ELSEVIER

EVALUATION OF THE EARLY CONCEPTION FACTOR (ECF™) TEST FOR THE DETECTION OF NONPREGNANCY IN DAIRY CATTLE

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ABSTRACT

The ability to detect conception and/or conception failure in cattle would be beneficial to producers in formulating reproductive management plans. A new diagnostic test, the early conception factor (ECF) test, has been developed for this application yet the accuracy of this test has not been adequately determined. The objectives of this study were to evaluate the effectiveness of the ECF test for detecting the nonpregnant cow, and to compare the reliability of serum versus milk ECF tests relative to actual pregnancy rates. In Trial I, Holstein heifers were synchronized, the animals were bred (timed-AI), and serum ECF tests were performed 72 h later. Heifers exhibiting a negative ECF test after AI were re-synchronized, bred again, and re-tested for ECF for up to three services. Relative to actual pregnancy rates, a negative ECF test was correct (i.e., true negative) 38.5% of the time over the three services. In Trial II, Holstein heifers were bred (AI) after observed estrus and serum ECF tests conducted between Days 1 and 3 and Days 7 and 9 after AI. In this trial, only 44.4% and 55.6% of the confirmed nonpregnant heifers were identified correctly by serum ECF analysis at Days 1 to 3 and Days 7 to 9 post-AI respectively. In Trial III, 40 lactating cows were synchronized, the animals were bred (AI), and serum and milk ECF tests were performed on Days 3, 9, 15, 21 and 30 after AI. Pregnancy diagnosis (ultrasound on Day 30 and palpation on Day 51) confirmed that 50% of the cows were pregnant to AI, while serum and milk ECF analysis indicated a 100% and 37.5% predicted pregnancy rate, respectively, at 30 d post-AI. Moreover, results of the serum and milk ECF tests disagreed with one another 36.9% of the time overall, while agreement between ECF and actual pregnancy rates were 50.6% and 45.6% for milk and serum respectively. Additionally in Trial III, a negative ECF result only identified 5% and 28.8% of nonpregnant cows overall for serum and milk tests respectively (i.e., true negatives), with a high incidence of false positive ECF results noted (47.5% and 31.3% for serum and milk, respectively). Collectively, these data indicate that the current ECF test cannot accurately identify the nonpregnant cow with the precision needed by the dairy producer.

Key words: conception, pregnancy, cattle, early conception factor

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INTRODUCTION

Available techniques for the detection of pregnancy in cattle includes hormonal assays such as milk progesterone (P_4;16), pregnancy-specific protein B (PSPB; 21) and estrone sulfate (18), and applied reproductive management practices such as transrectal palpation and ultrasonography (19). While current methods for pregnancy diagnosis are very effective, they are usually performed after implantation has taken place and therefore can rarely be used to discriminate between fertilization failure, which results in a nonpregnant animal, and that which may be due to early embryonic loss post-conception. The ability to detect conception and conception failure post-breeding (< 21 days) would be beneficial to producers if such a test were specific to early embryonic development and could provide a timely and accurate diagnosis. Of the hormonal factors associated with early embryonic development that have been identified to date (2, 3), most are regulators of the local uterine environment and are not detectable in maternal circulation. Nevertheless, one class of factors that acts locally to enhance embryonic survival and is detectable in the maternal host is early pregnancy factors (EPF). At present, EPFs hold the greatest promise for development of a diagnostic test for detection of fertilization and conception failure.

The use of EPF as a diagnostic tool requires two components: EPF-A, which is produced by the oviduct during proestrus and estrus; and EPF-B, which is produced by the ovary once a local signal from the fertilized ovum is present (ovum factor; 9). The apparent role of EPF is as an immunomodulator which may aid in defending the embryo from immunological rejection by the maternal host (9, 10), and is present as early as 24 hours after mating until parturition (10, 12). When embryo/fetal mortality occurs, or if the fetus is removed, EPF decreases within hours (14), thus illustrating the specificity of EPF to conception and pregnancy. The EPF glycoprotein has been identified to be present in most pregnant mammalian species investigated to date (9, 10). The current method for detecting EPF uses the rosette inhibition test (RIT) which, while accurate, can be difficult to maintain and is not suitable for high-throughput diagnostic applications (10 19). To replace the RIT with a more user-friendly assay, a new test was developed for rapid detection of EPF in the serum and milk of cattle within 1 to 15 days after breeding (21, 24). This test, referred to as the Early Conception Factor test, works on the principle of lateral flow assays that use monoclonal and polyclonal antibodies incorporated into nitrocellulose membranes in which an antibody-gold conjugate is used to mark the presence of the EPF glycoprotein (24). This diagnostic technology is analogous to other currently available lateral flow qualitative tests for hCG, LH, and other hormonal indicators or disease reactants (1).

