 billion and generates about 3.2 million direct and indirect jobs. The equine, in the economic aspect, performs the functions of saddle, load and traction. From the second half of the twentieth century onwards, sports and leisure activities stand out in the social aspect, as well as hippotherapy for the treatment of people with cognitive, psychomotor and socio-affective difficulties (LIMA *et al*., 2006, VIEIRA, et.al., 2015). Also noteworthy in equine agribusiness are the various suppliers of inputs, products and services for breeding, such as medicines, feed, saddles and accessories, shoeing, veterinarians and

trainers, equine transport and teaching and research. In the agricultural complex, the equine segment used in various sports activities moves values of around R$ 705 million and employs about 20,500 people, with the estimated participation of 50 thousand athletes (LIMA *et al*., 2006).

In the area of research, science and technology, according to Lima et al. (2006), in 2004 there were about 34 research groups registered with CNPq, with the participation of 666 researchers. Currently, about 60 research groups are registered with CNPq. Of these groups, 48 are related to research in Veterinary Medicine, including horses, 10 groups are related to Animal Science, including horses, 1 group in Biochemistry and 1 in Microbiology, with studies in horses.

In Brazil, as in other countries, investments in equine research are related to the perspectives of the equine industry segments in the country. Research can be broken down as being in production and management, genetics and breeding, nutrition and feeding, reproduction, medicine and surgery, diseases, health and health defense. There is also research in areas related to equestrianism, involving sports physiology, horsemanship and equine training. Finally, there are also studies on the national and international production and commercialization of horses, equine meat and by-products (LIMA *et al*., 2006).

The blood count is one of the most requested tests of the clinical routine because it serves as a "mirror" of the animal's health at the time of collection. It is routinely used to clinically monitor the development and performance of animals, since this examination enables both the investigation of pathological alterations and the monitoring of health, through a diversity of information provided through the analysis of the constituents of blood tissue (MORY, *et. al*., 2004). Therefore, the vascular system is indispensable for the maintenance and performance of all systems of the body, including the reproductive tract. Because it fulfills the metabolic need and assists in the regulatory transport of substances, this system is directly related not only to the hormonal supply, but also to the physiological variations of the reproductive tract (DUKES and REECE, 2006, AKAHIRO, 2011).

During pregnancy, physiological adaptations occur, such as increased blood volume, uterine expansion, increased need for energy, proteins, and other nutrients due to fetal development, and formation of maternal structure such as placenta, mammary glands, and blood (GRAVENA *et al*., 2010, FRADINHO *et al*., 2014). Reproductive efficiency is closely related to body condition, which has an effect on the duration of seasonal anestrus, the transition period to cyclic activity, the interovulatory period, IGF-1 eleptin secretion, the number of cycles/conception and the rates of pregnancy, early embryonic loss and abortion (BENDER, *et al*., 2014). Thus, the knowledge of the alterations in the hematological constituents in pregnant mares is indispensable, since they will contribute decisively to the clinical veterinarians who provide services to this species. Considering the importance of the blood count as a routine test in the evaluation of the health status and in the monitoring of the diseases of the animals, associated with the modernization of hematological

techniques through the use of automated equipment, we sought to characterize the hematological profile and evaluate possible hematological alterations between pregnant mares and non-pregnant mares from the casuistry of the University Veterinary Hospital and the farms contracted with the *Campus* University Teacher Cinobelina Elvas-CPCE.

MATERIAL AND METHODS

The samples were collected at the outpatient clinic of the University Veterinary Hospital of UFPI/CPCE, at Haras Helio Rosa and Haras Bispo, both properties located in the municipality of Bom Jesus-PI. The samples were processed at the Clinical Pathology Laboratory of the HVU, at the Federal University of Piauí, Professor Cinobelina Elvas Campus. Blood samples were collected from 20 healthy mares (evidenced by physical examination and blood count) with a mean age of 10 (±5.0) years and a mean weight of 402.5 (±50.1) kg, separated into two groups: control group (non-pregnant) and group 01 (pregnant women).

The animals were submitted to the same nutritional management and climatic conditions. In pregnant mares, rectal palpation and ultrasound examinations were performed to confirm gestational conditions. Samples were collected by venipuncture of the jugular vein. After being collected, the material was sent to the veterinary clinical pathology laboratory of the veterinary hospital (HVU-CPCE), where in an interval of 24 hours the blood count was completed, which was aimed at the global count of the number of red blood cells, determination of globular volume, hemoglobin content, absolute hematimetric indices such as Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC). overall and differential leukocyte count, platelet count, and determination of total plasma protein concentration. The total number of red blood cells was counted in a Neubauer chamber. For this purpose, the cells were diluted in a proportion of 1:200, using a semi-automatic pipette of 20 microliters. To determine the globular volume, the microhematocrit technique was used, in which homogeneous capillary tubes of 75 millimeters in length and 1 millimeter in diameter were used. On the other hand, the determination of the hemoglobin content in the blood was made by the cyanomethaemoglobin method with previous dilution in Drabkin's solution.

