

Serum amyloid A protein (SAA), haptoglobin (Hp) and selected hematological and biochemical parameters in wild mares before and after parturition

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Abstract

The aim of the study was to evaluate physiological changes in hematological and biochemical parameters in mares in perinatal period. Blood samples were collected from 24 pregnant Polish Konik breed mares which were divided into two groups. The first group (Group – I, n=12) comprised mares living in the wild, in the reserve. The second group (Group – II, n=12) consisted of mares kept in stables. The blood was collected 2 weeks prior to the parturition, then 24 hours after the delivery, and then at the 7th and 21st day after foaling. When comparing the two groups before the parturition, no significant differences in terms of WBC, RBC, and Hb were found, however, there was a significant difference in MCV, MCH, LYM, NEU and SEG NEU ($p \leq 0.05$). In Group II, 24 hours after the parturition and at the 21st day after foaling, a significant raise in WBC, NEU and SEG NEU ($p \leq 0.05$) was detected. No significant differences in serum concentrations of proteins such as TP, Alb or Glb were observed. As to acute phase proteins, significant rise in SAA and Hp ($p \leq 0.05$) was found in the two examined groups 24 hours after the parturition. Yet, this rise remained within physiological range. The study revealed a certain degree of fluctuations in hematological parameters, in serum concentrations of acute-phase proteins and total proteins in the mares in the perinatal period. However, these changes remained still within physiological ranges and thus they do not indicate potential susceptibility to disorders of perinatal period.

Key words: mare, pregnancy, acute phase response, hematology, biochemistry

Introduction

Perinatal period in mares is characterized by some degree of lability. Hematological and biochemical parameters may undergo certain dynamic changes both before and after the parturition. This is caused mainly by hormonal changes, yet mares age and breed is not without significance, either. The most apparent fluctuations in hematological parameters were observed after parturition because of changes in hormonal profile, due to the beginning of lactation and to growing energy demand (Vivrette 1994, Taylor-Macallister et al. 1997, Ousey 2004, Berg et al. 2007, Orozo et al. 2007). Furthermore, in this period a modulation of mare immune system and of immune response is observed (Moffett and Loke 2004). Stress caused by the parturition as well as the risk of post-partum infections influence hematological, biochemical and immunological parameters, and therefore may be of key importance for mares immunity in this period (Wong et al. 1992, Padgett and Glaser 2003). Yet, it is still controversial whether during pregnancy and in post-partum period there are in fact significant changes in hematological parameters and what would be the cause of them and finally what influence they could have on mares internal homeostasis. Studies performed by Aoki and Ishii (2012) indicate that in mares, at the end of pregnancy and in post-partum period, there are certain changes in WBC distribution and in serum concentrations of proteins. However, these authors did not find significant changes in terms of red blood cells (RBC) count, in hemoglobin (Hb) and hematocrit (Ht) level or in mean corpuscular volume (MCV) and in mean corpuscular hemoglobin (MCH) values. Some other studies report that in pregnancy a moderate anemia can develop (Harvey et al. 1994, Satue and Domingo 2008, Satue et al. 2012).

It seems that acute phase proteins (APPs) play a very important role in the immunity and in monitoring of pregnancy. Acute phase response (APR) is the reaction induced in the animals in the response to tissue lesion (Jacobsen and Andersen 2007, Coutinho da Silva et al. 2013). In horses, APR is observed in several pathologic processes including bacterial, viral and parasite infections, arthritis, burns, surgical interventions, early embryonic death or stress (Jacobsen and Andersen 2007, Krakowski et al. 2011, Coutinho da Silva et al. 2013, Satue et al. 2013). The major APPs in horses include SAA (serum amyloid A) and haptoglobin (Hp). They can act as opsonins and activate the complement system (Jacobsen and Andersen 2007). Serum concentrations of these proteins reflect activity of immune system and could be one of criteria in evaluation of mare healthiness during pregnancy and in post-partum period. Evaluation of these pro-

teins enables differentiation of acute and chronic inflammations and therefore could be very helpful in monitoring mares health condition (Jacobsen and Andersen 2007, Coutinho da Silva et al. 2013). Repeated control of serum APP concentration performed as a part of broadly defined prophylaxis could be a sensitive indicator of internal body homeostasis imbalance, particularly when clinical symptoms are not present. The aim of the study was to evaluate selected acute phase proteins, serum proteins and hematological parameters before and after parturition in mares living in the natural conditions.

