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Original Research

Use of a Qualitative Horse-Side Test to Measure Serum Amyloid A in Mares With Experimentally Induced Ascending Placentitis

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ABSTRACT

The objectives of this study were to assess (1) changes in serum amyloid A (SAA) detected by a lateral flow device (LFD) in mares with experimental placentitis and (2) to assess the correlation of this assay with a quantitative immunoassay. Horse mares carrying normal pregnancies were assigned to a control group (n = 12) and to a group with experimentally induced ascending placentitis (n = 13). Blood samples were obtained immediately before inoculation and/or initial sampling for control mares and then daily for 12 days or until abortion. Concentration of SAA was analyzed using a turbidometric immunoassay. Retrospectively, samples −9, −6, −3, and 0 days from abortion (DFA) were analyzed. Day 0 was defined as the day of abortion for mares in the placentitis group and the last sampling day for mares in the control group. Based on the LFD results, there were significant differences between mares in the control group and mares in the group with experimentally induced placentitis ($P < .0001$). There was a progressive increase in SAA from −6 to 0 DFA determined by the LFD. There were no significant differences among examiners ($P > .05$). The results obtained using the commercial LFD were moderately correlated ($\rho = 0.51$; $P < .001$) with the concentrations of SAA determined by the turbidometric assay. Five of 91 LFDs (i.e., 5.5%) failed to provide any reading. The LFD for determination of SAA appears to be a useful tool for quick assessment of whether inflammation is present; however, quantitative evaluation may still be warranted.

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1. Introduction

Placental diseases impose significant losses to the horse industry. Among these diseases, bacterial placentitis has been identified as the leading cause of losses from abortion and neonatal death (≤24-hour postpartum) [1–3]. Bacterial placentitis has been commonly associated with *Streptococcus equi* subspecies *zooepidemicus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Leptospira interrogans* (multiple serovars), *Crossiella equi*, and *Amycolatopsis* species (*A. kentuckyensis*, *A. lexingtonensis*, and *A. pretoriensis*) [2,4]. These bacterial agents can cause four different morphologic types of placentitis: ascending, focal mucoid (nocardioform), diffuse (hematogenous), and multifocal [4]. Overall, ascending placentitis is the most frequent, whereby beta-hemolytic streptococci predominate [2,5].

The diagnosis of ascending placentitis is based on clinical signs (i.e., vulvar discharge, premature udder development, and lactation) and transrectal ultrasonography [3,6]. Although transrectal ultrasonography is a

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valuable clinical tool to diagnose and manage placentitis [3,6], the technique is prone to false-positive diagnosis if improperly applied, and early cases of the disease can be missed. Thus, our group has been focused on identifying new diagnostic tests for equine placentitis. To date, several markers (e.g., acute-phase proteins, fetoplacental steroids, alpha-fetoprotein) have been investigated in experimental cases, and some appear to be useful tools to detect experimentally induced placentitis [7–11]. Among these markers, acute-phase proteins, particularly serum amyloid A (SAA), appear to be a sensitive but nonspecific marker for placentitis [7–11].

Serum amyloid A is a major positive acute-phase protein in the horse [12] mainly produced by the liver and lungs [13]. In clinically normal horses, SAA is present in low concentration (i.e., 20 mg/L) and upon inflammation is rapidly increased several fold [12]. Additionally, contrary to fibrinogen, SAA does not remain elevated when the inflammatory process has subsided [14]. Two of eight mares with experimentally induced ascending placentitis that received treatment (i.e., antibiotics, altrenogest, and pentoxifylline) had an elevation in SAA [15]; however, all five mares with experimentally induced placentitis that were untreated had an elevation in SAA and aborted thereafter [15]. In a recent study in our laboratory, we investigated whether SAA could be used as diagnostic tool for mares with experimentally induced bacterial placentitis [11]. Mares with experimentally induced placentitis had elevated SAA concentration starting 2 days after inoculation, and SAA remained elevated until abortion [11]. In addition, foaling did not induce an elevation on SAA levels [11].

