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Hematological and biochemical profiles of Mangalarga Marchador mares in the transition period bred on pasture

[*Perfis hematológico e bioquímico de éguas da raça Mangalarga Marchador no período de transição mantidas a pasto*]

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ABSTRACT

The present study aimed to evaluate the effects of the transition period on hematological and biochemical constituents in Mangalarga Marchador mares. Forty-eight mares were used to form a maintenance group (MG) and transition group (TG), formed by pregnant mares and, after delivery, infants. Blood samples were collected at the following times: T-60 (60 d pre-delivery), T-30 (30 d pre-delivery), T-15 (15 d pre-delivery), T0 (first 6h post-delivery), T15 (15 d post-delivery), T30 (30 d post-delivery), and T60 (60 d post-delivery). The TG had lower values ($P< 0.05$) of red blood cells, hematocrit and hemoglobin at T0, T15, T30 and T60 times than MG. The mean corpuscular volume was lower in MG ($P< 0.05$) than in TG (T0, T15, T30 and T60) and mean corpuscular hemoglobin concentration was higher ($P< 0.05$) in MG than in TG (T15, T30 and T60). On the other hand, the diameter distribution of red blood cells presented a lower value ($P< 0.05$) in MG than in TG (T15 and T30). Mares in transition period presented regenerative anemia. The results demonstrate physiological metabolic variations of different intensities during pregnancy, delivery and early lactation.

Keywords: equine, blood count, lactation, pregnancy, peripartum

RESUMO

O presente estudo teve como objetivo avaliar os efeitos do período de transição em constituintes hematológicos e bioquímicos em éguas Mangalarga Marchador. Foram utilizadas 48 éguas para formar um grupo de manutenção (GM) e um grupo de transição (GT), composto por éguas gestantes e, após o parto, lactentes. Amostras de sangue foram coletadas nos seguintes tempos: T-60 (60 dias pré-parto), T-30 (30 dias pré-parto), T-15 (15 dias pré-parto), T0 (seis primeiras horas pós-parto), T15 (15 dias pós-parto), T30 (30 dias pós-parto) e T60 (60 dias pós-parto). O GT apresentou valores menores ($P<0,05$) de hemácias, hematócrito e hemoglobina, nos tempos T0, T15, T30 e T60, do que o GM. O volume corpuscular médio foi menor no GM ($P<0,05$) do que no GT (T0, T15, T30, T60) e a concentração corpórea de hemoglobina corpórea foi maior ($P<0,05$) no GM do que no GT (T15, T30, T60). Por outro lado, a distribuição do diâmetro dos eritrócitos apresentou um valor menor ($P<0,05$) no GM do que no GT (T15 e T30). Éguas em período de transição apresentam anemia regenerativa. Os resultados demonstram variações metabólicas de diferentes intensidades durante a gestação, o parto e o início de lactação.

Palavras-chave: equino, gravidez, hemograma, lactação, periparto

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INTRODUCTION

The transition period comprises the last third of pregnancy, parturition, and early lactation (Manso Filho *et al*., 2008), also called the peripartum period (Aoki and Ishii, 2012; Mariella *et al*., 2014). During this period, physiologic adaptations are observed in hematological constituents (Hadden and Mclaughlin, 2009). In the last trimester before delivery, there is an increase in the nutritional demand of the mare, making it more vulnerable to the development of diseases (Bazzano *et al*., 2014a). These metabolic changes are essential in order to meet maternal demands, ensure the development and proper growth of the fetus, and provide an energy reserve and necessary substrates after birth (Hadden and Mclaughlin, 2009). This phase may be characterized by intense anabolic activity: already during delivery and lactation there is increased catabolic activity, with much of the metabolism directed to lactation.

Periods of pregnancy and lactation influence blood elements (Milinković-Tur *et al*., 2005). Studies with different equestrian breeds have been carried out in order to describe the main hematological characteristics of mares during this period (Bazzano *et al*., 2014b; Mariella *et al*., 2014), however, few Brazilian studies have addressed this topic, especially in the Mangalarga Marchador breed, which has great importance for the equine culture of Brazil and specifically for the state of Minas Gerais, in which activities involving equines have significant social and economic dimensions.

