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Hematologic and biochemical profiles in Standardbred mares during peripartum

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ABSTRACT

The purposes of this study were to determine physiological changes occurring in hematologic and biochemical parameters in mares between the last month of gestation and the first week after parturition. If a significant change was observed with respect to the reference interval of an adult horse, a further aim of the study was to establish different reference intervals. Blood samples were collected from 62 healthy pregnant Standardbred mares. Seventeen nonpregnant and nonlactating mares were used as a control group. In pregnant mares, blood sampling was conducted every three days from 1 month before the expected foaling date (335 days after the last mating), at parturition, and 7 days after foaling. The barren mares in the control group were sampled once. Results from samples collected 20 and 10 days before parturition, at parturition, and 7 days after were considered in the statistical analysis. A parametric method for all the parameters studied was used to establish reference intervals. Results were compared by repeated measures ANOVA. When significant differences were observed in relation to sampling time, a post hoc analysis was performed (Tukey test). The one-way ANOVA test followed by Dunnett's test was performed to evaluate the presence of a significant difference between each sampling time and the control group. Any significant difference in the blood count parameters at different sampling times was observed by repeated measure ANOVA. Hemoglobin ($P < 0.01$) and hematocrit ($P < 0.01$) 7 days after parturition and white blood cell count ($P < 0.01$) at parturition were significantly different from the control group. Erythrocyte indices and platelet count were within the normal reference intervals as established in the control group. In the biochemical panel, gamma-glutamyltransferase, creatinine, glucose, biliar acids, total protein, albumin-to-globulin ratio, and calcium were significantly different at different sampling times. Moreover, serum concentration of creatine kinase, aspartate aminotransferase, creatinine, blood urea nitrogen, glucose, lactate, total protein, albumin, albumin-to-globulin ratio, calcium, magnesium, sodium, chloride, potassium, and total, direct, and indirect bilirubin was different from that of the control group. Remarkable changes were not observed in alkaline phosphatase, triglyceride, and fibrinogen concentrations. Temporal changes in the hematologic and biochemical parameters observed in the present study in the peripartum and the differences with reference intervals made up for nonpregnant and nonlactating mares could be used to better evaluate the conditions of periparturient mares.

1. Introduction

Each animal species needs specific reference intervals of hematologic and biochemical parameters for an appropriate interpretation of the results obtained from blood

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samples. Moreover, each analyte could have a distinct reference interval for age, breed, and reproductive status. The hematologic and biochemical changes that occur throughout pregnancy could make the interpretation of laboratory results susceptible to misinterpretation. In women, it is well-known that, during pregnancy, reference intervals are different from the nonpregnant state [1,2].

Hematologic and biochemical changes in peripartum mares were studied little [3–6], although they could be useful to correctly identify metabolic diseases such as hyperlipidemia, and subclinical hepatopathy, reproductive disorders caused by malnutrition, emergencies during foaling, and postpartum diseases such as puerperal fever and metritis. Those studies evaluated the entire period of pregnancy: In one study, pregnancy was divided into three periods of similar duration and the third period ranging from 231 days to the end of gestation [6]; in other studies, the hematobiochemical analysis was repeated once a month [3–5]. No one of those studied thoroughly investigated the last month of pregnancy when most mares received the last veterinarian examinations before parturition.

The purposes of this study were to determine whether significant changes occur in hematologic and biochemical parameters during the last month of gestation, at parturition, and at 7 days of lactation, and whether changes are substantial enough to establish specific reference intervals.

2. Materials and methods

2.1. Animals

Blood samples were collected from 62 Standardbred mares. Thirty mares were housed at Scuderia Trio (Ozzano dell'Emilia, Bologna, Italy) and 32 were hospitalized at the Equine Perinatology Unit "Stefano Belluzzi" of the Department of Medical Veterinary Sciences, University of Bologna, during the 2006 to 2009 foaling seasons. The mares were hospitalized because the owners requested an attended parturition. The farms were located in the same geographic area (district of Bologna); therefore, the mares were in similar pastures and received similar quality of hay, although no attempt was made to strictly standardize feeding procedures and amounts. The experimental design was approved by the Ethic-Scientific Committee for Experiments on Animals of the University of Bologna, in accordance with Decree Law 116/92, and approved by the Ministry of Health. Orally informed consent for mares' participation was given by the owners. The age of the mares ranged from 6 to 21 years. The mares received a complete clinical examination at admission and were diagnosed as clinically normal. All the mares foaled spontaneously, and remained healthy and clinically free of disease during the study period.