The ECF test is marketed currently as a "cow-side" test to diagnose nonpregnant cows after natural or artificial matings. However, independent examinations confirming the effectiveness of this test for application to reproductive management programs in dairy or beef cattle have not been widely published, and existing reports are conflicting in findings of ECF test accuracy (24, 25). To this end, the objectives of the present studies were to evaluate the effectiveness of the commercially available ECF test for detecting the nonpregnant dairy cow, and to compare the reliability of serum versus milk ECF tests relative to actual pregnancy rates.

MATERIALS AND METHODS

Trial I

Holstein heifers (n = 18) maintained on the Mt. Mint Dairy (St. Croix, USVI) were used in this first preliminary trial. The objective of this trial was to evaluate the use of the ECF (ECF™, Concepto-Diagnostics, Knoxville, TN) test in conjunction with AI and estrous synchronization on a group of Holstein heifers to gain experience with the ECF assay. Heifers that were observed to previously express estrus were treated with prostaglandin $F_{2\alpha}$ (PG, 25 mg im; Lutalyse®, Pharmacia-UpJohn, Peapack, NJ), and then KMAR (KMAR Inc., Springs, CO) patches were applied to each heifer to aid in the detection of estrus (Day 0). Since all heifers exhibited estrus after just one dose of PG, a second injection of PG was not needed to synchronize the first breeding by AI (AI-1). Artificial insemination was performed 12 h after standing estrus (AM/PM rule) using frozen semen from one of two Holstein bulls for up to 3 services after synchronization (AI-1, AI-2, or AI-3). At 72 h after each AI, a blood sample was collected via coccygeal vessel puncture from all heifers that were inseminated. The blood samples were then centrifuged, the serum was harvested and freshly collected serum was used for ECF analysis. Serum was applied to the ECF lateral flow dipsticks according to the manufacturer's instructions at the time of this first trial (ECF™, Concepto Diagnostics, Knoxville, TN; Lot # C98007-2). Briefly, the ECF test was performed by adding 1 drop of serum, using the plastic dropper provided by the manufacturer, followed by 4 drops of buffer wash solution. The ECF cassettes were then incubated at room temperature for 2 h and the results were recorded (positive = two red lines; and negative = one red line as per the manufacture's instructions at the time of testing). For the 18 heifers examined in this study, a total of 26 ECF tests were performed as follows: Animals exhibiting a negative ECF test 72 h after AI-1 (n = 6 of 18) were treated with PG again 6 d later (Day 9 after AI-1) and bred 12 h after detected estrus (AI-2). Diagnostic ECF tests were then performed 72 h after AI-2, and 2 of the 6 heifers bred at AI-2 were observed to have negative ECF tests. These remaining heifers (n = 2) were given PG 6 d later (Day 9 after AI-2), bred again after estrus (AI-3), and retested for ECF 72 h later. For heifers exhibiting positive ECF tests, palpation per rectum for pregnancy diagnosis was performed at 45 d after AI-1, AI-2, or AI-3. All AI were performed and ECF test readings were conducted by one technician, and palpation results were obtained by two qualified technicians. The accuracy of the ECF tests and confirmation of palpation results were based on actual calving dates for the heifers.

Trial II

Holstein heifers with observed estrus activity (n = 15) were randomly selected from the Mississippi State University Bearden Dairy Research Center heifer unit (Mississippi State, MS) to be used in this second preliminary study. The objective of this trial was to evaluate the effectiveness of the ECF test for the detection of nonpregnant heifers using fresh versus frozen serum. This was done to examine whether the same results could be expected when stored samples (-20°C) are retested at a later date. Heifers were bred by AI 12 h after observed standing estrus using the AM/PM rule. Blood samples were collected via jugular venipuncture 24 to72 h after breeding, and then again on Days 7 to 9 post-AI. Early Conception Factor tests (Lot #s 99STCOM1625 and 99STCOM 1626) were performed using fresh serum obtained after centrifugation of blood samples on the day of collection. After completion of the fresh serum ECF tests, the serum was then frozen (-20°C) and stored for a minimum of 72 h. The frozen serum was thawed and ECF tests were

conducted and the results were compared to ECF test results on the fresh serum samples. A total number of 60 ECF tests were performed in this trial: 15 fresh versus 15 frozen serum tests at 24 to 72 h post-AI and 15 fresh versus 15 frozen serum tests at 7 to 9 d post-AI. Effectiveness of the ECF test to detect the nonpregnant heifer was evaluated relative to actual pregnancy status as determined by ultrasonography at Day 30 and 45, rectal palpation at Day 60, and verified by actual calving dates. The ECF tests were conducted according to the manufacturer's instructions at the time of this second trial as described previously.