During pregnancy, increased activity occurs in the renin-angiotensin system, which promotes aldosterone secretion, results in increased body fluid and plasma volume, yielding a reduction in hematocrit levels due to dilution of the red blood cell mass (Talbot and Maclennan, 2016). These authors reported an increase of approximately 30% in erythropoiesis. Mariella *et al*. (2014) recorded that on the seventh day after delivery there was a decrease in hematocrit and hemoglobin values.

Therefore, knowledge of the variations that occur in hematological and biochemical constituents of mares at this stage is fundamental, not only for the identification of diseases, but moreover to establish a prognosis, defining and monitoring treatments (Bazzano *et al*., 2014b). The present study aimed to evaluate the effects of the transition period on hematological and biochemical profiles of Mangalarga Marchador mares bred on pasture.

MATERIAL AND METHODS

The research was conducted at a Farm located in the municipality of Itaperuna, in the state of Rio de Janeiro, Brazil. The project was previously approved by the Ethical Committee in the Use of Animals of the Universidade Federal de Viçosa (CEUA/UFV) under protocol number 95/2015. Forty-eight healthy 5 to 15 year-old mares of the Mangalarga Marchador breed were used; twenty-two mares in maintenance (maintenance group – MG) and 26 pregnant (subsequently lactating) mares (transition group – TG). Transition group mares were carefully chosen by their calving dates. The mares were bred on pasture, extensive rearing system, remaining in pastures containing Tifton grass (*Cynodon* spp.), water, and commercial mineral salt (Coequi Plus, Tortuga) *ad libitum*. Two weeks before delivery until after delivery, mares were housed in maternity paddocks, with no change in diet. After delivery, the mares were transferred to a pasture under the same initial conditions.

Blood sample collections were always performed in the morning. MG mares were evaluated only one time, at T-60 (first collection for all animals). TG mares were evaluated at seven different times: T-60 (60 d pre-delivery), T-30 (30 d pre-delivery), T-15 (15 d pre-delivery), T0 (6 h post-delivery), T15 (15 d post-delivery), T30 (30 d post-delivery), and T60 (60 d post-delivery).

In order to perform the blood count, blood samples were collected without prior fasting, after antisepsis, by venipuncture of the jugular, using disposable and weak vacuum needles containing EDTA K2 anticoagulant 4mL (Ethylenediamine tetraacetic Acid, Labor Import, SP, Brazil). The flasks were kept in ice water at a temperature of approximately 2–8°C. The hematological analysis was divided into an erythrogram, in which the number of erythrocytes (RBC), hematocrit (Hct), hemoglobin concentration (Hb). Absolute blood count indexes were calculated as mean

corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and erythrocyte diameter distribution (RDWcv). Hematocrit (Hct) was measured using the microhematocrit method (Benfer Products Digital Benfer DHM_6, Benfer Products of laboratory, SP, Brazil) using 75mm capillary tubes centrifuged at 700g for 5min.

The global leukocyte count and differential leukocyte count were measured according to Oliveira (2013). Moreover, the number of platelets (Plt) was measured. The erythrogram, global leukocyte and platelet counts were performed using an automatic blood cell count (Humacount Plus - HUMAN Gmbh, Max Planck-Ring 21, Wiesbaden, Germany).

Serum was obtained after collection of blood in siliconized vacuum flasks without anticoagulant, which were kept at room temperature (25°C) until clot retraction and then centrifuged at 1500 × g (Centrifuge Excelsai 2206, Fanem, SP, Brazil) for 5min. After collection, the serum samples were aliquoted into microtubes and immediately frozen at -20°C until analysis. The total protein (colorimetric method, Biuret, In Vitro Diagnóstica Ltda, Itabira, MG, Brazil) and albumin (colorimetric method, VBC-Green, Bioclin - Quibasa Química Básica Ltda, MG, Brazil) were measured in a HumaStar 300 (HUMAN, distributed by In Vitro Diagnóstica Ltda, MG, Brazil), globulin (obtained by the difference in serum protein and albumin values), and iron (colorimetric method, Cromazurol B, Bioclin - Quibasa Química Básica Ltda, MG, Brazil). The concentration of fibrinogen was obtained by the thermal precipitation method and measured by refractometer.

