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Serum protein electrophoresis profile during late pregnancy and early post partum period in mares

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The aim of the study was to determine how the physiological adjustments occurring during late pregnancy and the early post partum period affect mares' serum protein profile. Ten pregnant mares (Group A) were monitored from the 34th week of pregnancy until the 3rd week after foaling, ten non-pregnant mares (Group B) were used as the control. Blood samples were collected every 3 weeks, from -16 to -4 weeks preceding parturition, and then every week until the 3rd week after foaling. Additional blood samples were taken within 24±12 h from foaling. The statistical analysis revealed a significant increase in albumin and α2-globulin concentrations obtained from Group A during the experimental period. Dunnet's test also revealed significantly higher concentrations of α1-globulins, α2-globulins and γ-globulins in group A than in group B. The following results showed that the serum proteins differed in periparturient mares when compared to non-pregnant mares and significant changes in some protein fractions occurred over the experimental period. Focusing on the peripartum period, our study provides specific information about mare's serum protein profile that could help equine practitioners to better interpret clinical data and promptly diagnose pathological conditions that might compromise the health status of the mare and, as consequence, also her foal.

KEY WORDS: mares / post partum / pregnancy proteins / serum

Serum electrophoresis is a common technique of laboratory diagnosis in veterinary medicine. However, although it provides useful information concerning the protein fractions, serum electrophoresis is not commonly used in equine medicine [Carapeto *et al*. 2006]. Serum protein electrophoresis from healthy horses is characterized by the

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absence of the prealbumin region, and six different bands can be observed: albumin, α1-globulins, α2-globulins, β1-globulins, β 2-globulins, and γ-globulins [DeNicola *et al*. 2004, Morris and Johnston 2002]. Albumin is synthesized from the liver and represents the most osmotically active serum protein and an important protein carrier that plays a role in the transport of free fatty acids, bile acids, bilirubin, calcium, hormones, and drugs [Thrall 2004]. Globulins are a heterogeneous group of proteins classified as α, β, and γ on the basis of their electrophoretic mobility [Thrall 2004]. The α-globulins are mainly synthesized by the liver except for α1-fetoprotein, which is synthesized by the foetal liver cells. The α fraction also includes lipoproteins, α2-macroglobulin, haptoglobin, ceruloplasmin, and amyloid A [Kaneko 1997]. The β fraction includes important protein-like complements, hemopexin, transferrin, ferritin, and C-reactive protein, although some immunoglobulins IgM and IgA extend from the β2 to the γ regions [Kaneko 1997]. The γ fraction includes all types of immunoglobulins that are produced in the lymphoid tissues in response to antigenic stimulation [Thrall 2004]. Several factors can cause a shift in albumin and globulin concentrations, and their quantification by electrophoresis can be important in order to diagnose diseases, including problems in liver function and the immune system [Alberghina *et al*. 2010]. However, abnormalities of serum protein fractions must be interpreted in light of many influences not associated with pathological conditions. Normal physiological variations within an individual are relatively constant over a considerable period of time and even minor changes in the SPE may be of significance [Kaneko 1997]. Therefore, well-established relative and absolute reference values are essential to maximize the diagnostic value of the serum protein electrophoresis [Riond *et al.* 2009]. Physiological changes of serum protein concentrations occurring in healthy horses have been studied by several authors [Piccione *et al*. 2012, Riond *et al*. 2009]. However, only one study [Agricola et al. 2008] dealt with changes in serum protein fractions in ten periparturient Lusitano mares. Recent studies conducted on periparturient mares focused on the last quarter of pregnancy and the first month after foaling, showing that significant changes in hemostatic [Bazzano *et al.* 2014a, 2015], metabolic [Bazzano *et al*. 2014b] and hematologic [Bazzano *et al*. 2014c] profiles occur at this time. Therefore, we aimed to study whether the physiological adjustments occurring during late pregnancy and the early postpartum period affect mares' serum protein profile.

