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Original article

Leukocyte modifications during the first month after foaling in mares and their newborn foals

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Abstract

During early post-partum period both neonatal foals and peripartum mares are most susceptible to diseases. The aim of this study was to establish physiologic modifications of leukogram during the first month after foaling in mares and their newborn foals. To this end blood samples were collected from nine mares and nine foals (T0-T10), every three days from the 1st day until the 30th day after foaling. Samples were analysed for white blood cell (WBC) count and differential leucocyte counts.

Two-way repeated measure analysis of variance (ANOVA) showed, in postpartum mares WBC showed significant higher values at T0 (9.02 ± 0.76) in respect to other time points, and at T2 (8.08 ± 0.53) and T3 (7.92 ± 0.59) compared to T1 (6.98 ± 0.43), whereas in foals lower WBC values at T0 (6.11 ± 0.49) compared to other experimental periods except T1 (6.90 ± 0.94), and at T1 compared to T8 (7.95 ± 0.61) and T10 (7.90 ± 0.36) were observed.

The differential leucocyte counts showed significant modifications in the percentage of neutrophils ($p < 0.001$) and lymphocytes ($p < 0.001$) both in postpartum mares and in foals during the experimental period.

Furthermore ANOVA showed significant differences between postpartum mares and foals ($P < 0.01$) in all studied parameters, and between postpartum mares and control mares in WBC and neutrophils values.

The obtained results provide suitable information about the influence of foaling on leukogram of periparturient mares and reveal WBC dynamics in newborn foals during the first month post-partum.

Key words: foals, growing, haematological parameters, leukocytes, mares

Introduction

The early post-natal period is characterized by an increased susceptibility to infectious diseases both in neonatal foals and peripartum mares (Dolente 2004, Knottenbelt et al. 2004). The neonatal period is considered a transitional phase from fetal to extrauterine life. At this time, physiological and metabolic organs

and systems have to meet the new challenges of extrauterine environment (Rossdale 2004). At birth the oral nutrient intake becomes the sole source of nutrition; the colostrum's intake shortly after birth is critical for newborn survival as it ensures the transfer of passive immunity (Knottenbelt et al. 2004). Foals live in an environment heavily populated by bacteria, many of which are capable of causing disease

Table 1. Mean values \pm SD of physiological, haematological and biochemical parameters obtained from newborn foals.

Physiological Parameters				
<i>Body temperature (°C)</i>	<i>Heart Rate (beats/min)</i>		<i>Respiratory Rate (breath/min)</i>	
38 \pm 0.5	115 \pm 6		36 \pm 4	
Haematological Parameters				
<i>RBC (10⁶/μL)</i>	<i>Hb (g/dL)</i>	<i>Hct (%)</i>	<i>Plt (10³/μL)</i>	<i>WBC (10³/μL)</i>
9.58 \pm 0.49	14.71 \pm 1.00	39.90 \pm 2.80	41.61 \pm 1.17	6.11 \pm 0.49
Biochemical Parameters				
<i>Total Proteins (g/dL)</i>	<i>Albumin (g/dL)</i>	<i>Globulins (g/dL)</i>	<i>Urea (mmol/L)</i>	<i>Creatinine (μmol/L)</i>
4.62 \pm 0.17	2.59 \pm 0.09	2.21 \pm 0.10	3.7 \pm 0.2	200 \pm 42
<i>Total Bilirubin (μmol/L)</i>	<i>Triglycerides (mg/dL)</i>	<i>AST (U/L)</i>	<i>ALT (U/L)</i>	<i>Glucose (mg/dL)</i>
28.5 \pm 10	101.81 \pm 8.30	14 \pm 6	221 \pm 30	148 \pm 10

(Giguère and Polkes 2005). Microbial infections constitute a large threat to health in young foals and have been estimated to account for 33% of foal deaths during the first two months of life (Platt 1973, Haas et al. 1996). The haematological profile represents a useful screening and diagnostic test that can promptly provide valuable information about the health status (Bazzano et al. 2014). In horses, age-related changes in hematological parameters preclude the use of adult reference values in the evaluation of foals. The objective of this study was to evaluate the leukocyte count modifications during the first month after foaling in mares and their newborn foals.