To analyze the data, the Statistical Analysis System (SAS) was used (Sas, 2002). The data were evaluated using the Kolmogorov-Smirnov and Bartlett's tests to verify the normality of the errors and homogeneity of variances, respectively, and the variables that did not satisfy the ANOVA assumptions were submitted to the square root transformation (y ^'= √ (y +0.5)).

The data for pregnant mares were analyzed in linear mixed models with repeated plots in time using PROC MIXED (Sas, 2002), with autoregressive and animal covariance structure as random effect (Littell *et al*., 2006) according to the mathematical model: $Y_{ij} = X_{ijk}\beta_k + Z_{ijk}b_{ik} + e_{ij}$. For the comparison of means (least square means), the t-test with Tukey-Kramer adjustment was used for the unstable variables (CV < 15%), and the means were adjusted by the t-test for unstable variables (CV > 15%). In addition, the means of the times were compared to the maintenance group using the Dunnett's test. The level of significance was α = 0.05.

RESULTS

During the experimental period, the mean ambient temperatures were 17.4°C and 28.6°C (minimum and maximum, respectively), and the mean air relative humidity was 49%. The mares remained healthy, and all deliveries were eutocic and occurred during the night without human assistance. All newborns foals were healthy. The number of red blood cells in MG was higher (P< 0.05) than that in TG at T0, T15, T30 and T60, with the lowest value observed in the lactating mares at T30 (5.3±0.8). Comparison of the TG animals demonstrated a significant difference (P< 0.05) over time (Tab. 1). Pregnant mares in the T-60 group had a higher number of erythrocytes (P< 0.05) as compared to mares at calving (T0) and lactation (T15 and T30).

Similar results were observed for Hct values, in which the highest means obtained (P< 0.05) in MG mares (34.7±3.7) as compared to TG at T0, T15, T30 and T60. In TG animals, there was a difference over time (P< 0.05). T-60, T-30, T-15 and T0 presented higher average values as compared to T15, T30 and T60, with the lowest mean values observed at T30 (27.1±3.6). Hb also differed (P< 0.05) between the MG and TG (T0, T15, T30 and T60). The MCV differed (P< 0.05) in MG as compared to GT (T0, T15, T30 and T60), with a lower concentration in MG (45.1±4.2) and a higher concentration in the TG at 30 d postpartum (53.4±4.2). Over time, there was also a difference (P< 0.05) in the TG between the animals.

Regarding the MCH values, no differences were found between groups or over time (P> 0.05). For MCHC, the mares in maintenance differed (P< 0.05) from those with foals (T15, T30 and T60), with the highest values obtained in the MG (33.4±1.2) and TG at 60 d postpartum

(27.1±5.6). The results demonstrate that the RDWcv differed between the MG and TG (T30 and T60). In TG, the values were different (P< 0.05) during gestation and delivery. Higher means were observed at T15, T30 and T60, as illustrated in Table 1.

Table 1. Mean values and standard deviations of erythrocyte concentrations (RBC - $x10^6/\mu L^3$), hematocrit (Hct - %), hemoglobin (Hb - g/dL), mean corpuscular volume (MCV - fL), mean corpuscular hemoglobin (MCH - %), mean corpuscular hemoglobin concentration (MCHM - %) and erythrocyte diameter distribution (RDWcv - %) of mares in the transition group (TG) and mares in the maintenance group (MG)