Although SAA testing has been widely available in some countries for several years, in other countries, laboratory testing for SAA is not widely available, or when available may require overnight shipment, and the results may be available only one or two business days later. Costs with shipment and waiting time for results have limited the value of SAA testing in countries where the test is not available in referral laboratories. Thus, having a horse-side test for SAA available would potentially solve part of these limitations and increase the use of SAA testing. Different commercial kits have been marketed over the last 2 years; however, research comparing horse qualitative tests against well-established quantitative tests has not been performed. The Eiken assay has been the most widely studied method to quantify SAA in the horse [11,14,16]. Therefore, the objectives of this study were to assess (1) changes in SAA detected by a lateral flow device (LFD) in mares with experimentally induced placentitis and (2) to examine the correlation of this assay with a quantitative immunoassay.

2. Materials and Methods

Mares of different ages and light breeds were enrolled in this experiment. Mares were maintained at the Maine Chance Farm, Department of Veterinary Science, University of Kentucky, Lexington, Kentucky. All the experimental protocols were approved by the Institutional Animal Care and Use committee at the University of Kentucky (project #2010-0769). All the animals were kept in paddocks and supplemented with hay, grain, and water ad libitum along with trace minerals. Immediately before abortion or before normal parturition, the mares were kept in individual stalls (16×16 feet) during the night and turned out in pasture during the day.

Clinically healthy adult horse mares carrying normal pregnancies (260–280 days of gestation) were assigned to a control group ($n = 12$) or to a group with induced ascending placentitis ($n = 13$). As part of another study [10], six mares within the control group had transabdominal ultrasound-guided fetal fluid sampling at 0 (i.e., the day of inoculation or commence of sampling for mares in the control group), 5, and 12 days after inoculation or until abortion. Similar methodology has been described elsewhere [17]. In a previous publication, we demonstrated that fetal fluid sampling had no effect on the concentrations of SAA [11].

Ascending placentitis was induced via intracervical inoculation of *Streptococcus equi subspecies zooepidemicus* [11]. The experimental model for induction of bacterial placentitis in the mare was modified from that previously described [18]. Sham inoculation of control mares was not performed in the present study as this procedure may induce placentitis in an unpredictable manner, as noted by previous publications. The bacterial inoculum was deposited midway intracervically with a semiflexible artificial insemination pipette [11]. The inoculum was contained in a 0.5-mL straw and was deposited with the use of a stylet [11]. The bacterial strain used was isolated from a placenta from a mare diagnosed with spontaneous ascending placentitis by the Veterinary Diagnostic Laboratory at the University of Kentucky. The bacterial isolate was preserved in cryovials containing skim milk at −80°C until use. Before inoculation, bacterial cultures were prepared and bacterial counts performed in standard fashion using blood agar plates (incubated at 37°C), serial dilution, and plating were used to determine the number of colony-forming units (cfu) [11]. The bacterial inoculum, containing five million cfu, was suspended in 0.5 mL of phosphate-buffered saline [11]. Once inoculation was carried out, a swab was obtained from the leftover in the cryovial and cultured in similar conditions to assure that the bacteria inoculum was still viable [11].

Blood samples were obtained immediately before inoculation and/or initial fetal fluid sampling and then daily for 12 days or until abortion. Immediately after collection, the blood was centrifuged at 600*g* for 10 minutes at 5°C, and plasma was harvested and preserved at −20°C until further analysis. Concentration of SAA was analyzed using a turbidometric immunoassay (Eiken Chemical Co, Ltd, Nagoya Naka Ward, Aichi Prefecture, Japan) [16]. Reported intercoefficient and intracoefficient of variations were 5.4% and 2.8%, respectively [14]. For the turbidometric immunoassay previously reported sensitivity was 53% and specificity was 94% [14]. Retrospectively, only samples at −9, −6, −3, and 0 days from abortion (DFA) in mares with experimentally induced placentitis were analyzed in the present study. For mares in the control group, day 0 was defined as the last sampling day, similarly the remaining three sampling days (−3, −6, and −9) were also analyzed.

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