Trial III

Forty lactating Holstein (n = 30) and Jersey (n = 10) cows maintained at the Mississippi State University Bearden Dairy Research Center (Mississippi State, MS) were bred by AI (Day 0) after synchronization using a modified Ovsynch (PG/GnRH; 25) protocol beginning at 68 ± 8 d postpartum. Using the experience gained from the previous two Holstein heifer trials, the objective of Trial III was to evaluate the accuracy of the ECF test on different days post-breeding for detecting the nonpregnant cow in a commercial setting representative of a dairy production operation. Blood samples were collected via jugular venipuncture daily for the first 15 d after breeding, and then on alternate days until Day 30 post-AI. Serum was harvested from blood samples after centrifugation. Milk samples were collected at the morning milking (0400) using in-line milk fat samplers (Westfalia-Surge, Inc., Naperville, IL) to obtain a uniform milk sample for ECF analysis. Fresh serum and milk ECF tests (Lot #s 99STCOM1630 and 00STCOM1633) were performed on Days 3, 9, 15, 21 and 30 according to the manufacturer's instructions at the time of sampling as described previously; (i.e., 1 drop of serum or 3 drops of milk followed by 4 drops buffer wash solution). Ultrasonography for pregnancy diagnosis was performed on Day 30, and confirmed by palpation on Day 51. Finally, all serum samples were analyzed for concentrations of progesterone (P_4) to support ultrasound and palpation findings of pregnancy status. Concentrations of P_4 were measured using a commercially available radioimmunoassay (DSL 3900, Diagnostic System Laboratories, Webster, TX) modified for use in the bovine as follows: 1) incubating the serum samples at 4°C for 18 to 20 h as opposed to 37°C for 60 to 70 min (manufacturers instructions); and 2) extending the working range of the standard curve to encompass a P_4 range of 0.075 to 60 ng/mL. The intra- and inter-assay coefficients of variation (CV) for the P_4 assays were 6.12% and 14.21%, respectively. Finally, actual calving dates were used to ultimately confirm ultrasound, palpation and P_4 determinations.

Statistical Analysis

Statistical analysis was performed using ANOVA and the Student's T-test employed for mean separation of P_4 values between pregnant and nonpregnant animals and serum vs. milk ECF results where appropriate (SAS®, Cary, NC; 23). McNemar's test statistic was used to test the proportion of agreement between ECF predicted pregnancy rates versus actual pregnancy rates (i.e., agreement versus disagreement; Trials I, II and III). Data are presented as the ability of the ECF tests to detect the nonpregnant cow or heifer since the ECF test is marketed specifically for this application. However, while true positive results are not described, false positive results are reported as this represents a misdiagnosis of a nonpregnant animal.

RESULTS

Trial I

Actual pregnancy rates of heifers at AI-1, AI-2, and AI-3 were 33.3%, 0% and 50% respectively, with a total pregnancy rate for the entire group (n = 18) after 3 services of 38.9% (determined by palpation and confirmed by calving dates). The ECF test-predicted pregnancy rate differed ($P < 0.05$) from the actual pregnancy rate 61.5% of the time (n = 26 ECF determinations), with ECF predicted pregnancy rates of 61.1%, 33.3% and 100% for AI-1, 2 and 3, respectively. True negative ECF test results were observed 38.5% of the time, while false negative results were seen only 3.8% of the time for all ECF tests performed. Overall, the ECF test misdiagnosed 30.8% of the heifers as pregnant (false positive) when in fact they did not conceive to AI.

Trial II

In Trial II, our objective was to complement Trial I with further analysis of the use of ECF in heifers, and to determine whether the ECF test would perform similarly when fresh versus frozen serum was used. We reasoned that frozen serum would be beneficial for later ECF analysis if re-testing was needed, or for the shipping of samples for diagnostic tests to be performed elsewhere. The ECF predicted pregnancy rate, agreement between ECF results and actual pregnancy rate, and agreement between fresh versus frozen serum ECF results are shown in Table 1. Based on methods

Table 1. Early conception factor (ECF) test-predicted pregnancy rate, ECF test agreement with actual pregnancy rate, and agreement between fresh versus frozen serum (%; Trial II).

Day/serum sample	ECF-predicted pregnancy rate	ECF agreement with actual pregnancy rate*	Ability of ECF to detect nonpregnant heifers
1 to 3			
Fresh	66.7[a]	46.7[a]	33.3
Frozen	66.7[a]	73.3[a]	55.6
7 to 9			
Fresh	53.3[a]	60.0[a]	55.6
Frozen	96.7[b]	53.3[a]	22.2

Superscripts differ within column within day between fresh versus frozen serum, [ab]$P < 0.05$.

