Hematological and biochemical features of postpartum fever in the heavy draft mare

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Heavy draft mares potentially have a higher risk of suffering from postpartum fever (PF) than light breed mares. The purpose of this study was to compare hematological and biochemical features between clinically healthy mares (n=40) and PF-affected mares (n=16). Mares were classified as having PF when their rectal temperature rose to >38.5°C within 96 hr of foaling. The numbers of leukocytes, lymphocytes, and neutrophils and the serum magnesium level were significantly lower in PF-affected mares. The serum SAA and NEFA levels were significantly higher in PF-affected mares. Strong inflammation responses, fat mobilization associated with negative energy balance and possibly endotoxin participate in hematological and biochemical features of PF in heavy draft mares.

Key words: biochemistry, heavy draft horse, hematology, postpartum fever

In equine medicine, sequelae of postpartum metritis vary from a delay in uterine involution to the development of systemic acute metritis (SAM) and life-threatening laminitis [3]. SAM and laminitis are commonly observed in heavy draft mares but rarely occur in Thoroughbreds and other light breeds [15].

Postpartum fever (PF) is a general term for febrile diseases during the puerperal period, and it is often associated with symptoms of SAM [3]. PF is a useful indicator for early detection of both SAM and laminitis. In our preliminary investigation conducted in a large animal clinic, 66 out of 68 heavy draft horses that were diagnosed with PF (rectal temperature >38.5°C) received their first medical treatment within 96 hr after foaling, and 7 horses died or were euthanized because of severe laminitis.

The purpose of this study was to compare hematological and serum biochemical characteristics between clinically healthy foaling mares and PF-affected mares and reveal the clinicopathological features of equine PF.

The peripartum events of 78 foaling mares were studied from January 2012 to June 2013. Mares (9 Percherons and 39 crossbred heavy draft horses, crosses between the Percheron, Belgian, and Breton) were bred on three stud farms. Prepartum mares showing signs of foaling were monitored, and foaling events were recorded. General condition and rectal temperature were recorded twice a day during the puerperal period. We defined a mare as having PF when its rectal temperature rose to >38.5°C within 96 hr of foaling in this study. Mares that developed PF were treated by uterine irrigation using several 10 l saline bags and administration of antibiotic agents, NSAIDs, and corticosteroid as needed. The following mares were excluded from the study: mares that foaled in the absence of witnesses (n=10), mares that were treated by veterinarians for diseases other than PF (n=11), and a mare that died after giving birth (n=1).

Blood samples were obtained on a weekly basis from approximately 1 month prior to the expected foaling date (11 months after the last mating) and then 1 hr, 12 hr, 1 day (24–48 hr), 2 days, 4 days, and 7 days after foaling. We defined the sample collected within 1 week before parturition as the prepartum sample. Peripheral blood was collected into 10 ml vacuum tubes (Venoject II VP-P100K, Terumo Corp., Tokyo, Japan) and 5 ml vacuum tubes containing...
EDTA (Venoject II VP-NA050K, Terumo Corp.) by jugular venipuncture using 21 G × 1.5" needles (MN-2138MS, Terumo Corp.). All blood samples were stored on ice until transfer to the laboratory and processed within 2 hr. Samples containing EDTA were used for complete blood counts and leukocyte differentiation. Plain tubes were centrifuged for 12 min at 3,000 rpm after incubation (37°C, 60 min). Serum was withdrawn and frozen at −30°C for serum amyloid A (SAA) and other biochemical analyses at a later date.

The numbers of total leukocytes, and erythrocytes; hemoglobin concentration; hematocrit; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and platelet count were measured using an automated hematology analyzer (Celltac alpha MEK-6358, Nihon Kohden Corp., Tokyo, Japan). Thin blood smears for differential leukocyte population counts were air-dried and stained using a Hemacolor staining kit (Merck KGaA, Darmstadt, Germany). Altogether, 200 cells, including neutrophils, basophils, eosinophils, monocytes, and lymphocytes, were counted under a microscope at ×400 magnification.

In each sample, the levels of lactate, nonesterified fatty acid (NEFA), total cholesterol, triglyceride, total protein, albumin, globulin, urea nitrogen, creatinine, aspartate aminotransferase, gamma-glutamyltransferase, alkaline phosphatase, creatine kinase, lactate dehydrogenase, iron, calcium, inorganic phosphate, magnesium, sodium, potassium, and chlorine were measured using an automated clinical chemistry analyzer (TBA-120FR, Toshiba Medical Systems Corp., Otawara, Japan) [1].