Materials and Methods

Nine mares (7-9 years) with their respective newborn foals (5 females and 4 males) and 7 healthy non-pregnant non-lactating mares (control group, 6-10 years) of different breeds (Italian Saddle, Anglo-Arabian) were enrolled in the study with the informed owner consent. All foals were born full term between February and April 2014. The mares' mean gestation length was 340 \pm 10 days. Animals were housed in individual straw-bedded boxes (4.0 x 3.5 m) at the same breeding centre and were kept under natural environmental conditions. At birth and during the experimental period all foals underwent a clinical examination (evaluation of body temperature, heart rate, respiratory rate, auscultation of lungs and heart, external palpation of the umbilicus), moreover routine haematology and plasma biochemistry testing were assessed at the beginning of the study (Table 1). Mares were subjected to daily clinical examination over the first three days after foaling (evaluation of body temperature, heart rate, respiratory rate, external genitalia, vagina, cervix, uterus and ovaries). During post-partum, transrectal ultrasound exams were weekly performed to ensure the normal involution of

the uterus using the M-Turbo[®] ultrasound system (SonoSite, London, United Kingdom).

Both postpartum mares and control mares were fed twice a day (7.30 am; 5.00 pm) and water was available *ad libitum*. Diet consisted of hay (first cut meadow hay, sun cured, late cut; 6.9% crude protein on average) and 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%) differed for two mares groups in quantity: postpartum mares received 6 \pm 1 kg/day hay and 5 \pm 0.5 kg/day concentrates while control mares received 5 \pm 0.5 kg/day hay and 2 \pm 0.5 kg/day concentrates. Foals had access to mares' breast milk *ad libitum*.

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.

Sampling was performed in the morning (08.00 am) at 11 time points (T0-T10), every three days from the 1st (12-24 h from foaling) until the 30th day after foaling. Blood was collected by jugular venipuncture into vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) (Terumo Co., Tokyo, Japan). All blood samples were refrigerated and analysed for complete blood count within 6 h from the collection. All samples were tested for complete blood counts and leukocyte differentiation. The number of total leukocytes was measured using an automated haematology analyzer (HeCo Vet C, SEAC, Florence, Italy). For leukocyte identification and counting, a manual analysis was performed on all samples. Two peripheral blood smears were performed for each sample. After air-drying, the slides were stained through „Dif-Stain” kit (Titolchimica srl, Rome, Italy). The same laboratory professional has later performed the microscopic analysis of blood films by using an optical microscope (Nikon Eclipse e200, Nikon Instruments Europe BV, Amsterdam, Netherlands). A manual 200-cell differential count was performed on each