Material and methods

Animals and study design

Twenty healthy mares (10 pregnant mares, 10 non-pregnant mares) of three breeds (Italian Saddle, Thoroughbred, Standardbred) and age (4-17 years) were enrolled in the study. Before starting the sampling period, all the mares were subjected to clinical examination (evaluation of body temperature, heart rate, respiratory rate), routine biochemistry, and transrectal ultrasound examination of the reproductive system, in order to confirm their health status. Animals (under natural photoperiod) were housed

at the same breeding centre (latitude 37.46 N; longitude 14.93 E) in individual straw-bedded boxes (4.0 x 3.5 m), and were moved to paddocks during the day (10.00 AM – 04.00 PM). During the study animals were fed twice a day (07.30 AM; 05.00 PM) and water was available *ad libitum*. Pregnant mares received 6±1 kg/day hay and 5±0.5 kg/day concentrates (crude protein 16%, crude fat 6%, crude fibre 7.35%, ash 10.09%, sodium 0.46%, lysine 0.85%, methionine 0.35%, omega-3 0.65%), while non-pregnant mares received 5±0.5 kg/day hay and 2±0.5 kg/day concentrates. Ten pregnant mares, of mean body condition score (BCS) 6.0-7.5, were monitored from the 34th week of pregnancy until the 3rd week after foaling (Group A). Ten non-pregnant, non-lactating mares, mean BCS 5.5-7.0, were used as the control (Group B).

Mares of group B with known medical history were selected from the same breeding centre. Group A was subjected to daily clinical examination (evaluation of body temperature, heart rate, respiratory rate, vulvar discharge) over the first three days after foaling. Transrectal ultrasound exams were weekly performed to monitor uterine involution and ovarian activity using the M-Turbo® ultrasound system (FUJIFILM SonoSite, London, United Kingdom). All the pregnant mares delivered between March and mid-May and the mean gestation length was 340±10 days.

Blood sampling and analysis

Blood samples were collected every 3 weeks, in the morning (07.00 AM), from -16 to -4 weeks preceding parturition, and then every week until the 3rd week after foaling. Additional samples were taken from each mare within 24±12 h from foaling (F). Blood samples were collected by jugular venipuncture into 9 ml vacutainer tubes containing clot activators (Terumo Corporation, Tokyo, Japan). Blood samples were processed at the laboratory within 30 min from the collection. The tubes were centrifuged at 3,000 rpm for 10 min and the obtained sera were stored at -25°C until analysis. The concentration of serum total proteins was determined by the biuret method using an automated analyzer UV Spectrophotometer (SEAC, Slim, Florence, Italy). The protein fractions were assessed using an automated system (Sel Vet 24, SELEO Enginering, Naples, Italy) according to the procedure used by Alberghina et al. [2013] for equine species. For each sample, 25 μL of serum were applied to numbered sample wells. Each holder accommodated up to 24 samples. Films were electrophoresed for 28 min at 450 V. After electrophoresis, films were simultaneously fixed using an automated system, stained in a red stain acid solution for 10 min, and then dried at 37°C. After destaining in acetic acid and drying completely for 15 minutes films were scanned on a densitometer, displaying electrophoretic curves plus related quantitative specific protein concentrations for each sample. Relative protein concentrations within each fraction were determined as the optical absorbance percentage; then the absolute concentration (g/dL) and albumin/globulin ratio (A/G) were calculated using the total protein concentration. The major protein fractions were divided into albumin, α1, α2, β1, β2, and γ-globulins, from the cathode to the anode, according to the recommendation by the manufacturer.

All treatments, housing and animal care met the requirements of the standards

recommended by the EU Directive 2010/63/EU for animal experiments.

Statistical analysis

Data were tested for normality using the Shapiro-Wilk test. One-way analysis of variance was applied to assess significant effects of the experimental period on the serum protein profile. When significant differences were found, Duncan's post hoc comparison was applied. Dunnett's test was also performed to evaluate whether significant differences were found between pregnant and control mares.. The statistical analysis was performed using the STATISTICA software package (STATISTICA 7 Stat Software Inc., Tulsa, Oklahoma).