The total number of leukocytes was counted in a Neubauer chamber, and the blood samples were diluted in a ratio of 1:20, using Turk's fluid as diluting solution. Fresh blood smears were produced for leukocyte counts. In each blood smear, 100 leukocytes were differentiated, classified and read under a microscope at 1000x magnification, according to their morphological and dyeing characteristics, into neutrophils, eosinophils, basophils, lymphocytes and monocytes. The determination of Total Plasma Proteins (TPP) was performed by refractometry, after centrifugation at 12,000 rpm/min of the blood in a microhematocrit capillary. The data obtained were submitted to

statistical analysis using the analysis of variance method (ANOVA). Differences were considered statistically significant when P<0.05.

RESULTS AND DISCUSSION

Regarding the erythrocyte profile, the variables analyzed were not significantly influenced (P<0.05) by the pregnant group compared to the non-pregnant group, as shown in Table 1.

Table 1 – Mean values ± standard deviations of erythrogram of pregnant and non-pregnant mares.

Erythrogram	Pregnant	Non-pregnant	Reference values*
Hematias (x10⁶/ul)	6.32 ± 0.87	7.02 ±1.04	6.8 -12.9 (x10⁶/ul)
Hemoglobina (g/dL)	10.5 ± 1.16	11.65 ±1.51	11-19 (g/dL)
Hematocrit (%)	30.3 ± 3.59	34.1 ± 4.93	32-53 (%)
MCV¹ (fL)	48.19 ± 3.86	48.67 ± 3.58	37-59 (fL)
CHCM² (%)	34.27 ± 1.192	34.12± 1.47	31-39 (%)

*WEISS & WARDROP (2010). 1 Mean globular volume. 2 Mean Globular Hemoglobin Concentration.

The values found in the red blood cell count (6.352 10⁶/ul), hemoglobin (10.5g/dL), and hematocrit (30.3%) of the group of pregnant mares presented values below the standard values for the species, according to Weiss & Wardrop (2010). In the opinion of Souza et al. (2002), the values are decreased due to the greater expansion of the plasma volume in relation to the expansion of the erythrocyte mass during pregnancy, resulting in a decrease in hemoglobin and hematocrit concentrations, decreasing blood viscosity.

The reference values for the non-pregnant group did not change and remained within the reference values for the species (WEISS & WARDROP, 2010).

The values of MCHC and MCV did not show significant difference (P<0.05) during the physiological reproductive periods analyzed, as shown in Table 1.

In the analyses of the group of non-pregnant mares in the different periods analyzed, there was no significant difference in the variables (P<0.05), as shown in Table 2.

Table 2 – Mean values ± standard deviations of erythrogram of pregnant mares in different gestation periods

Erythrogram	0-30 days of gestation	60-90 days of gestation	120-180 days of gestation	Reference values*
Hematias (x10⁶/ul)	7.03 ± 0.13	5,78 ± 0,89*	6.85 ±0.16	6,8 -12,9 (x10⁶/ul)
Hemoglobina (g/dL)	11.5 ± 0.28	9.6 ± 0.94*	11.33 ± 0.35	11-19 (g/dL)
Hematocrit (%)	33 ± 0	28.2 ± 3.34*	32 ± 3.60	32-53 (%)
MCV¹ (fL)	47.1 ± 3.54	49.54 ± 3.60	46.66 ± 5.05	37-59 (fL)
CHCM² (%)	34.85 ± 0.91	34.58 ± 2.32	33.37± 1.85	31-39 (%)

*WEISS & WARDROP (2010). 1 Mean globular volume. 2 Mean Globular Hemoglobin Concentration. *The red cells indicate that the value found is below the reference value for the species.

However, for pregnant women between 60 and 90 days of age, the values of red blood cells, hemoglobin and hematocrit were lower than the reference values, in agreement with Couto (2010), in a study carried out with native mares, concluding, in the evaluation of the erythrogram, a decrease in the values of erythrocytes, hemoglobin and globular volume in mares with 90 days of gestation, justified by the expansion of plasma volume in response to the increase in the concentration of renin in the plasma and consequent slight reduction of levels of atrial natriuretic peptide, and it is also linked that the increase in plasma volume is the result of systemic vasodilation and increased vascular capacity.

In mares less than 30 days of gestation there were no changes in the reference values. This is justified by fetal recognition, which occurs only after 30 days of gestation, therefore, there are no sudden changes in these values.

In the group of pregnant women between 120 and 180 days, erythrogram values were within the reference values. Cunninghan (2008) states that the total erythrocyte count is closely linked to the body's need for oxygen, that is, as the demand for oxygen increases, the amount of red blood cells increases, due to the release of erythropoietin resulting from the release of progesterone and placental chorionic somatotropin, which act mainly at the end of pregnancy, raising red blood cell levels. This justifies the increase in erythrocyte value between the periods of 30 to 60 days and 120 to 180 days.