SAA level was determined using commercially obtained ELISA kits (Tridelta Phase range kit, Tridelta Development Ltd., Kildare, Ireland) according to the manufacturer’s instructions.

Non-normally distributed parameters were logarithmically transformed to approximate a normal distribution before statistical analysis. The sequence of postpartum data was analyzed by repeated-measures analysis of variance (ANOVA). Significant differences between the two groups in each sampling period were determined using the Student’s *t*-test. Results with *P*-values of <0.05 were considered significant.

Forty mares were diagnosed as clinically healthy (CH group; mean age ± SD, 9.2 ± 4.0 years; median, 9.5 years; range, 3 to 17 years), and 16 mares were affected by PF (PF group; mean age ± SD, 10.3 ± 4.1 years; median, 10.5 years; range, 3 to 16 years). Five mares developed a fever between 12 hr and 1 day postpartum, and others developed fever between 1 and 2 days postpartum. The first treatment was performed in all 16 affected mares between the day 1 and 2 sampling time points. No significant differences between groups were observed in any parameter during the prepartum period. There were significant differences between the groups during the postpartum period with regard to the numbers of total leukocytes, neutrophils, and lymphocytes and the SAA, magnesium, and NEFA levels. The numbers of total leukocytes, lymphocytes (12 hr and 1 and 2 days postpartum), and neutrophils (1 and 2 days postpartum) and the serum magnesium level (1, 2, and 7 days postpartum) were significantly lower in the PF group than in the CH group (Table 1). The SAA (1, 2, 4, and 7 days postpartum) and NEFA (2 days postpartum) levels were significantly higher in the PF group than in the CH group (Table 1).

The present hematological study clearly demonstrated that significant decreases in the numbers of leukocytes, lymphocytes, and neutrophils occur in postpartum heavy draft mares when they have a fever of >38.5°C. These hematological findings were in good agreement with the increased serum SAA and NEFA levels in PF-affected mares, probably due to the strong inflammation responses caused by metritis and fat mobilization.

Neutropenia occurs when the rate of emigration of neutrophils from the vasculature into the tissues exceeds the rate of replacement of these cells in the blood by the bone marrow. Typically, this occurs during an overwhelming or severe acute inflammatory disease. If associated with endotoxemia, margination of neutrophils may be a dominant change in neutrophil kinetics [13, 18]. In equine medicine, the relationship between postpartum acute metritis and endotoxemia remains unproven [3]. Blanchard *et al.* reported that infusion of *E. coli* endotoxin into the uteri of normal foaling pony mares on days 1 and 4 postpartum failed to result in a detectable presence of endotoxins within the blood, and neutropenia did not occur [4]. Although leukopenia was observed in the PF-affected mares in the present study, we could not distinguish the two possible causes, i.e., 1) mobilization of peripheral leukocytes to the injured uterus or 2) margination of neutrophils (adhesion to the vascular endothelium) by endotoxins.

SAA, an acute phase protein, is clinically utilized for the diagnosis of inflammatory diseases [7, 8]. Macrophages and neutrophils, accumulated at the site of challenge, produce a wide range of mediators including the pro-inflammatory cytokines. Hepatic biosynthesis of SAA is upregulated by these pro-inflammatory cytokines, and the circulating levels can increase up to 1,000-fold during an acute phase response [9]. In equine medicine, SAA is detected at very low levels in healthy horses, but the levels rapidly increase when horses are affected with an inflammatory disease [11, 12]. The SAA level increases even after normal parturition [11]. In the present study, the serum SAA levels in clinically healthy mares also increased after foaling. Those of the PF group showed higher values than those of the CH group after 1 day postpartum. This result suggests that more
damage occurred to the uterus and/or birth canal during foaling in the PF group than in the CH group.

The serum magnesium level depends on dietary intake and is regulated by mineralocorticoids and parathyroid hormone. Hypomagnesemia is sometimes complicated by hypoproteinemia, because approximately 30% of serum magnesium is bound to negatively charged sites on proteins [5, 14]. However, prolonged anorexia or hypoproteinemia were not observed in this study. According to a recent equine study, during experimental endotoxemia, the serum magnesium level was decreased. The change occurred quickly, and it occurred because of the movement of ionized magnesium from plasma to cells [16]. The hypomagnesemia observed in mares of the PF group might have been associated with endotoxemia, although this hypothesis remains unproven.