Materials and Methods

Experimental animals

The study was performed in the Roztocze National Park, at the Centre of Conservative Breeding in eastern Poland. The study was approved by the Local Ethic Committee on Experiments on Animals at the University of Life Sciences in Lublin, agreement No. 103/2013 of December 2013. The study was carried out on 24 Polish Konik breed mares which were in the third trimester of pregnancy. All examined mares were at the age from 4 to 14 years with body weight ranging from 300 to 400 kg. The mares were divided into two groups. The first group (Group I, $n = 12$) were the mares living in the Reserve. The second group (Group II, $n = 12$) were the mares maintained under conventional conditions in the stables. Mares from Group I who lived in the Reserve did not receive any feed; their food was the only vegetation found in their refuge in the National Park. In turn, the animals from Group II were fed in a standard manner, they were given hay and oats and had ad libitum access to water. All mares included in the study were dewormed following the program used in the Reserve. Mares living in the wild were in a constant contact with their carer, which greatly facilitated their stress-free examination and blood collection. All the mares included in the experiment were naturally mated with stallions of the same breed, and their pregnancies were confirmed by USG examination with the camera (Aloka SD 500 Mitaka-shi, Tokyo, Japan) using a rectal probe with a frequency of 3.5-7 MHz. The study was performed in perinatal period. At the beginning of the study, the mares were clinically healthy and did not demonstrate any signs of systemic homeostasis disorders. The study included clinical observation, with particular emphasis on the final period of pregnancy, parturition course, leaving the placenta and appearance of foaling heat. Meanwhile, the blood samples were collected from the two

examined groups of mares in order to evaluate hematological and, biochemical parameters and to determine selected acute phase proteins. The blood was collected first 2 weeks before parturition (trial -0), and then within the first 24 hours after delivery, and then at the 7th and 21st day after foaling. Blood was collected from the jugular vein into sterile tubes type Vacuette of 9 ml volume (containing heparin, Greiner Labortechnik GmG, Austria). Thus collected blood was delivered to the laboratory within 2 hours. Blood with coagulation activator was centrifuged and the serum obtained was frozen in the temperature -86°C

Hematological analyses

The number of white blood cells (WBC), red blood cells (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean cell volume (MCV), mean cell hemoglobin (MCH) and platelet count (PLT) were measured by using an automated hematology analyzer (MS9-5s Melet Schloesing, Osny, France). Thin blood smears for differential leukocyte population counts were air-dried and stained with Hemacolor staining kit (Merk KGaA, Darmstadt, Germany). Altogether, 200 cells, including neutrophils, basophils, eosinophils, monocytes, and lymphocytes were counted under a microscope at 400 x magnification.

Biochemical analyses

Total protein (TP) and albumin (Alb) were measured using an automatic clinical chemistry analyzer (Mindray BS-130 Chemistry Analyzer, Shenzhen Mindray Bio-medical Electronics Co., Ltd, ShenZen, China). The concentration of globulin (Glb) was calculated as the difference of TP to Alb. Serum concentration of SAA was measured by ELISA test using the commercial kit (Equine SAA; Tridelta Development Ltd, Kildare, Ireland) with double determinations for each sample. The absorbance was read using the ELISA ELX 800 (Bio-Tek Instruments Inc., Winooski, VT, USA) at the wavelength of 450 nm. Concentration of Hp was determined using the colorimetric method with the commercial Haptoglobin Assay (Tridelta Development Ltd). The absorbance was read using the ELISA ELX 800 (Bio-Tek Instruments Inc.) at the wavelength of 450 nm.

Statistical analysis

Statistical analysis was performed using STATISTICA software (version 6.0). The values are presented

as arithmetic means (X) and standard deviations \pm SD. The differences between the mean values of the examined groups were compared using t-Student test for unconnected variables. Probability value of ($p \leq 0.05$) was accepted as the limit of statistical significance.