Table 3 – Individual erythrogram values of each mare belonging to the group of pregnant mares and their respective gestation time.

Gestation time	Animal	Erythrocytes	Hemoglobin	Haematocrit	VCM	CHCM
30 d gestation	Tordilla	6,65*	11,7	33	49,6	35,5
30 d gestation	Lorinha	7,4	11,3	33	44,6	34,2
60 d gestation	Ravika	6,05	10,8	31	52,2	38,4
60 d gestation	Successor	6,2	10	31	50	32,2
60 d gestation	Girl	5,7	9,1	27	47,4	33,7
60 d gestation	India	4,3	8,3	23	53,5	34,8
90 d gestation	Crazy	6,65	9,8	29	44,6	33,8
120 d gestation	Mega sena	6,67	11	31	46,5	35,5
120 d gestation	Queen	6,95	11,7	36	51,8	32,5
180 d gestation	Brysa	6,95	11,3	29	41,7	32,1
Reference values*		6,8 -12,9 (x10⁶/ul)	11-19g (g/dL)	32-53 (%)	37-59 (fL)	31-39 (%)

*WEISS & WARDROP (2010).1 Mean Globular Volume. 2 Mean Globular Hemoglobin Concentration. *The red cells indicate that the value found is below the reference value for the species.

Table 3 shows the erythrogram values of the pregnant mares, classified according to the gestational period at the time of collection. All animals pregnant between 60 and 90 days had normocytic normochromic anemia. Souza et al. (2002) reported that this "physiological anemia" in the first third of pregnancy occurs due to blood hemodilution, since the expansion of the plasma

volume is greater than the expansion of the erythrocyte mass. The authors also state that erythrocytes should remain this way until the puerperium, around 6 to 8 weeks postpartum.

Some animals aged 30 and 120 to 180 days pregnant had red blood cells/hematocrit values below the reference values, but they do not qualify as truly anemic.

Regarding the leukogram, there was no significant difference (P<0.05), except for monocyte counts, which were higher for pregnant women, but without clinical significance, since they remained within the reference values (WEISS & WARDROP, 2010).

For the other variables analyzed between the groups, there was no statistical difference, as well as Couto (2010), who demonstrated that no significant difference was observed between the leukograms of empty mares, with three, six and ten months of gestation.

Table 4 – Mean values ± standard deviations of leukocyte count of pregnant and non-pregnant mares.

Leukogram	Pregnant	Non-pregnant	Reference values
Total Leukocytes	10.515 ± 3377,5	10.168 ± 3.172,33	5.400-14.300 ul
Rod neutrophils	0 ± 0	0 ± 0	0-1.000 ul
Segmented neutrophils	6.802,60 ± 2751,24	6.363,8± 2218,57	2.260-8.580 ul
Eosinophils	377,60 ± 463,28	619,3 ± 870,15	0-1.000 ul
Basophils	23,20 ± 37,61	61,9 ± 95,24	0-290 ul
Monocytes	422 ± 240.08 **A**	204.6 ± 126.12 **b**	0-1.000 ul
Lymphocytes	4341,90 ± 5285,87	2947,7 ± 1059,43	1.500-7.700 ul

ab Equal letters represent equal means, different letters indicate statistical difference, P<0.05.*WEISS, D.J. & WARDROP, K.J. (2010). For both groups, all the means of the variables analyzed in the leukogram were within the reference values for the species (WEISS & WARDROP, 2010).

In the leukogram of the group of pregnant mares, only two animals present leukocytosis due to the increase in the number of segmented neutrophils. In both cases, the animals were less than 60 days pregnant.

In the group of non-pregnant mares, only one mare had leukocytosis due to the considerable increase in the number of eosinophils.

Table 5 – Mean values ± platelet standard deviations and total plasma protein count of pregnant and non-pregnant mares.

	Pregnant	Non-pregnant	Reference values
Platelets	249,8 ± 54,86	287,8 ± 30,32	100-350 ul
Total plasma proteins	7.08 ± 0.44	6,94 ± 0,34	5.8-8.7 g/dL

*WEISS, D.J. & WARDROP, K.J. (2010).

The values of total plasma protein and platelet did not show significant difference (Table 3), being within the reference values (WEISS & WARDROP, 2010).

The results are in line with Bazzano et al. (2014), who analyzed the plasma concentrations of pregnant mares and observed a significant increase in parameters close to calving. Like the above-mentioned author, Campelo (2008) also found differences between the concentrations of PPT in the middle and final third of gestation of the animals analyzed.

CONCLUSION

The analyzed variables, erythrogram, leukogram, platelet count and total plasma protein did not show significant difference between the groups of pregnant and non-pregnant mares. However, all mares that were in the gestation period between 60 and 90 days presented a picture of normocytic normochromic anemia, justified mainly by blood hemodilution, characterizing the stage of gestation that requires more attention by owners and veterinarians.

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