The blood NEFA level is used as an indicator of lipid degradation in adipose tissue. If an animal needs to mobilize stored energy from adipose tissue, the blood NEFA level will increase [6]. Higher NEFA levels in the PF group indicate that fat mobilization increased as an energy source. Although fat mobilization was only a temporary condition in this study, previous reports state that persistent and excessive fat mobilization leads to hyperlipidemia and that its predispositions are pregnancy, lactation, and obesity [2, 10]. The mortality rate of equine hyperlipemia is approximately 70% [17]. When treating equine PF, preventive treatments for hyperlipidemia might be required in addition to typical therapies for SAM.

In conclusion, it is suggested that strong inflammation responses, fat mobilization associated with negative energy balance, and possibly endotoxin participate in hematological and biochemical features of PF in heavy draft mares.

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### Table 1.

The numbers of total leukocytes, neutrophils, and lymphocytes and the levels of serum SAA, magnesium, and NEFA in clinically healthy mares and postpartum fever-affected mares

| Time after foaling | Before foaling | 1 hr | 12 hr | 1 day | 2 days | 4 days | 7 days |
|--------------------|---------------|------|------|-------|--------|--------|-------|
| **Leukocytes (10^3/µl)** | | | | | | | |
| CH | 840 (2.5) | 102.3 (3.1) | 92.8 (2.2) | 81.6 (2.8) | 74.3 (3.3) | 77.3 (1.6) | 92.0 (2.1) |
| PF | 83.4 (3.6) | 100.4 (6.4) | 79.2 (3.9) ** | 34.8 (2.5) ** | 46.9 (6.5) ** | 71.9 (4.6) | 93.9 (7.9) |
| **Neutrophils (10^3/µl)** | | | | | | | |
| CH | 57.3 (2.5) | 80.8 (3.2) | 67.3 (2.4) | 52.7 (2.5) | 45.7 (3.0) | 49.8 (1.6) | 61.7 (1.9) |
| PF | 58.4 (3.6) | 82.1 (5.6) | 59.2 (3.5) | 19.1 (2.3) ** | 28.6 (6.0) ** | 43.1 (4.1) | 66.8 (6.4) |
| **Lymphocytes (10^3/µl)** | | | | | | | |
| CH | 24.9 (1.1) | 20.2 (1.2) | 24.2 (1.3) | 26.3 (1.2) | 26.2 (1.2) | 25.3 (1.0) | 27.1 (1.3) |
| PF | 23.2 (1.7) | 17.2 (1.5) | 19.1 (1.3) * | 14.3 (1.3) ** | 17.2 (1.5) * | 26.8 (2.6) | 25.1 (2.0) |
| **SAA (log102 µg/ml)** | | | | | | | |
| CH | 0.35 (0.06) | 0.58 (0.13) | 1.00 (0.15) | 1.13 (0.16) | 0.92 (0.16) | 0.52 (0.13) | 0.17 (0.07) |
| PF | 0.35 (0.08) | 0.80 (0.16) | 1.46 (0.21) | 1.81 (0.24) * | 2.60 (0.22) ** | 2.24 (0.29) ** | 0.94 (0.27) ** |
| **Magnesium (mg/dl)** | | | | | | | |
| CH | 2.01 (0.03) | 2.04 (0.04) | 1.92 (0.03) | 1.85 (0.04) | 1.87 (0.05) | 1.99 (0.05) | 1.99 (0.04) |
| PF | 1.94 (0.09) | 1.92 (0.06) | 1.86 (0.06) | 1.61 (0.05) ** | 1.60 (0.07) ** | 1.95 (0.10) | 1.82 (0.06) * |
| **NEFA (log102 µEq/l)** | | | | | | | |
| CH | 2.11 (0.04) | 2.22 (0.04) | 2.00 (0.04) | 2.03 (0.05) | 2.01 (0.04) | 1.93 (0.04) | 1.89 (0.03) |
| PF | 2.10 (0.08) | 2.24 (0.06) | 2.10 (0.06) | 2.11 (0.06) | 2.20 (0.07) * | 2.06 (0.08) | 2.02 (0.07) |

CH group, n=40, PF group, n=16. Results are expressed as means (standard error of mean). Asterisks indicate significant differences between the groups, *P*<0.05, **P*<0.01.
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