Results

The results of examinations of blood cells counts in the mares are presented in Table 1. The results indicate that before the parturition, the hematological parameters like WBC, RBC, Hb and Ht did not differ significantly and were comparable with physiological values for this species. However, significant differences were found in the platelet count, in MCV and MCH value, in the number of lymphocytes (LYM), neutrophils (NEU), segmented neutrophils (SEG NEU) and band neutrophils (BAND NEU). In group I, PLT count, MCV and MCH values were significantly higher than those found in group II whereas in group II there was a significantly higher lymphocytes' percentage. Furthermore, in group II a significant difference in the percentage of NEU and SEG NEU was observed. Table 1 presents hematological parameters 24 hours after the parturition. In group II, there was a significant rise in WBC, MCV, NEU and (SEG NEU) compared to group I, whereas the percentage of LYM and MON in group II was significantly lower than in group I. In the first week after the parturition, there were no significant differences in terms of hematological parameters between the examined groups of mares. The only exception were MCV and MCH values which were significantly higher in group II. Three weeks after the parturition, in group II when compared to group I, a significant rise of WBC, MCV, NEU, SEG NEU, BAND NEU and EOS was found, whereas lymphocytes percentage was significantly lower. When comparing hematological parameters in respective periods of blood collected for each group separately, in group I twenty four hours after the parturition there was a significant rise in LYM and N-p percentage; at the same time a significant decline in MON, NEU and SEG NEU was found. Seven days after the parturition, these parameters returned to its pre-foaling values. No significant differences were detected between the 7th and 21st day after the parturition. In group II, the hematological parameters presented slightly differently. Twenty four hours after the parturition, there was a significant decline in LYM and rise in NEU percentage. In the remaining periods of blood collection, rise in LYM percentage and significant NEU decline was observed. Median TP, Alb and Glb values are presented in Fig. 1. No significant

Table 1. Hematological parameters in pregnant mares before and after parturition.

Parameters	Before delivery		24 h after delivery		7 days after delivery		21 days after delivery	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
WBC ($10^9/L$)	11.01±3.62	10.01±2.03	9.67 ^a ±1.75	13.03±3.57	11.28±1.84	11.32±2.01	9.74 ^a ±1.26	11.78±1.22
RBC ($10^{12}/L$)	7.14±1.30	7.61±0.32	8.07±1.09	6.86±1.51	7.23±0.12	6.81±1.36	7.55±0.30	7.35±0.52
HGB (g/dl)	12.71±1.14	12.54±1.05	13.07±1.65	12.32±0.90	11.15±0.34	12.50±2.49	12.96±0.70	13.45±1.39
HCT (%)	35.18±4.33	35.51±2.12	35.57±5.15	33.98±2.07	32.88±1.37	34.64±5.55	35.02±1.43	36.53±2.93
PLT ($10^9/L$)	174.44 ^a ±54.95	138.29±47.34	177.0±93.53	210.83±29.21	216.0±65.45	190.20±22.37	190.60±75.51	185.50±17.25
MCV (fL)	51.33 ^a ±5.28	46.69±21.27	48.29 ^a ±0.90	50.17±2.25	47.0 ^a ±1.72	50.80±1.41	46.20 ^a ±0.75	49.75±1.17
MCH (pg)	18.47 ^a ±2.53	16.50±7.64	17.51±0.68	18.42±1.14	16.95 ^a ±0.44	18.32±0.75	17.46±0.40	18.28±0.97
LYM (%)	26.32 ^a ±10.50	38.14±17.72	31.99 ^a ±4.12	15.82±5.98	23.37±4.82	21.46±2.57	28.84 ^a ±4.98	26.03±0.63
MON (%)	3.37±1.26	3.48±1.96	2.90 ^a ±0.55	2.28±0.28	2.22±0.22	2.50±0.59	2.84 ^a ±0.31	2.45±0.73
Total Neutrophils	70.32 ^a ±11.47	57.66±25.70	65.84 ^a ±4.52	81.83±5.97	74.17±4.90	76.04±2.96	68.18 ^a ±4.90	71.93±0.47
Segments (%)	62.0 ^a ±12.31	51.33±9.55	55.0 ^a ±7.24	72.83±15.90	66.0±6.55	64.20±5.69	58.80 ^a ±7.76	57.25±5.71
Bands (%)	0.11±0.37	0.14±0.05	0.57±0.75	0.67±0.73	0.83±0.49	0.40±0.90	0.20±0.75	1.20±0.40
Eosinophils (%)	4.43±3.20	4.33±1.26	4.14±5.25	5.50±1.64	4.50±3.54	7.80±3.30	2.80±6.43	8.50±1.94
Basophils (%)	0.33±0.54	0.86±1.16	0.43±0.76	0.50±0.49	1.0±0.98	0.80±1.15	1.20±0.19	0.50±0.19

Mean ± standard deviation, (a) significant differences between the two groups ($p \leq 0.05$).

differences between the examined groups in the proteins fractions were detected either before or after the parturition. Only a significant rise in TP was found on the 7 th day after the foaling when comparing group I to group II. However, no significant differences in the parameters between the groups were found in terms of timing of blood collection. When analyzing changes in proteins fractions for each of the examined groups, in group I, twenty four hours after the parturition, there was a significant decline in Alb and Glb, whereas in the remaining periods of blood collection, Alb and Glb concentrations remained at the same level. However, in group II a significant decrease in TP was observed 7 days after the foaling in comparison to the 1st day after the parturition. Concentrations of acute phase proteins are presented in Fig. 1. In prenatal period, the concentrations of SAA and Hp were maintained at the level which is physiological for this species. Twenty four hours after the foaling, a significant rise in SAA and Hp was observed in both groups. Yet, the proteins' concentrations were also maintained within physiological range. On the 7 th and 21st day after the parturition, there was a significant decline in SAA and Hp level returned to the values observed before the foaling.

