TÜBİTAK

Case Report

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Prevention of predicted neonatal isoerythrolysis with jaundice foal agglutination test in a newborn foal

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Abstract: This case report aims to highlight the utility of the jaundice foal agglutination (JFA) test used in the prevention of neonatal foal death caused by equine neonatal isoerythrolysis. The colostrum and the serum of a Friesian mare with repeated neonatal foal deaths and the whole blood of her newborn foal were tested for 5 days with the JFA test to detect a probable incompatibility. For the first 2 days, the JFA test gave a significantly positive reaction, indicating the existence of antibodies in the colostrum of the mare against the foal's erythrocyte antigens. The foal was not allowed access to her mother's colostrum and was fed with the frozen colostrum of other mares. At day 5, JFA test results were negative, indicating the disappearance of maternal antibodies from the colostrum.

Key words: Jaundice foal agglutination test, neonatal isoerythrolysis, foal

Introduction

Neonatal isoerythrolysis (NI) is an acute immune hemolytic disease in which red blood cells are destroyed in newborn foals. This occurs when colostrum contains antibodies against the foal's red blood cells (RBCs) (1,2). Another similar condition is erythroblastosis fetalis in humans, where Rh blood group system incompatibility occurs (3). In horses, there are over 30 blood group factors distributed in 7 systems (4). Factor Aa (A system) and factor Qa (Q system) are the most common factors involved in NI (1,2). In cases of NI, the foal has alloantigens inherited from the stallion and the mare is synthesized against these RBC antigens during a previous pregnancy, after a blood transfusion, or during a vaccination (2). In this condition, the affected foals are healthy at birth but develop clinical signs (lethargy, tachycardia, tachypnea, and icterus) within hours to days (1–3). The detection of maternal antibodies requires laboratory methods. There are a few tests that are based on lytic and agglutination methodology. However, many tests require a complement such as rabbit serum. Using the commercial laboratory tests, it is not always possible to detect antibodies produced against surface antigens of the foal's RBCs. It has been reported that the jaundice foal agglutination (JFA) test is the most suitable test because it does not need a complement (2).

To the authors' knowledge, this is the first report describing the prevention of NI in a foal in Turkey.

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This report aims to demonstrate the usability of the JFA test as a preventive measure against NI in foals of suspected mares with unsuccessful foaling histories.

Case history

A 9-year-old pregnant Friesian mare with suspected foal loss was selected for the study. According to the owner's description, all of this mare's previous foals died after birth except for her last foal, which showed signs of convulsion, tachycardia, and tachypnea for 5 days after parturition and recovered with supportive care, which included isotonic saline administration and antimicrobial therapy. Because of this, the selected mare was observed closely in her latest pregnancy. The foal was muzzled quite soon after birth and was not allowed to receive the mare's colostrum. Instead, the foal was given freeze-stored colostrum collected previously from other mares.

Serum and colostrum from the mare and anticoagulated blood from the foal were obtained in order to test for the existence of colostral and seral antibodies for 5 days after birth. The probability of the occurrence of NI was determined by using a JFA test. Briefly, colostrum (9 mL) and serum were collected from the mare. An anticoagulated blood sample (2 mL) was also collected from the foal. Six tubes were labeled as the control and dilutions of 1:2, 1:4, 1:8, 1:16, and 1:32 for both the serum and colostrum. Isotonic sodium chloride (1 mL) was added into all tubes, including the control tubes. Colostrum or serum (1 mL) was added into tubes labeled as 1:2 and pipetted gently to mix, and then 1 mL was taken from the 1:2 tubes and pipetted into the 1:4 tubes. The same process continued until all dilutions were prepared. At the end, 1 mL of sample from the 1:32 tubes was discarded. Whole foal blood (50 μL) was added into all tubes and mixed gently. All tubes were centrifuged for 3 min at 1600 rpm. After centrifugation, each tube was inverted (but contents were not allowed to flow down completely through the tube) and the bottom of the tubes was observed. If agglutination occurs, the cells form large clumps at the bottom of the tube. The cells form smaller clumps and flow easily down the side of the tube when agglutination is weak or absent. The clumping should not occur in the control tube. When the bottom of the tubes were being observed, a drop of supernatant was discarded, and then the tubes were mixed again to observe whether agglutination occurred or not. In addition, agglutination can also be determined in a microscope because rouleaux formation in the horse is more prominent than in other species (4). The test can be considered significant when a positive reaction occurs at dilutions of 1:16 or greater (2).

Results and discussion

No agglutination occurred in either control tube (Figure 1A, for colostrum), whereas marked agglutination was determined for both colostrum (Figure 1B) and serum (Figure 1C) for the first 2 days of the JFA test. The agglutination reaction gradually decreased over the 5 days. While there was a slightly positive reaction for serum, no agglutination was determined for colostrum on day 5 of the JFA test. This suggested that maternal antibodies were no longer present in the colostrum. Plasma levels of immunoglobulin G (IgG) and γ-glutamyltransferase (GGT) in the foal were 278 mg/dL and 38 IU/L, respectively.

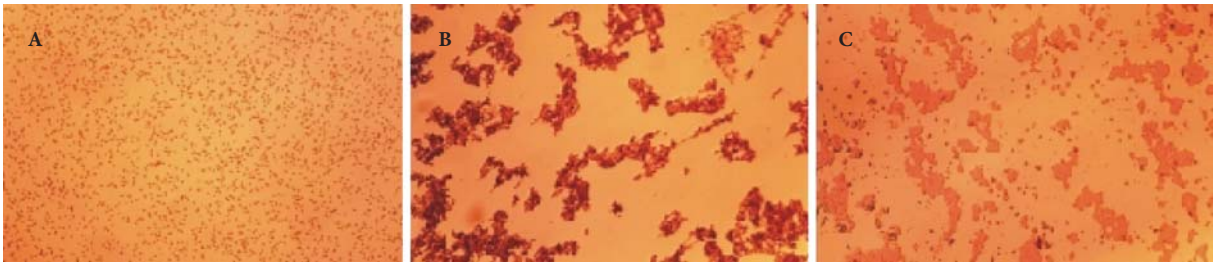

Figure 1. Microscopic appearance of agglutination in JFA test using A) control colostrum, B) colostrum, and C) serum, 100× magnification.

Newborn foals are born essentially agammaglobulinemic and acquire passive immunity by the ingestion and absorption of colostrum from the dams. The colostral antibody level is 10-fold higher than the blood antibody level and declines gradually to baseline levels within 3 days after birth. Failure of the newborn foal to ingest and absorb colostral antibodies may result in disorders of passive immunity. Therefore, a newborn foal should be fed by colostrum immediately or within the first few hours of life. In this case, however, the foal was not permitted to suck colostrum from the dam for 5 days; instead, colostrum was provided from fresh frozen colostrum collected from other mares. Levels of GGT and IgG indicated a failure of passive transfer in the foal (5–7). However, the foal continued to live and no infection was detected.

The results of the JFA test suggest that clinical use of the JFA test is an important tool in the prevention of foal deaths and that this test may help to accurately diagnose equine NI.

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