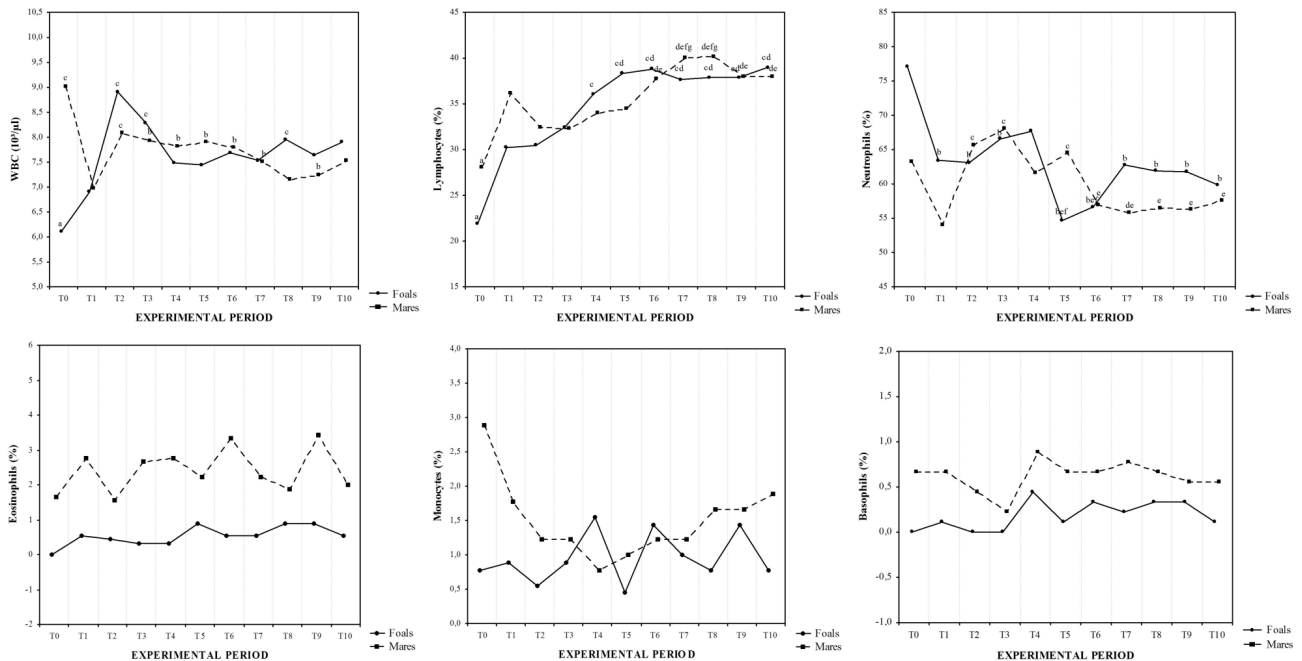


Fig. 1. The trend of mean values together with the relative statistical significances of leukocyte parameters obtained every three days during the first month post-partum (T0-T10). Significances (TIME): a vs all, b vs T0, c vs T1, d vs T2, e vs T3, f vs T4, g vs T5.

blood film. For each animal, the leukocyte differential count was calculated by averaging of the data recorded from each blood film of the same sample.

Two-way analysis of variance (ANOVA) for repeated measures was applied to evaluate changes in the immunologic parameters over time (within-subjects), differences between foals and postpartum mares, and between postpartum mares and control mares. *P* value <0.05 was considered statistically significant. Data were analyzed using statistical software STATISTICA 7 (StatSoft Inc., Tulsa, OK, USA).

Results

Two-way ANOVA showed a statistically significant effect of time ($p < 0.001$) on WBC count both in postpartum mares and foals (Fig. 1). No significant effect of time was observed in control mares.

In particular in postpartum mares WBC showed significant higher values at T0 (9.02 ± 0.76) respect to other time point, and at T2 (8.08 ± 0.53) and T3 (7.92 ± 0.59). compared to T1 (6.98 ± 0.43). In foals Bonferroni's post hoc test showed significantly lower WBC values at T0 (6.11 ± 0.49) compared to other experimental periods except T1 (6.90 ± 0.94), and at T1 compared to T8 (7.95 ± 0.61) and T10 (7.90 ± 0.36).

The differential leucocyte counts showed significant modifications in the percentage of neutrophils ($p < 0.001$) and lymphocytes ($p < 0.001$) both in postpar-

tum mares and in foals during the experimental period (Fig. 1). Lymphocytes count (%) in postpartum mares was lower at T0 (28.11 ± 1.45) in respect to all time points, at T2 (32.44 ± 2.55) and T3 (32.33 ± 2.87) compared to T6-T10, and at T4 (34.00 ± 2.91) and T5 (34.44 ± 2.35) compared to T7 (40.00 ± 2.50) and T8 (40.11 ± 2.89); in foals lymphocytes showed lower values at T0 (21.88 ± 2.02) compared to other time point, at T1 (30.22 ± 2.33) and T2 (30.44 ± 2.69) compared to T4-T10 and at T3 (32.44 ± 2.50) compared to T6-T10. Neutrophils (%), in postpartum mares, showed significantly increased values at T2 (65.66 ± 10.82) as compared to T1 (63.22 ± 8.01) and T7 (55.77 ± 6.15), at T3 (68.00 ± 3.80) as compared to T1, T6-T10; at T5 (64.44 ± 1.42) as compared to T1; foal's neutrophils assumed higher values at T0 (77.11 ± 3.51) in respect to other time point and decreased at T5 (54.66 ± 3.93) and T6 (56.66 ± 5.56) in respect to T3 (66.56 ± 7.56) and T4 (67.66 ± 4.60).