Blood Parameters	Times - Transition Group (TG)							Maintenance Group
	Pregnancy			Delivery		Lactation		
	T-60	T-30	T-15	T0	T15	T30	T60	MG
RBC	7.0±0.8^{a}	7.0±0.9^{a}	6.6±0.9^{ab}	6.3±1.0b^{A}	5.6±0.9^{Ac}	5.3±0.8^{Ac}	6.4±1.2^{Aab}	7.3±1.0
Hct	33.5±2.7^{a}	33.0±2.7^{a}	32.6±3.1^{a}	31.8±3.6^{Aab}	28.3±3.7^{Acd}	27.1±3.6^{Ad}	29.4±3.6^{Abc}	34.7±3.7
Hb	11.0±1.05^{a}	10.6±1.1^{ab}	10.1±1.05^{b}	10.1±1.1b^{A}	8.5±1.1^{Acd}	8.1±1.0^{Ad}	8.9±1.1^{Ac}	11.0±0.9
MCV	48.6±3.1^{cde}	46.5±3.5^{de}	45.6±4.7^{e}	51.5±7.4^{Aabc}	49.2±6.2b^{Acd}	53.4±4.2^{Aa}	52.1±3.7^{Aab}	45.1±4.2
MCH	15.6±0.9^{a}	16.0±3.5^{a}	15.3±1.1^{a}	16.3±2.4^{a}	15.1±1.1^{a}	15.4±1.6^{a}	6.2±7.6^{a}	15.0±1.2
MCHC	32.0±0.9^{a}	31.7±5.8^{a}	32.9±1.1^{a}	31.8±2.3^{a}	31.1±3.7^{Aab}	29.1±3.2b^{Ac}	27.1±5.6^{Ac}	33.4±1.2
RDWcv	7.0±0.8^{a}	7.0±0.9^{a}	6.6±0.9^{ab}	6.3±1.0b^{A}	5.6±0.9^{Ac}	5.3±0.8^{Ac}	6.4±1.2^{Aab}	7.3±1.0

Means followed by lower case letters on the same line differ (P< 0.05) by the Tukey test. Means followed by capital letters in the same row differ (P< 0.05) in the group maintenance by Dunnet's test.

There was no difference (P> 0.05) between groups and or between times in global leukocyte values and segmented neutrophils and rods. The concentration of platelets was similar (P> 0.05) among the groups, and over time differed (P< 0.05) only at T-60. There was a difference in total protein concentrations (P< 0.05) between the MG and TG (T-15). In the transitional mares, T-15 and T0 differed (P< 0.05) from T15 and T30, with the lowest concentrations observed at T30 (7.3±0.7).

There was also a difference (P< 0.05) in fibrinogen concentrations between the MG and TG (-30) and throughout the evaluated times. The highest amount (P< 0.05) was observed at T-30 (484.6±212.9) and the lowest at T-15 (223.0±86.2), as may be observed in Table 2. In the MG, the albumin concentration differed (P< 0.05) from the TG only at time T-15, where the highest mean value was observed (3.9±0.2). In the TG, lower values were obtained (P< 0.05) at T0 and T-30. The amount of globulin was similar among the groups (P> 0.05). The TG had a higher amount of globulin at T0 (4.2±1.1) and differed (P< 0.05) from that at T15 (3.6±0.7). There was no difference in iron levels between the mares in the maintenance group and those in the transition group (P> 0.05) or within the transition group (Tab. 2).

Table 2. Mean values and standard deviations of the iron concentrations (Fe - µg/dL), total protein (TP - g/dL), albumin (Alb - g/dL), globulin (Glo - g/dL) and fibrinogen (Fib - mg/dL) of mares in the transition group (TG) and mares of the maintenance group (MG)

Blood Parameters	Times - Transition Group (TG)							Maintenance Group
	Pregnancy			delivered		Lactation		
	T-60	T-30	T-15	T0	T15	T30	T60	MG
Fe	145.7±107.5^{a}	102.0±24.0^{a}	109.6±20.2^{a}	131.0±63.8^{a}	100.0±19.2^{a}	97.0±20.7^{a}	75.5±36.2^{a}	108.2±63.7
TP	7.5±0.4^{bc}	7.7±0.5^{abc}	7.9±0.5^{Aa}	7.8±0.8^{ab}	7.4±0.6^{c}	7.3±0.7^{c}	7.5±0.5^{ab}	7.4±0.5
Alb	3.7±0.2^{ab}	3.6±0.2^{b}	3.9±0.2^{Aa}	3.6±0.6^{b}	3.8±0.5^{ab}	3.7±0.3^{ab}	3.6±0.2^{ab}	3.5±0.2
Glo	3.7±0.5^{ab}	4.0±0.6^{ab}	3.9±0.6^{ab}	4.2±1.1^{a}	3.6±0.7^{b}	3.7±0.8^{ab}	3.8±0.5^{ab}	3.8±0.5
Fib	300.0±116.6^{bc}	484.6±212.9^{Aa}	223.0±86.2^{c}	392.3±183.1^{ab}	353.8±206.3^{ab}	407.6±222.5^{ab}	383.6±186.9^{ab}	300.0±123.4

Means followed by lower case letters on the same line differ (P< 0.05) by the Tukey test. Means followed by capital letters in the same row differ (P< 0.05) in the group maintenance by Dunnet's test.