* Actual pregnancy rate in Trial II, as determined by ultrasonography, palpation and calving dates, was 40%.

for pregnancy diagnosis (ultrasound and palpation), the actual pregnancy rate for heifers in Trial II was 40% (confirmed by calving dates). The actual pregnancy rate (40%) did not differ ($P > 0.10$) from the ECF predicted pregnancy rate (66.7%) on Day 1 to 3 regardless of whether fresh or frozen serum was used. Furthermore, the ECF predicted pregnancy rate did not differ ($P > 0.10$) between fresh versus frozen serum on Day 1 to 3. However on Day 7 to 9, while the actual pregnancy rate did not differ ($P > 0.10$) from fresh serum ECF results (40.0 vs. 53.3%), frozen serum ECF results were greater ($P < 0.05$) than the actual pregnancy rate (40.0 vs. 96.7%). In comparing the use of

fresh versus frozen serum on Day 7 to 9, the ECF predicted pregnancy rate differed ($P < 0.05$) by 43.4% (Table 1).

Agreement of ECF with actual pregnancy rates on Day 1 to 3 or Day 7 to 9 did not differ ($P > 0.10$) when fresh versus frozen serum was used for the ECF tests (Table 1). However, it should be noted that ECF results between fresh versus frozen serum only agreed with one another 80% of the time on Day 1 to 3 and 66.6% of the time on Days 7 to 9 (calculated by matching fresh with frozen positive ECF results, and fresh with frozen negative ECF results). Surprisingly, the use of frozen serum resulted in a proportionally greater ability to detect the nonpregnant heifer on Days 1 to 3 than on Days 7 to 9, while the converse was true for fresh serum (Table 1). These data demonstrate that additional variability of the ECF test can be expected when using fresh versus frozen serum, and that the ability to detect a nonpregnant animal was never greater than 56%. As the ECF test is marketed for use with fresh serum, further analysis in this study was conducted using fresh serum only. For heifers that were confirmed nonpregnant (9/15) by the ECF test (fresh serum), only 44.4% (4/9) and 55.6% (5/9) of the nonpregnant animals on Days 1 to 3 and Days 7 to 9, respectively (i.e., true negatives) were actually identified by ECF analysis. Similar to Trial I, the ECF test misdiagnosed 33.3% of the heifers as pregnant (false positive) when in fact they were confirmed as nonpregnant to AI.

Trial III

In Trial III we examined the effectiveness of the ECF test using milk and serum collected between Days 3 and 30 post-AI in mature cows. The actual pregnancy rate after synchronized AI was 50% (20/40) as confirmed by ultrasonography, palpation per rectum, and serum P_4 profiles. Serum concentrations of P_4 were greater ($P < 0.05$) in pregnant than nonpregnant cows on both Days 21 (5.24 vs. 1.84 ng/mL) and 30 (5.10 vs. 2.06 ng/mL) post-AI. Pregnancy detection methods were verified by actual calving dates. Comparisons of serum versus milk ECF tests were found to differ ($P < 0.05$) on each day tested, and varied between 12.5 and 90% when compared to actual pregnancy rates (Table 2). Moreover, milk and serum ECF tests disagreed with one another 72.5%, 82.5%, 65.6%, 80.0% and 37.5% on Days 3, 9, 15, 21 and 30, respectively (calculated by matching positive serum with positive milk ECF results, and negative serum with negative milk ECF results), regardless of correspondence with actual pregnancy rates. These data showed a failure of the milk and serum ECF test results to parallel one another in this study. Further analysis of independent serum and milk ECF determinations were as follows:

Serum ECF. The agreement between serum ECF-predicted pregnancy rate and the actual pregnancy rate (confirmed by ultrasound and palpation to be 50%) is shown in Table 2. Briefly, agreement between serum ECF and actual pregnancy rate was 45.0 to 52.5% between Days 3 and 21 after breeding. On Day 30, the serum ECF test identified all cows (100%) as pregnant to AI, yet 20 of the 40 cows were confirmed to be nonpregnant by ultrasound, palpation, serum concentrations of P_4, and ultimately actual calving dates. The possibility exists that between Days 9 and 30 post-breeding, ECF presence could have been the result of subsequent matings conducted between AI

Table 2. Early conception factor (ECF) test predicted pregnancy rate, agreement with actual pregnancy rate and the proportions of false positive, true negative, and false negative results at varying time intervals post-breeding (%; Trial III).

