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Original Research

Relation Among Neutrophil Enzyme Activity, Lipid Peroxidation, and Acute-Phase Response in Foal Heat in Mares

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ABSTRACT

Apart from functional abnormalities, genetic structural disorders and management problems endometritis is one of the major causes of infertility or subfertility in mares. However, the causes of postbreeding endometritis in foal heat have not been clearly resolved to date. The aim of this study was to search for the relationship between neutrophil activity, acute-phase proteins, and oxidative status to indicate the parameters, which can influence fertility in cold-blooded mares in foal heat. The blood for the experiment was collected from 16 cold-blooded mares at five time points: 6–8 days before parturition, 24 hours after parturition, at the first postpartum breeding on the ninth day, 24 hours after breeding, and 48 hours after ovulation. The obtained samples were assigned for hematological tests, assays of neutrophil activity, plasma malondialdehyde (MDA), and fibrinogen concentrations. We estimated that in susceptible mares during persistent postbreeding endometritis, neutrophil activity increased together with MDA and fibrinogen plasma level. Elastase release in resistant mares before parturition was 48.91 ± 1.75%, whereas in susceptible animals, the value reached 45.57 ± 1.9% of the maximal release. Myeloperoxidase release in resistant mares before parturition reached 13.95 ± 2.1%, then increased at three consecutive measurements, and returned to a value from before parturition at the last measurement. Myeloperoxidase level in susceptible mares was slightly lower than in resistant ones, then these values augmented at all measurements, reaching the maximum at the fourth one. The obtained results may help to indicate the predisposition to persistent postbreeding endometritis in cold-blooded mares bred at foal heat.

1. Introduction

In mares, in comparison with other species, the first postpartum estrus, commonly called as foal heat, has some unique features. It is characterized by the very early onset, is an ovulatory estrus, visible without a preceding progesterone phase, and the uterine involution has not been completed. The pregnancy rates in foal heat have usually been lower than in subsequent estrus periods, but there is no practical way of diagnosing which mares are ready to be bred in foal heat and which ones are not [1]. Moreover, the factors which could influence the susceptibility to endometritis as a cause of lower fertility were not fully recognized.

Among important factors causing lower fertility is postbreeding endometritis, an infective and inflammatory condition of the uterus characterized by the presence of neutrophils in endometrium [2]. When endometrial tissue

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receives a stimulus such as semen or bacteria, an early inflammatory process begins with neutrophil migration, fluid accumulation, and the presence of inflammatory mediators. However, if the inflammation persists, the intrauterine environment is not able to support a pregnancy [3,4].

It was estimated before that breeding in normal resistant mares results in a transient neutrophilic endometritis that usually resolves within 48–72 hours [5] often, but not always, with the presence of accumulated intrauterine fluid, which is resolved until 12 hours after mating. In susceptible mares, in turn, a persistent postbreeding endometritis develops, resulting in prolonged accumulation of inflammatory fluid within the uterus. Although the affected mares do not show clinical signs associated with systemic disease, the increase of acute-phase proteins may indicate an inflammatory process with activation of circulating neutrophils [6].

Neutrophils are vital for host defense and for the initiation and control of inflammation and immunity. Among neutrophil products, elastase and myeloperoxidase (MPO) are toxic mediators involved in killing of bacterial pathogens [7]. However, if neutrophil secretory response is excessive or prolonged, it may be detrimental and may cause tissue injury. It has been reported previously that excessive neutrophil function related to coagulation disorders leads to severe reproductive complications in dairy cows [8].

The secretory activity of circulating neutrophils as a causative agent in postpartum endometritis in mares has not been fully clarified to date. Separately, oxidative stress has been proposed as a potential factor involved in the pathogenesis of endometritis leading to low fertility [2]. However, interactions between neutrophil secretory activity, oxidative status, and acute-phase response in predisposition to infertility have not been investigated to date. The aim of our study was to characterize the parameters involved in susceptibility to persistent endometritis in cold-blooded mares and relate these parameters to the ability of the mares to become pregnant during postpartum heat.

2. Materials and Methods

2.1. Animals and Study Design

The study was conducted from March to June 2012 on 16 cold-blooded mares, aged 3–7 years. All mares were kept in one stable. The mares were fed oats and hay and were watered ad libitum. The body weight of mares ranged from 600 to 800 kg. The mares involved in the experiment were in the third trimester of pregnancy and were normal on clinical, gynecologic, and hematological examination before the start of the experiment. Blood samples from the jugular vein were obtained at five time points: 6–8 days before parturition (first measurement), 24 hours after parturition (second measurement), at the first postpartum breeding on the ninth day (third measurement), 24 hours after breeding (fourth measurement), and 48 hours after ovulation (fifth measurement). The obtained blood samples were assigned for hematological tests, isolation of neutrophils for in vitro assays, and plasma malondialdehyde (MDA) and fibrinogen concentrations.

All mares included in the experiment were repeatedly examined ultrasonically with Aloka 500 ultrasound with 7.5-MHz linear probe for detection of foal heat, ovulation, and presence of uterine fluid after breeding. The first examination was on the ninth day after parturition, at that time the uterine edema and follicle size were taken into consideration. Consecutive examinations were conducted every 24 hours until detection of ovulation. All mares were bred naturally with the same stallion, when the follicle reached the size of >35 mm. Then, ultrasonography was done 72 hours after detected ovulation for detection of intrauterine fluid. Pregnancy diagnosis was performed with ultrasound 16 days after ovulation. Scans were then repeated at the 45th-day of gestation to confirm the presence in the uterus of an apparently healthy developing conceptus.

Cytology samples were analyzed at the ninth day postpartum and 72 hours after ovulation. The samples were collected with cytology brushes (Minitube Abfull-und Labortechnik GmbH, Germany), smeared on glass slides, dried at room temperature, and stained with Diff-Quick. Polymorphonuclear neutrophils (PMN) were counted in five fields (×400 magnification), and the mean number per field was calculated.

At the previously mentioned time points, the samples for bacteriologic assay were also obtained with uterus culture swabs (Minitube Manual GmbH, Germany). The uterine swabs were streaked on blood agars (5% ovine blood) and incubated aerobically for 24 hours at 37°C. Culture results were recorded as *Escherichia coli*, beta-hemolytic streptococci, staphylococci, other uterine pathogens, or no growth. Mares with mean PMN counts >2 in five fields, together with positive bacteriologic tests, were considered to have inflammation.

Two mares were excluded from the experiment immediately after parturition because of inflammatory complication after the death of neonate foals. The remaining mares were classified for the experiment as resistant (n = 6) or susceptible to endometritis (n = 8) based on history, clinical evaluation, ultrasonography, cytology, bacteriologic assay, uterine fluid retention, and the ability to fertilize.

2.2. Laboratory Assays

Hematological tests including red blood cells, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, differential white blood cell (WBC) count, and platelets count were done using hematology analyzer ABC VET-HORIBA.

Neutrophils were isolated from peripheral blood. After red blood cells had been lysed by the addition of 0.83% ammonium chloride at the ratio of 3:1, the remaining pellet was washed twice with phosphate-buffered saline (Biomed, Lublin, Poland). After isolation, the viability of PMN cells was determined by trypan blue exclusion. After cell counting and differentiation (>85% of neutrophils on May-Grunwald-Giemsa–stained preparations), cell suspensions were adjusted to a final concentration of 2×10^6 cells/mL.

Neutrophil activity was determined on the basis of elastase and MPO release and nitric oxide (NO) generation.