Discussion

Hematological and biochemical parameters in blood of mares during pregnancy and in post-partum period undergo metabolic self-regulation which is caused by this different physiological state and they are influenced by several external and internal factors. Diagnostic laboratory examinations aim at the monitoring of mares health condition during pregnancy and at detecting potential subclinical states in clinically healthy subjects. Pregnancy causes adaptive changes in all systems including blood which are caused by the presence of fetus and placenta and growing demand of fetal-placental unit for oxygen and nutritional substances (Harvey et al. 1994, Satue et al. 2012). This leads to changes in biochemical homeostasis of the body and several diagnostic parameters do not maintain within physiological range. These changes do not imply yet the disease, however they may serve as an indication of possible development of disorders which will jeopardize mother's and fetal health. Our study revealed that in the examined group of mares no deviations from normal in blood parameters including RBC, Hb or Ht were found either before or after the foaling. Only some fluctuations

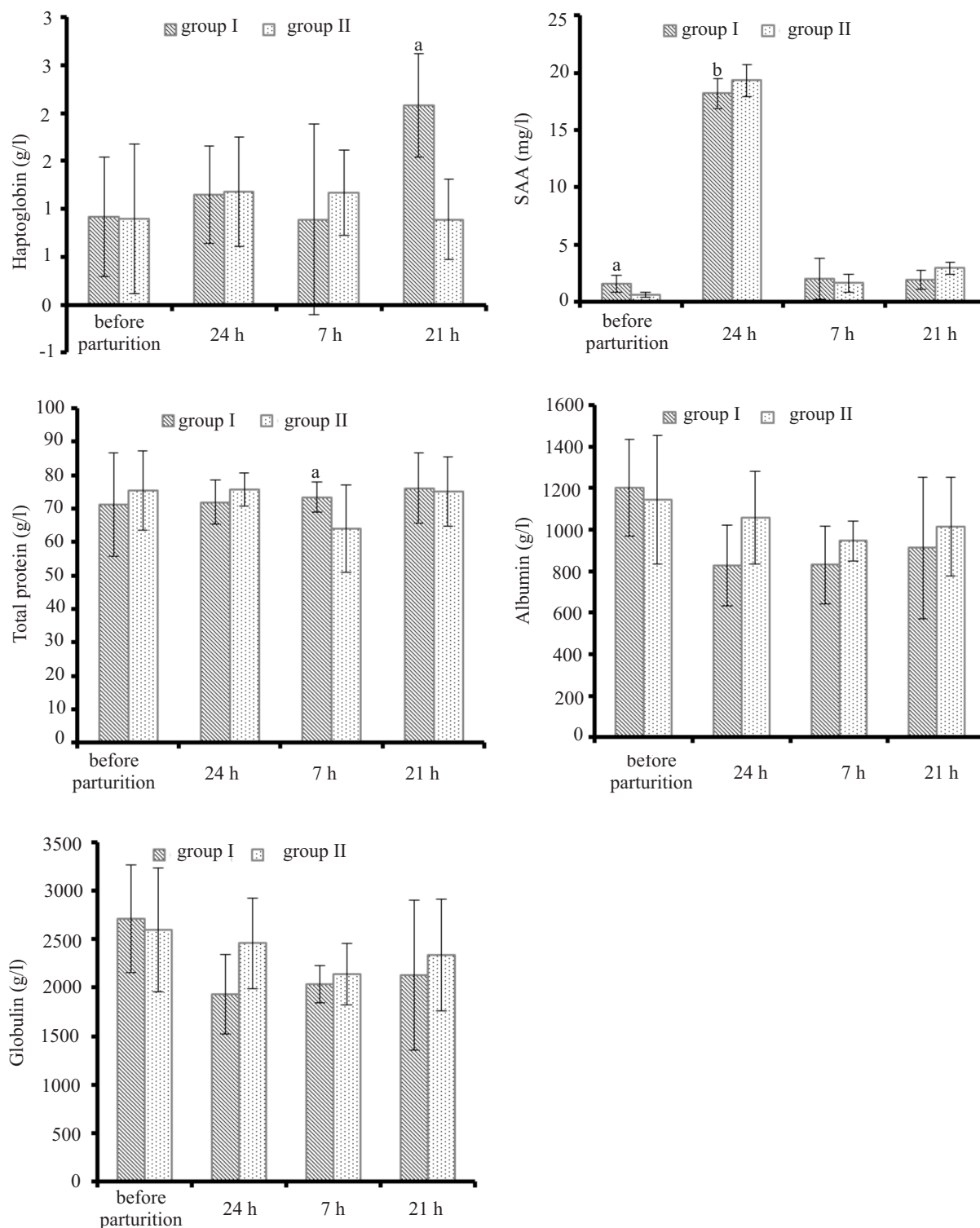


Fig. 1. SAA, Hp and serum proteins in pregnant mares before and after parturition, (a) Significant differences between the two groups ($p \leq 0.05$), (b) Significant differences between the two groups, before and 24 h post parturition ($p \leq 0.05$).

in PLT, MCV and MCH values were observed. Significant differences in these parameters between the examined groups were detected before and after the parturition. As to the complete WBC count in the examined mares, there were certain differences. Contrary to the results obtained by other authors

(Da Costa et al. 2003, Aoki and Ishi 2012, Mariella et al. 2014, Tomenendalova et al. 2014 Meliani et al. 2015), our study revealed that before the parturition, WBC count was rather high with and amounted to 11.01 ± 3.62 ($\times 10^3/\text{mL}$) and 10.01 ± 2.03 ($\times 10^3/\text{mL}$) for the mares from group I and group II, respectively.

Twenty four hours after the parturition, in group I there was a slight decline in WBC count, whereas in group II a significant rise in WBC count with simultaneous rise in neutrophils percentage and significant lymphocytes decline was observed. Interestingly, in the first week after the parturition, in both groups of mares, the total WBC count increased and was comparable to the values determined prior to the parturition. In the 2nd week after the parturition, in group II, again the rise in total WBC count was found. We think that WBC count fluctuations observed after the parturition were due to the clearing of the uterus, to its involution and to appearance of post-foaling estrus. Interestingly, no significant changes in serum concentrations of proteins such as