DISCUSSION

Significant variations were detected in the hematological and biochemical constituents of Mangalarga Marchador mares in the transition period maintained on pasture. These changes are similar to those found in other studies on mares in different stages of pregnancy, parturition, and lactation (Orozco *et al.*, 2007; Aoki *et al.*, 2013; Meliani *et al.*, 2014). Despite these similarities, it

is important to evaluate this horse breed during this period and under this type of management.

Blood and body fluids present changes during pregnancy to meet the requirement of fetal and placental development, with important variations in blood volume and its components (Carlin and Alfirevic, 2008). During the last trimester, more intense anabolic activity (Vincze *et al*., 2015) has been detected by virtue of the higher nutritional demand of fetal growth. This study found a progressive reduction in the number of RBC in mares during the transition period, mainly in the times evaluated from delivery to the first thirty days of lactation. At T60, there was a small increase in the numbers of RBC, though not significant, as compared to the means during gestation and delivery times (Tab. 1). This finding indicates that near this period (T60), recovery of the hematimetric indices of Mangalarga Marchador mares begins to occur.

Hct and Hb presented a similar behavior to erythrocytes. In mares, at this stage, blood volume expansion of approximately 30 to 50% occurred. This may be caused by factors such as the activation of the renin-angiotensin-aldosterone system, which promotes an increase in fluid volume in the body (Cheung and Lafayette, 2013), resulting in a rise of renal sodium reabsorption and water retention (Bazzano *et al*., 2014b). This system plays an important role in the cardiovascular, hematological, and hemodynamic demands imposed by fetal and placental development, and has a direct influence on fetal viability and birth weight (Satué and Domingo, 2011). However, during this time, there is an increase in the liquid part of the blood without a proportional increase in erythrocyte mass, resulting in hemodilution. Consequently, there is a reduction in the number of RBC, in the Hct, and in the concentration of Hb, generating an anemia by dilution, as described by Chandra *et al*. (2012). These statements elucidate the changes in erythrocyte values detected in this research.

Decreased values of Hct and Hb in mares during the transition period were moreover obtained by Bazzano *et al*. (2014b). It should be noted that the lowest values obtained were recorded postpartum, confirming the results of the present study. According to these authors, this was probably due to the higher intake of water by animals in the final third of gestation generated by the addition of liquid to the placenta and fetus. This finding differs from that quoted by Cheung and Lafayette (2013). Similar results to the present assay were obtained by Hunka *et al*. (2015).

In a study carried out with pregnant mares at different stages of gestation and the onset of lactation, Nagel *et al*. (2016) described the occurrence of hemoconcentration around labor. According to these authors, this may be due to extravasation of fluids during labor, which differs from the results of the present study. The slight increase in RBC, Hct and Hb values recorded at T60 may be related to the reduction in energy requirements required for milk production. Therefore, part of the energy route that was used in this way will be directed to the production of red blood cells.

Equine are animals that do not exhibit presence of reticulocytes in peripheral blood as a hematopoiesis sign, but rather with the presence of macrocytosis and anisocytosis. According to Tvedten (2010), MCV > 53 fl in horses is indicative of a response in hematopoiesis. In the animals of the present study, an increase in the value of MCV at T30 was observed, signaling that there was a response of the organism to the reduction of erythrocyte numbers. Thus, it may be inferred that the small increase in the number of RBC at T60 has the participation of the medullary response.

