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Early conception factor lateral flow assays for pregnancy in the mare

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Abstract

The ECF™ lateral flow assay test is marketed to detect non-pregnancy in mares. The objectives of the present study were to determine the accuracy of the ECF test, the accuracy of the electronic reader accompanying the ECF test, and agreement between two human readers and the electronic reader. Serum samples were collected from anestrus, cycling but not inseminated, and inseminated mares, and were evaluated with the ECF™ test (EDP Biotech Company, Knoxville, TN, USA) at The Ohio State University and at the EDP Biotech Laboratory. Specificity ranged from 0.07 to 0.16, the negative predictive value ranged from 0.15 to 0.33, and accuracy ranged from 0.43 to 0.52. The electronic reader did not add improve the accuracy or predictive values of the test. Based on the electronic reader, 80.0% of the serum samples collected from the anestrus mares were false positives; Readers 1 and 2 had 60.0 and 33.3% false positives, respectively. For samples collected during the estrous cycle, 83.9% were false positives by the electronic reader, whereas Readers 1 and 2 had 43.7 and 26.4% false positives. We concluded that, regardless of whether the test strips were evaluated by a human or electronic reader, this assay was not accurate for determination of the non-pregnant mare. Published by Elsevier Inc.

Keywords: Pregnancy diagnosis; Early conception factor; Mare; Equine

1. Introduction

A transrectal examination (manual palpation or ultrasonography) is the most reliable method of early pregnancy diagnosis in the mare. Unfortunately, these methods do not allow reliable detection of the non-pregnant mare much earlier than the onset of spontaneous luteolysis in these mares (approximately 13 or 14 d after ovulation). However, if a non-pregnant mare could be accurately identified 6 d after ovulation, she could be immediately given prostaglandin ($PGF_{2\alpha}$) to initiate luteolysis [1], thereby shortening interestrus intervals and increasing opportunities for breeding [1].

The discovery of early pregnancy factor (EPF) and the rosette inhibition test enabled detection of pregnancy soon after breeding [2]. However, this method was time consuming, expensive, and tedious [2], and could not be readily performed under field conditions [2]. More recently, the early conception factor (ECF™) test, originally released in 1998 by Concepto Diagnostics, Knoxville, TN, USA, was marketed as an immunoassay capable of diagnosing the non-pregnant cow within 12–48 h after ovulation

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[3]. Although this test was reported as inaccurate [3–6], the company released a similar test in 2003 for horses. The manufacturer claimed that the test could detect the ECF glycoprotein in bred mares between 3 and 30 d after ovulation. According to the manufacturer, this is a lateral flow assay that uses monoclonal and polyclonal antibodies with a colloidal gold indicator. There is apparently only a single report regarding the specificity and sensitivity of this test; in that report, it was suggested that the test was inaccurate [5].

The present study examined the improved equine ECF test (marketed in 2006) to determine if changes made to the previous marketed test (2004) improved the specificity, accuracy, and negative predictive value, and to determine if the added electronic reader (2006) improved the results of the test. The electronic reader values were also compared to two human ''readers''. The objectives of this project were to determine the accuracy of the ECF test, the accuracy of the electronic reader accompanying the ECF test, and agreement between two human readers and the electronic reader.

2. Materials and methods

2.1. Horses

This study was performed during the 2007 breeding season (February 1 to May 31), using 61 Standardbred mares housed at a farm in central Ohio. Data regarding age, parity, previous breeding history, and lactation were not specifically recorded.

2.2. Experimental groups

There were three experiments that varied by reproductive status. In these three experiments, mares were anestrus, cycling but not inseminated, and cycling and inseminated. For these experiments, the objectives were to determine if anestrus or cycling but non-bred mares would yield false positive results, and to determine the reliability of the test to detect pregnant mares.

2.2.1. Experiment 1

Eleven anestrus mares were used. Anestrus was defined as no follicles >20 mm in diameter and no luteal tissue was detected in the ovaries by transrectal palpation and ultrasonography (Pie Medical Scanner 480, 5 MHz, linear-array transducer; Pie Medical Imaging, Netherlands) conducted every Monday, Wednesday, and Friday for three consecutive weeks. Blood samples were collected every Monday.

2.2.2. Experiment 2

Fifteen mares that had ovulated and had at least one apparently normal estrous cycle (with continued cyclicity) were used. For two consecutive weeks, transrectal examinations (to monitor ovarian activity) were done as described in Experiment 1 and blood collection was done twice weekly (Monday and Friday). None of the mares were bred until after all blood samples had been collected.

2.2.3. Experiment 3

This experiment used 61 cycling mares, including the 26 previously used in Experiments 1 and 2. All mares in estrus with an ovarian follicle ≥35 mm in diameter were artificially inseminated with fresh extended semen. Mares were bred every 48 h until ovulation occurred; most were bred at least twice and some three times. Ovulation (Day 0) was confirmed by transrectal palpation or ultrasonography after insemination. Blood samples were collected on Day 6 (range, Days 4–7), Day 10 (range, Days 10–12), Day 14 (range, Days 13–15), Day 18 (range, Days 17–19), and Day 35. Day 6 was chosen as the critical time to give $PGF_{2\alpha}$ to induce premature luteolysis and estrus in non-pregnant mares [7]. Pregnancy diagnosis was conducted with transrectal ultrasonography on Day 18; mares that were pregnant on Day 18 were re-confirmed as pregnant on Day 35. The Day-35 blood samples were collected only on mares deemed pregnant on the basis of a transrectal ultrasonographic examination on Day 18.

2.3. Blood samples

Samples were collected (jugular venipuncture) with a 20-ga, 38 mm vacutainer needle into a 10-mL red top vacutainer tube containing no anticoagulant (Becton Dickinson, Franklin Lakes, NJ, USA). The tubes were kept cool from collection to centrifugation (refrigeration of the blood samples did not affect test results [5]) and were centrifuged (2800 × *g* for 10 min) within 12 h after collection.

2.4. Test procedure

Testing was done immediately after centrifugation. Serum and test kits were allowed to reach room temperature before the tests were performed. The ECF lateral flow assay test was performed in accordance with the manufacturer's instructions.

Lateral flow assays are designed to rapidly detect a particular molecule; typically the molecule of interest is isolated based on binding to a specific antibody, and this