Results and discussion

Animals included in the study showed no clinical signs of disease throughout

Table 1. Means and standard errors (in parentheses) of serum total proteins (TP), albumin, α1, α2, β1, β2, and γ-globulins recorded during the study from pregnant (Group A) and non-pregnant mares (Group B). Mean concentrations of each parameter are expressed as g/dL. Time is expressed as weeks before (-16 to -1) and after (+1 to +3) foaling (F)

Item	TP	Albumin	α1	α2	β1	β2	γ
Group A							
-16	7.10 (0.15)	3.38 (0.38)	0.20 (0.03)	0.66 (0.13)	0.82 (0.19)	0.45 (0.15)	1.43 (0.20)
-13	7.08 (0.20)	3.33 (0.37)	0.24 (0.07)	0.67 (0.16)	0.87 (0.23)	0.47 (0.14)	1.44 (0.31)
-10	7.02 (0.16)	3.40 (0.43)	0.22 (0.04)	0.64 (0.08)	0.82 (0.14)	0.50 (0.15)	1.44 (0.18)
-7	7.05 (0.16)	3.38 (0.25)	0.22 (0.04)	0.67 (0.09)	0.80 (0.06)	0.43 (0.04)	1.45* (0.31)
-4	7.18 (0.19)	3.54 (0.29)	0.24 (0.06)	0.64 (0.09)	0.82 (0.05)	0.41 (0.08)	1.41 (0.28)
-3	7.38 (0.17)	3.66 (0.54)	0.23 (0.08)	0.70 (0.17)	0.92 (0.08)	0.47 (0.08)	1.41 (0.33)
-2	7.48 (0.24)	3.62 (0.33)	0.27* (0.07)	0.80* (0.16)	0.90 (0.15)	0.48 (0.14)	1.51* (0.23)
-1	7.55 (0.21)	3.71 (0.63)	0.24 (0.04)	0.78* (0.15)	0.86 (0.17)	0.45 (0.12)	1.52* (0.32)
F	7.59 (0.17)	3.99 (0.58)	0.26 (0.04)	0.70 (0.08)	0.83 (0.12)	0.41 (0.09)	1.40 (0.31)
+1	7.25 (0.18)	3.58 (0.43)	0.22 (0.04)	0.65 (0.09)	0.90 (0.07)	0.43 (0.09)	1.46* (0.28)
+2	7.26 (0.13)	3.49 (0.39)	0.22 (0.03)	0.63 (0.11)	0.81 (0.12)	0.41 (0.09)	1.40 (0.34)
+3	7.30 (0.12)	3.44 (0.25)	0.23 (0.04)	0.68 (0.08)	0.90 (0.07)	0.47 (0.09)	1.49* (0.30)
GroupB	6.92 (0.19)	3.62 (0.53)	0.21 (0.03)	0.60 (0.08)	0.86 (0.13)	0.47 (0.08)	1.08 (0.30)

Significance: * vs. Group B ($P<0.05$).

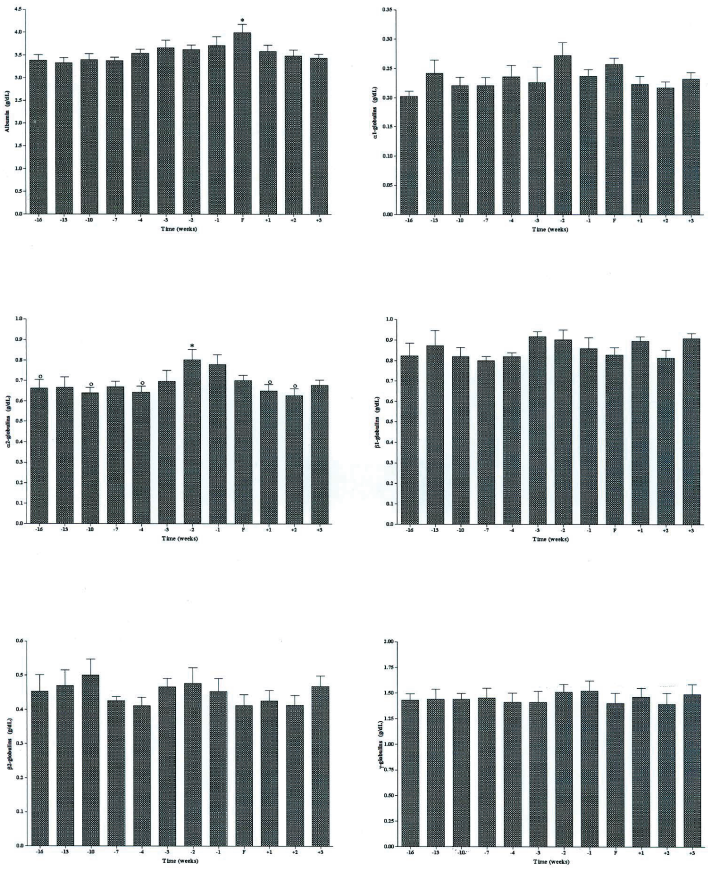

Fig. 1. Means and standard errors (in parenthesis) of serum total proteins (TP), albumin, α1, α2, β1, β2, and γ-globulins recorded during the study from pregnant mares. Time is expressed as weeks before (-16 to -1) and after (+1 to +3) foaling (F).

