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Original Research

Age-Related Developmental Clotting Profile and Platelet Aggregation in Foals Over the First Month of Life

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ABSTRACT

This study aimed to evaluate how selected clotting parameters and platelets' responsiveness change in healthy full-term foals during the first 4 weeks after foaling. Blood samples were collected from each animal every 3 days from day 1 until day 30 after foaling and were tested for prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fb) concentration, platelet (Plt) count, percentage of aggregation and slope. One-way repeated measures analysis of variance, followed by Bonferroni post hoc comparison test, was applied to determine statistical effect of day of life on studied hemostatic parameters. Significant effect of time was found for PT ($P < .0005$), APTT ($P < .0001$), and Fb concentration ($P < .05$). No statistical significant effect of days of life was found for Plt ($P = .19$), whereas platelets aggregation changed ($P < .0001$) during the experimental period. The results obtained in the present study improves the knowledge about blood coagulation and platelet responsiveness in foals during the first month of life showing how hemostatic parameters change at this stage and provide specific information on routine coagulation tests that may support monitoring foal's hemostatic profile during neonatal period.

1. Introduction

Hemostasis is a dynamic physiological process that begins in utero and evolves into the adult system throughout postnatal period [1]. The neonatal period is a critical stage when the newborn has to adapt almost all the body systems to extrauterine life. The time during which these changes take place is known as the adaptive period. At this time, foals are metabolically instable and susceptible to a number of both congenital and acquired diseases that can compromise the health conditions of the newborn and its odd of surviving. Among body functions, the hemostatic system plays a critical role to guarantee foal survival.

The hemostatic system is profoundly influenced by age, and the concentrations of many hemostatic proteins are dependent on both the gestational and postnatal stages [2]. Effectively, it is known that during neonatal period homeostatic mechanisms complete their maturation allowing the foal's adaptation to extrauterine life [3]. Despite homeostatic mechanisms that function to maintain blood parameters within physiological levels, some changes are likely to occur [4]. In particular, it was demonstrated that hemostatic factors are reduced in several animals [2,5] including foals [6,7].

Compared with the adult, the healthy equine neonate possesses unique alterations in hemostatic and fibrinolytic indices [6,8] because placental transfer of coagulation or fibrinolytic factors does not occur, and the fetus's coagulation system evolves independently [6].

These differences are dynamics, and adult values are achieved by 1 month of age [6]. The clinical relevance of these age-related changes becomes important when

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attempting to distinguish clinically normal foals from those with coagulopathies or other associated disorders [9]. Therefore, the establishment of normal values for the components of hemostasis during the early postnatal period is required to provide a basis for the correct diagnosis of heritable clotting disorders in newborn and for recognition of alterations associated with certain disease processes [6,9,10]. However, laboratory diagnosis of coagulation disorders in newborn may be difficult to establish because of the need to adapt all coagulation assays and analytic instruments for small amounts of blood and for the age-related changes of hemostatic system characterizing the postnatal period [1,6].

Some aspects of foals' physiology during postnatal period including endocrine [4], metabolism [11], biochemical [11,12], and hematological [12,13] profiles were investigated. Few studies dealt with modification of clotting profile in healthy newborn foals [6,10,14], whereas, to our knowledge, no research on platelet functional activity in equine species are reported during the early postpartum period.

Therefore, the aim of this study was to investigate whether physiological changes in hemostatic system, including platelets' responsiveness, occur in healthy full-term foals during the first month after foaling.

2. Materials and Methods

All treatments, housing and animal care were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments.

Six Italian Saddle foals (four females and two males, body weight at birth 39.00 ± 3.50 kg) were enrolled in the study with the informed owner consent.

All deliveries occurred between March and April 2014. Each foal was born full term, spontaneously without human assistance, and their dams shed a normal and intact placenta within 2 hours.

All foals were kept in a box with their dams under natural environmental conditions at the same breeding center located in Sicily, Italy (latitude 38.18°N; longitude 15.55°E).

At birth, each foal was subjected to clinical examination (heart rate, respiratory rate, and rectal temperature), routine hematology, and plasma biochemistry testing. All the foals considered healthy were included in the study.

From each foal, blood sampling was performed every 3 days from day 1 (24 hours after birth) until day 30 after foaling. Blood samples were collected in triplicate by the same operator. Samples were collected by jugular venipuncture into 2-mL vacutainer tube (Terumo Corporation, Tokyo, Japan) containing EDTA for the assessment of platelet (Plt) number and into two 3.6-mL vacutainer tubes (Terumo Corporation) containing 3.8% of sodium citrate (1 part citrate: 9 parts of blood) to assess prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fb) concentration, and percentage of aggregation and slope.

Plt number was assessed using an automated hematology analyzer (HeCo Vet C; SEAC, Florence, Italy).

For the evaluation of PT, APTT, and Fb, citrated plasma were obtained by centrifugation, within 15 minutes from collection, at 2,500 rpm for 15 minutes (Thermo Scientific CL10 centrifuge; Thermo Fisher Scientific Inc, Waltham, MA). Commercial standard kits made especially for Clot 2 automatic coagulometer (SEAC) were used. The PT kit was based on the assay principle that the addition of an adequately calcified amount of tissue factor (factor III) to citrated plasma activates factor VII, which induced the formation of a stable plug. The assay procedure was performed by placing 200 mL of tissue factor (PT reagent) in a test tube preheated to 37°C and subsequently adding 100 mL of citrated plasma. On the addition of test plasma, a stopwatch was started, and the clotting time was measured. The time in seconds from plasma-reagent mixing to visual clot formation was defined as the PT.

The APTT kit was based on the addition of a platelet substitute (phospholipids and ellagic acid as a soluble activator) and calcium chloride, which induced the formation of a stable plug. The assay procedure was performed by placing 100 mL of citrated plasma and 100 mL of aPTT reagent (preheated to 37°C) in a test tube preheated to 37°C, followed by an additional incubation for 3 minutes at 37°C, and then adding 100 mL of calcium chloride that had been preheated to 37°C. On the addition of calcium chloride, a stopwatch was started, and the clotting time was measured. The time in seconds from calcium chloride addition to visual clot formation was defined as the APTT.

The standard kit for the quantitative determination of Fb was based on the addition of a relatively large amount of thrombin to diluted citrated plasma, ensuring that the clotting time depended on only the fibrinogen contained in the sample. The assay procedure consisted of placing 200 mL of diluted plasma (diluted 1:10 by the combination of 100 mL of plasma + 900 mL of buffer) in a test tube preheated to 37°C, incubating for an additional 2 minutes at 37°C, and then adding 100 mL of the fibrinogen reagent. On the addition of fibrinogen reagent, a stopwatch was started, and the clotting time was measured. The time (seconds) until clot formation was automatically converted into mg/dL by the automated mechanical endpoint coagulation instrument.

To assess platelet aggregation on all samples, platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared at room temperature. The PRP was obtained by centrifugation, within 15 minutes after collection, at 300 rpm for 20 minutes at room temperature. The upper two third of the PRP was carefully removed, using a plastic transfer pipette, and was transferred into plastic containers. Successively, the PPP was prepared by further centrifugation of the remaining blood at 3,000 rpm for 10 minutes.

Platelet aggregation was measured by adding adenosine diphosphate (ADP) as an agonist to promote platelet activation. The final concentrations of ADP were 1 and 0.5 μM.

Platelet aggregation responses were evaluated using the following two parameters: the maximum degree of aggregation and the initial velocity of aggregation.

The maximum degree of aggregation was determined by measuring the maximum height of the aggregation wave over a 4-minute period, beginning at the onset of