TP, Alb or Glb were observed either before the parturition or after the foaling. The only exception was significant rise in total protein concentration in the first week after the parturition but it concerned only group I. It is difficult to explain this rise as other biochemical and hematological parameters did not indicate the presence of potential inflammatory process (low level of SAA and Hp). May be the rise in total protein concentration was the effect of some undefined environmental factors present in the mares' from group I habitat of living. Evaluation of TP, Alb and Glb is very useful in clinical practice. A rise in total protein concentration can indicate acute or chronic inflammatory process or severe dehydration of the body (Kaneko 1989, Mariella et al. 2014). On the other hand, a significant decline in TP may be indicative of internal hemorrhage or of nephropathy (Kaneko 1989). Quite interesting are the results concerning concentrations of acute-phase proteins in the examined mares. SAA and Hp are considered inflammatory processes biomarkers and high concentrations of them can indicate inflammation at the absence of clinical symptoms (Jacobsen and Andersen 2007, Satue et al. 2013). Our study revealed that only in the 1st day after the foaling there was a significant rise in SAA and Hp. However, these values remained within normal range. In the 1st and in the 2nd week after the parturition, a decline in concentrations of these proteins was observed which can probably indicate that there was no post-partum inflammation and that the clearing and involution of the uterus proceeded properly, without any complication. Other authors also confirm appearance of high concentrations of SAA and Hp a few hours after the parturition (Nunokawa et al. 1993, Coutinho da Silva et al. 2013). APR is thought to be related to damage and deterioration of tissues during parturition (Coutinho da Silva et al. 2013). Lack of any complications during parturition and no case of fetal membrane retention in neither of the examined

mares can serve as evidence in support of this hypothesis. No symptoms indicating systemic homeostasis disorders of the body were found either.

Conclusion

To conclude, when comparing two groups of mares, no particularly important or significant differences in the examined parameters were found either before or after the parturition. This testifies that the two different living environments of mares did not influence significantly hematological or biochemical parameters. However, it has to be underlined that Polish Konik is a primitive breed of horses derived from wild horses and is one of the oldest breeds in Poland. They are characterized by resistance to diseases, high fertility, good maternal features, longevity and capability to adapt to extreme environmental and alimentary conditions.

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