the experimental period. All the pregnant mares delivered healthy, viable foals at full term gestation, without human assistance. They shed a normal placenta spontaneously within two hours and showed the complete involution of the uterus within two weeks after foaling. Table 1 presents means and standard errors of serum total proteins and their fractions recorded during the study. The statistical analysis revealed significant changes in albumin and α2-globulin concentrations during the experimental period (Fig. 1). In particular, albumin showed a statistically significant increase (P=0.02) during the last month of pregnancy (F vs -16 to -4 and +1 to +3d) as well as α2-

globulins that significantly peaked (P=0.01) at -2 (significances vs. -16 to -4 and +1 to +3) and -1 (significances vs. -16, -10, -4, +1 and +2). Dunnet's test revealed significant differences (Tab. 1) between groups A and B in terms of α1-globulins (at -2), α2-globulins (at -2 and -1) and γ-globulins (at -7, -2, -1, +1 and +3).

In the present study significant changes were recorded in albumin and α2-globulin concentrations in periparturient mares. The progressive increase in serum albumin during the last month of pregnancy might contribute to the increase in Hct values observed in periparturient mares [Bazzano *et al*. 2014c]. Effectively, albumin accounts for approximately 80% of the oncotic blood pressure, preventing water from diffusing from the blood into the tissues [Thrall 2004]. In late pregnancy the increase in blood volume together with the drop in blood viscosity represent important adjustments in order to keep up with the increase in blood flow to organs such as the uterus and kidneys [Harm *et al.* 2012]. The hypervolemia of pregnancy is also necessary to protect the mother and the foetus from the harmful effects of decreased venous return and to prevent the mother from suffering the adverse effects of blood loss during delivery [McMullin *et al*. 2003]. However, a study by Agricola *et al*. [2008] found no differences in albumin concentrations during pregnancy and post partum in Lusitano mares, while higher albumin levels in periparturient Standardbred mares were found by Mariella *et al*. [2014].

During two weeks preceding parturition, the α-globulin fraction significantly increased, so that α1 and α2-globulin concentrations were higher in pregnant mares than in the control. Proteins that migrate in the α-globulin region are the APPs [Morris and Johnston 2002]. The acute phase response is now considered to be a dynamic process involving systemic and metabolic changes, providing an early nonspecific defense mechanism against insult before specific immunity is achieved [Suffredini et al. 1999]. In addition to inflammatory conditions, APPs are also released in normal physiological conditions such as pregnancy [Kustritz 2005]. Previous studies on women [Sacks *et al.* 2004], bitches [Ulutas *et al*. 2009] and mares [Coutinho da Silva *et al*. 2012, Taira *et al.* 1992] dealt with changes in some APPs during the peripartum period, showing that significant increases in specific APPs are likely to occur at that time. Therefore, the higher α-globulin concentration which we found in the present study might be due to an increased APP production.

As previously observed by Agricola *et al*. [2008], no significant change was found in the γ-globulin fraction in pregnant mares during the experimental period. However, γ-globulin concentrations observed in pregnant mares during the experimental period were significantly higher than in the control mares. This difference in γ-globulin levels reveals a different immunological status in periparturient mares that might reflect physiological adjustments to the transfer of immunoglobulins from the maternal circulation to the mammary gland during colostrogenesis [Burns 2007].

In normal equine pregnancy, the foal is born functionally agammaglobulinemic and antibodies that protect the neonate from disease must be obtained from colostrum. The health status, nutrition and body condition score of the mare may be further

colostrum quality variation factors [Drogoul *et al.* 2008]. Because of the importance of protein functions in the body, changes in serum protein electrophoresis, when properly interpreted, can be one of the most useful diagnostic aids available to the clinician to ensure early detection of diseases [Kaneko 1997]. Therefore, monitoring the electrophoretic profile of pregnant mares, especially in the last two weeks preceding foaling, might help to preserve the health status of the mare and, as a consequence, also her foal.

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