Furthermore, ANOVA showed significant differences between foals and postpartum mares ($p < 0.01$) in all studied parameters (Fig. 2) and between postpartum mares and control mares in WBC and neutrophils values (Fig. 3).

Discussion

The present study shows how leukogram changes in postpartum mares and their newborn foals, but not

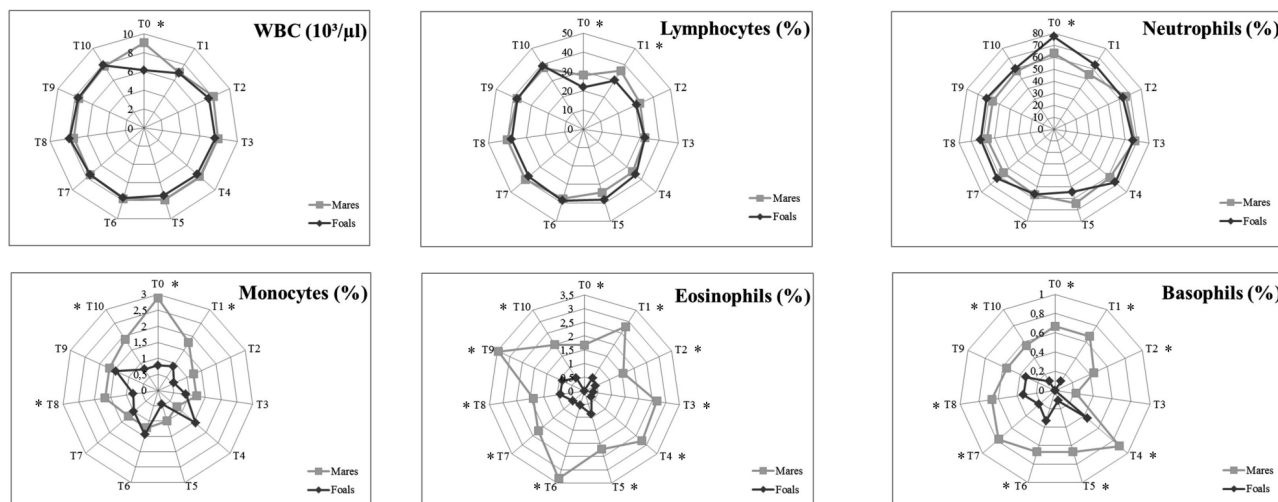


Fig. 2. Radar graph representing the mean values of leukocyte parameters in mares (—◆—) and foals (—■—). Each axis displays the proportion of each leukocyte subset within a given time point (T0-T10). Significances (GROUP): * vs Foals.