2.2. Sample collection and handling

In pregnant mares, jugular blood samples were collected every 3 days from 1 month before the expected foaling date (335 days after the last mating), at parturition, and 7 days after foaling. In mares in the control group, jugular blood samples were collected once. To reduce circadian variations, all samples were collected in the morning, between 9 and 11 hours, except for the one collected at parturition. Blood samples were divided into four aliquots collected in 2.5-mL tubes containing K3EDTA, in 5-mL tubes with gel clotting activator, in 5-mL tubes containing sodium citrate, and in 1.5-mL tubes containing sodium fluoride and potassium oxalate (NaF/KOx), respectively. We used EDTA tubes for hematologic studies, gel clotting activator tubes for clinical biochemistry, citrate tubes for fibrinogen, and NaF/KOx tubes for lactate (Lac). All the K3EDTA tubes were refrigerated (0 °C–4 °C) and analyzed within 24 hours from withdrawal. Gel clotting activator tubes and citrate and NaF/KOx tubes were centrifuged at 3000 rpm for 10 minutes after withdrawal. Citrate and NaF/KO_x plasma and serum were harvested and transferred into plastic tubes. Finally, the samples were stored at −20 °C and analyzed within 2 months after collection. All analyses were performed at the Veterinary Clinical Pathology Service of the Department of Veterinary Medical Sciences, University of Bologna.

2.3. Complete blood count

The K3EDTA tubes were first placed on Vortex (Reamix 2789; Hecht Assistent, Sondheim/Rhon, Germany); then, the samples were analyzed with an automated cell counter (CELL-Dyn 3500 R, Abbott Laboratories, Santa Clara, CA, USA). The red blood cell count ($\times 10^{12}$/L), hematocrit (Hct proportion of 1.0), hemoglobin concentration (g/L), white blood cell (WBC) count ($\times 10^{9}$/L), mean corpuscular volume (fL), mean corpuscular hemoglobin (pg), mean corpuscular hemoglobin concentration (g/L), platelet count ($\times 10^{9}$/L), and mean platelet volume (fL) were measured. Specimens containing clots or grossly hemolyzed were excluded. The quality control of the cell counter was performed every day.

2.4. Biochemical and fibrinogen analyses

In each sample, the concentrations of creatine kinase (CK; μkat/L), aspartate aminotransferase (AST; μkat/L), alkaline phosphatase (ALP; μkat/L), gamma-glutamyltransferase (GGT; μkat/L), creatinine (Cre; μmol/L), blood urea nitrogen (BUN; mmol/L), Lac (mmol/L), glucose (Glc; mmol/L), total, direct, and indirect bilirubin (Bt, Bd, Bi, respectively; μmol/L), biliar acids (μmol/L), triglyceride (mmol/L), total protein (TP; g/L), albumin (Alb; g/L), Alb-to-globulin ratio (A/G), ionized calcium (Ca; mmol/L), magnesium (Mg; mmol/L), sodium (Na; mmol/L), potassium (K; mmol/L), and chloride (Cl; mmol/L) were measured using an automated clinical chemistry analyzer (Chemistry Analyzer Olympus AU400, Beckman Coulter, Milan, Italy). Fibrinogen (μmol/L) was measured with a turbidimetric assay (Turbidimetric Fibrinogen, INstruchemie BV, Delfzijl, The Netherlands). Specimens containing clots or that were grossly hemolyzed were excluded.

2.5. Statistical analyses

Results from the samples collected 20 days (T − 20) and 10 days (T − 10) before parturition, at parturition (Tp), and 7 days (T + 7) after parturition were considered for