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Hemostatic profile during late pregnancy and early postpartum period in mares

M. Bazzano, C. Giannetto, F. Fazio, S. Marafioti, E. Giudice, G. Piccione*

Department of Veterinary Science, University of Messina, Messina, Italy

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ABSTRACT

Hemostasis is a physiological process that prevents excessive blood loss and represents a protective mechanism at the time of delivery. Peripartum hemorrhage is a recurring hazardous condition to mare's health; therefore, we aimed to study mares' hemostatic profile to investigate whether physiological adjustments occur during late pregnancy and early postpartum. Fifteen pregnant mares have been monitored from the 34th week of pregnancy until the third week after foaling. Fifteen nonpregnant mares were used as control group. Jugular blood samples were analyzed for platelet count (Plt), prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen (Fb). Platelet count showed significant changes at foaling ($P < 0.05$) and a negative correlation ($r = -0.968$; $P = 0.032$) with postpartum. Prothrombin time changed ($P < 0.05$) showing a significant correlation ($r = 0.675$; $P = 0.016$) with late pregnancy. Fibrinogen concentrations changed throughout the experimental period ($P < 0.0001$). The linear regression model revealed a positive correlation ($r = 0.9210$; $P < 0.0001$) between Fb and late pregnancy and a negative correlation ($r = -0.9583$; $P = 0.042$) between Fb and early postpartum. The shortening in PT recorded in the imminence of parturition along with the increase in Plt and Fb at foaling might reflect a physiological hypercoagulable state that constrains excessive bleeding, enhancing mares' odds of surviving. Our research improves the knowledge about blood coagulation in periparturient mares providing specific information on routine coagulation tests that may support in monitoring mare's hemostatic profile during late pregnancy and early postpartum.

1. Introduction

Pregnancy is a physiological condition that significantly influences animal metabolism [1]. Most adjustments occur during late gestation and postpartum after hormonal changes [2,3]. Although several studies dealt with the assessment of blood coagulation in pregnant women [4–6], few researches concerned with hemostatic profile in domestic species such as cows [7,8], sows [9], mares [10], and dogs [11–13] during pregnancy. Hemostasis is a physiological process that prevents excessive blood loss from damaged vessels and represents a protective mechanism at the time of delivery [14]. In women, normal pregnancy is often associated with major changes in blood coagulation and fibrinolysis causing hypercoagulability. This event is probably because of oestradiol-induced triglyceride changes and protects women from fatal hemorrhage during delivery [5]. Also in equine species, the peripartum hemorrhage is one of the most common problems. The rupture of the middle uterine, iliac, utero–ovarian, pudendal or vaginal artery usually occurs during or after parturition. However, few mares may hemorrhage during midgestation to late gestation or up to several days after delivery [15]. The vascular damage can lead the mare to a rapid and profound blood loss resulting in hypovolemic shock and death [15–18]. A few

* Corresponding author. Tel.: +39 0903503584; fax: +39 0903503975.
E-mail address: giuseppe.piccione@unime.it (G. Piccione).

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research dealt with physiological modifications in pregnant mares and it is well known that significant changes occur within this period [16,19,20]. Specific attention was paid to hormonal changes around parturition, and mare's hormonal profile is widely recognized [2,3,21]. Hematological and biochemical changes have also been studied during pregnancy [19], whereas little is known about mare's coagulation profile during the peripartum period [10]. Prothrombin time (PT), activated partial thromboplastin time (aPTT), and platelet count (Plt) are the minimum-recommended laboratory tests for hemostasis. Prothrombin time reflects the function of the extrinsic pathway and aPTT monitors the changes in the intrinsic system and common pathway [22]. We assessed fibrinogen (Fb) to obtain further information about blood coagulation as well.

Peripartum hemorrhage represents a recurring hazardous condition to mare's health; therefore, we aimed to study mares' hemostatic profiles to investigate whether physiological adjustments occur during late pregnancy and early postpartum.

2. Materials and methods

2.1. Animals

Thirty healthy mares of different breed and age (Table 1) were enrolled in the study with the informed owner consent. The study was realized from November 2012 to June 2013 in Sicily (latitude 37.46° N; longitude 14.93° E). Animals were housed in individual straw-bedded boxes (4.0 × 3.5 m) at the same breeding center and were kept under natural environmental conditions. Fifteen pregnant mares (group A) were monitored from the 34th week of pregnancy until the third week after foaling. Fifteen nonpregnant mares (group B) were used as control group. All deliveries occurred within March and midMay. Mares from group A were subjected to daily clinical examination over the first 3 days after foaling. During postpartum, transrectal ultrasound exams were performed weekly to ensure the normal involution of the uterus using the M-Turbo ultrasound system (FUJIFILM SonoSite, London, UK). Animals were fed twice a day (7 AM and 5 PM) and water was available *ad libitum*. Diet consisted of 6 ± 1 kg/day hay and 5 ± 0.5 kg/day concentrates (crude protein 16%, crude fat 6%, crude fiber 7.35%, ash 10.09%, sodium 0.46%, lysine 0.85%, methionine 0.35%, and omega-3 0.65%). Animals were allowed to go to pasture during the day (10 AM–4 PM) as well.

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.

2.2. Data collection

Sampling was performed weekly on the same day in the morning (8 AM) until the time of parturition. All mares delivered within a week (5 ± 2 days) from the last prepartum sampling. Therefore, we expressed each time point before foaling (BF) as weeks BF. Additional samples were taken from each mare within 24 ± 12 hours from foaling (F) and then 7, 14, and 21 days after foaling (AF). Blood samples were collected by jugular venipuncture into 3.6-mL vacutainer tubes containing 3.8% of sodium citrate (Terumo Corporation, Tokyo, Japan) and 3-mL vacutainer tubes containing EDTA (Terumo Corporation). Whole blood with sodium citrate was centrifuged at 1500 × *g* for 15 minutes within 30 minutes from the collection. Citrated plasma and EDTA whole blood samples were placed on ice, delivered to the laboratory, and processed within 2 hours. Platelet count was performed on EDTA whole blood samples using the HeCoVet automatic analyzer (SEAC, Florence, Italy). Sodium citrate plasma was analyzed for PT, aPTT, and Fb using standard kits for Clot 2 coagulometer (SEAC) as described by Casella, et al. [23].

2.3. Statistical analysis

All data are expressed as means ± standard error of the mean. Two-way repeated measures analysis of variance

Table 1
Breed and age (years) of experimental (group A) and control (group B) mares. Gestation length (days) and parity (+, multiparous; −, primiparous) have been indicated for group A.

No.	Breed		Age (y)		Gestation length (days)	Parity
	Group A	Group B	Group A	Group B	Group A	Group A
1	Selle Français	Holsteiner	17	12	337	+
2	Standardbred	Italian Saddle	4	4	360	−
3	Thoroughbred	Standardbred	12	6	346	+
4	Italian Saddle	KWPN	16	10	341	+
5	Paint	Italian Saddle	8	7	350	+
6	Italian Saddle	Thoroughbred	6	6	356	−
7	Thoroughbred	Thoroughbred	5	4	335	+
8	Thoroughbred	Standardbred	5	8	323	+
9	Italian Saddle	Quarter	9	7	333	+
10	Italian Saddle	Italian Saddle	10	18	344	+
11	Italian Saddle	KWPN	3	12	345	−
12	KWPN	Italian Saddle	17	4	328	+
13	Rheinlander	Standardbred	14	6	338	+
14	KWPN	Holsteiner	10	15	331	+
15	Italian Saddle	Italian Saddle	12	18	339	−