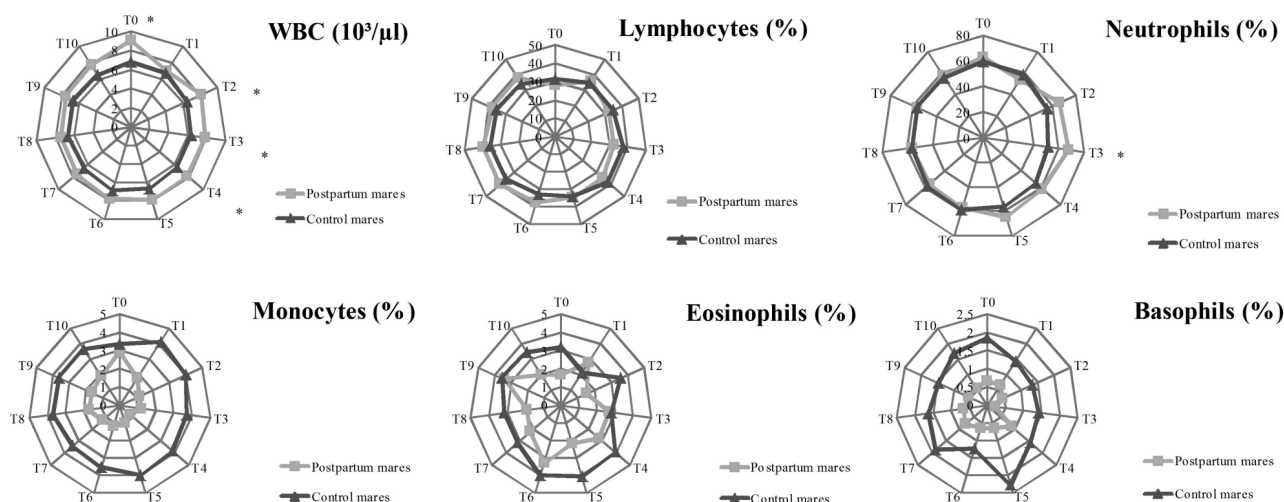


Fig. 3. Radar graph representing the mean values of leukocyte parameters in postpartum mares (—■—) and control mares (—▲—). Each axis displays the proportion of each leukocyte subset within a given time point (T0-T10). Significances (GROUP): * vs control mares.

in control mares. According to previous studies on periparturient mares (Harvey et al. 1994, Aoki et al. 2013, Bazzano et al. 2014, Mariella et al. 2014), our results showed a significant increase in WBC within 24h after foaling. The peak in WBC could be due to physical stress associated with delivery (Aoki et al. 2013) and consequent release of cortisol and catecholamine (Davies Morel 1993). A significant rise in blood cortisol concentrations was observed in pregnant mares in the imminence of parturition as well (Nagel et al. 2012).

It is known that these stress hormones induce significant changes in absolute numbers and relative

proportions of leukocytes in the blood. In fact, changes in blood leukocyte numbers were used as an indirect measure for changes in plasma cortisol before methods were available to directly assay the hormone (Hoagland et al. 1946), and numerous studies in humans have shown that glucocorticoid hormones induce significant changes in blood leukocyte distribution (Dhabhar et al. 1996). Studies have also delineated the rapid and significant effects of catecholamine hormones in mediating stress-induced changes in blood leukocyte distribution (Redwine et al. 2003).

At birth foals showed lower average values in total

leukocyte count. Between T3 and T10 a reduction in WBC was found. These variations are considered physiological and have been previously described in foals of other breeds (Harvey et al. 1994, Stoneham 2006, Aoki and Ishii 2012).

A significant increase in neutrophils was observed in the first 24 h of the foal's life. This increase is probably due to the large increase in circulating neutrophils in response to the peak level of cortisol in the fetal circulation that occurs in this phase (Stoneham 2006, Axon and Palmer 2008). This occurs only in healthy foals and it is directly related to endogenous steroid production, maturity of the adrenocortical system and consequently the foal's maturity at birth (Rossdale 2004). As previously found in newborn foals (Waelchli et al. 1994, Flaminio et al. 1999, Smith III et al. 2002, Aoki and Ishii 2012, Aoki et al. 2013), in this study a progressive increase in lymphocytes during the first month of life was observed. At birth, healthy foals have approximately 2.5 times more neutrophils than lymphocytes, variable number of monocytes, and no eosinophils (Welles 2010). Effectively, eosinophils are low during the first month of life, and slowly increase in number, probably in response to intestinal parasite exposure (Welles 2010). As reported by other researches (Harvey 1990, Curcio and Nogueira 2012) our results showed a low number of monocytes and basophils during the neonatal period.

In conclusion, obtained results provide suitable information about the influence of foaling on leukogram of periparturient mares and reveal WBC dynamics in newborn foals during the first month post-partum supporting clinicians to better interpret clinical data and diagnose equine neonatal diseases.

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