Similar to other studies on mares (Taylor-Macallister *et al*., 1997; Aoki and Ishii, 2012), the present study found that mean corpuscular hemoglobin (MCH) did not differ during the experimental phase. MCHC demonstrated variations, with decreased concentration ($P< 0.05$) at T30 and T60. When evaluating the reduction of this variable associated with the increase in the values of MCV and RDWcv at T30 and T60 (Tab. 1), the presence of hypochromia, macrocytosis, and anisocytosis were detected. As previously mentioned, progressive hemodilution occurs due to the expansion of blood volume in mares in the transition period, as confirmed in the present study. Answering for that alteration, fifteen days after delivery, the body begins to respond increasing hematopoiesis, which in equines is

characterized primarily by macrocytosis and anisocytosis in the peripheral blood.

With the presence of hypochromia, macrocytosis, and anisocytosis associated with a decrease in the number of RBC and the Hct, these results concluded that in this stage, macrocytic anemia, regenerative hypochromic of low intensity, or as it may be called, regenerative anemia occurs (Stockham and Scott, 2011), the concomitant presence of macrocytosis and anisocytosis support the presence of young red blood cells in the peripheral blood. In evaluating the number of platelets, monocytes, eosinophils, and lymphocytes, the variables remained in the reference range for the equine species and confirms that the alterations detected were discrete, indicating the lack of clinical significance for this finding. Orozco *et al*. (2007) also reported no change in leukogram values in mares at the end of gestation.

Protein and serum albumin shown an increase in mean values at T-15 and T0. This increase, detected in the final third of gestation, may be a result of hormonal variations (Milinković-Tur *et al*., 2005), such as glucocorticoids, which promote mobilization of extrahepatic proteins and transport of amino acids to hepatocytes. The amino acids mobilized in the liver cells are used in most part in glucose synthesis, which is the main source of energy for the embryo (Satué and Montesinos, 2013). These authors found similar results for the amount of protein in Spanish mares evaluated in the final third of pregnancy. The high values of serum protein may also be associated with globulin production, required to form colostrum. The amount of globulin did not differ between the groups, as in a study by Harvey *et al*. (2005), however, a higher mean value was observed at T0 in the mares in transition.

Aoki and Ishii (2012), in a study performed with mares before delivery time, at parturition, and throughout the first four weeks of lactation, observed higher amounts of protein, albumin, and globulin at calving time. The authors explained that a reduction in the amount of albumin and an increase in globulin causes variation in serum protein and that the temporary increase of albumin may be the result of dehydration at delivery, which was not observed in the present study. Nevertheless, a higher concentration of albumin was observed 15 days before delivery (T-15). Mean values were higher in the transition group; however, only T-15 differed from MG, similar to Mariella *et al*. (2014) results. This increase coincides with an increase in protein synthesis (Satué and Montesinos, 2013) in the liver (Milinković-Tur *et al*., 2005). Fibrinogen is a pro-coagulant acute phase protein that may increase in response to an inflammatory stimulus (Mariella *et al*., 2014) and, during pregnancy, its concentration remains high in comparison to the non-gestational period (Hill and Pickinpaugh, 2008).

In general, an increase in the amount of fibrinogen was observed during the transition period, however, these values remained within the reference range for the species. Bazzano *et al*. (2014c), in a study on mares in the gestation and postpartum period, obtained a higher concentration of fibrinogen around delivery. Gentry *et al*. (1991), in a study on healthy mares, observed that the values of fibrinogen increased gradually until delivery. Oliveira (2013) explained that an increase in the value of fibrinogen after calving may be the consequence of an inflammatory process that takes place in the uterus, however, in the present study, the mares did not present this kind of response.

Laboratory tests are aimed at monitoring animal health. The transition period generates changes in different systems of the body, to meet the demands of the fetus, placenta and to protect the mare. The extensive breeding system to which the mares were submitted and the impossibility of altering the management did not allow for a pre-harvesting fast or any type of nutritional supplementation. However, the authors do not believe that these factors interfered with the results obtained.

CONCLUSION

Mares in transition period, maintained on pasture, presented regenerative anemia. The results demonstrate physiological metabolic variations of different intensities during pregnancy, delivery and early lactation. Hematological and biochemical evaluations are necessary for the species, during the transition period, as they reflect the health of the animals, allowing possible interventions.

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