Sample/ day	ECF-predicted pregnancy rate	ECF agreement with actual pregnancy rate	True negative*	False positive*	False negative*
Serum[a]					
3	87.5	52.5	60.0	89.5	10.5
9	95.0	50.0	100.0	100.0	0.0
15	95.0	45.0	0.0	90.9	9.1
21	90.0	50.0	50.0	90.0	10.0
30	100.0	50.0	0.0	100.0	0.0
Milk[b]					
3	70.0	45.0	41.7	68.2	31.8
9	82.5	52.5	57.1	84.2	15.8
15	57.5	57.5	58.8	58.8	41.2
21	75.0	50.0	50.0	75.0	25.0
30	10.0	37.5	44.0	16.7	83.3

[ab]Comparisons between serum and milk ECF predicted pregnancy rates differed on respective day, $P < 0.05$; *True negative: a negative ECF test for a confirmed nonpregnant cow relative to the total number of nonpregnant animals; False positive: a positive ECF test acquired for a confirmed nonpregnant cow relative to the total number of disagreements between ECF testing and the actual pregnancy rate; and False negative: a negative ECF test acquired for a confirmed pregnant cow relative to the total number of disagreements between ECF testing and the actual pregnancy rate.

and the Day 30 sampling. To discount this possibility, farm records showed that half (10/20) of the confirmed nonpregnant cows were not bred after AI, and thus would have no confounding ECF levels. For the remaining cows (n = 10) that were re-bred before Day 30, only 2 of the cows were confirmed to have conceived during this time. Therefore, most of the animals (8/10) that were re-bred did not become pregnant to breedings before Day 30. This suggests that the possibility of ECF being present from subsequent conceptions was most likely not the case, and thus most of the nonpregnant animals should not have been diagnosed as ECF positive after repeated sampling. Of the negative ECF tests, correct results (i.e., true negatives) varied greatly depending on the day of sampling post-AI (Table 2). Irrespective of days in which correct negative ECF tests approached 60 to 100% (as a proportion of the negative ECF tests found), the ability of the ECF test to identify nonpregnant animals (i.e., a negative ECF test equaled a confirmed nonpregnant cow relative to the total number of nonpregnant animals) was only 0.0 to 15.0% (Day 3 = 15.0%; Day 9 = 10.0%; Day 15 = 0.0%; Day 21 = 10.0% and Day 30 = 0.0%). For those serum ECF tests that disagreed with actual pregnancy results, the proportions of false positive and false negative tests were calculated. In most cases, a greater proportion of false positive results were observed than false negatives (Table 2).

Milk ECF. The agreement between the milk ECF-predicted pregnancy rate and the actual pregnancy rate (50%) is shown in Table 2. Briefly, agreement between the milk ECF and actual pregnancy rate was 37.5 to 57.5% between Days 3 and 30 after AI. Of the negative ECF tests, correct results (i.e., true negatives) varied greatly depending on the day of sampling post-breeding (Table 2). Irrespective of a 40 to 60% correct negative diagnosis (Table 2), the ability of the ECF test to actually identify nonpregnant animals (as stated previously, a negative ECF test equaled a confirmed nonpregnant cow relative to the total number of nonpregnant animals) varied greatly from 20.0 to 70.0% (Day 3 = 25.0%; Day 9 = 20.0%; Day 15 = 50.0%; Day 21 = 25.0% and Day 30 = 70.0%). For those milk ECF tests that disagreed with actual pregnancy results, the proportions of false positive and false negative results were calculated. Similar to serum ECF findings, a greater proportion of false positive results were observed than false negative when milk was used in the ECF tests (Table 2).

DISCUSSION

The overall objective of the three trials reported here was to determine whether a new diagnostic test for the detection of EPFs was accurate for on-the-farm applications in the reproductive management of dairy cattle. Previously, the rosette inhibition test (RIT) had been used to detect early pregnancy factors after breeding in sheep (10), cattle (9), and swine (11) with an accuracy as high as 91.4%. However, the RIT is time-consuming, difficult to maintain and not suitable for high-throughput commercial applications as would be required for use in livestock (19). It has been suggested that a more advanced assay, coupled with a greater understanding of the chemical structures and biological actions of EPFs, was needed before the reliability of EPFs for conception or pregnancy detection could be verified (19). The lateral-flow ECF test represented the next generation of EPF assays for application as an applied (i.e., "cow-side") qualitative assay for nonpregnancy confirmation. While the use of this technology for the determination of EPF presence would represent a significant advancement toward a rapid diagnostic test for pregnancy or nonpregnancy detection, our findings indicate that the current commercially available EPF assay is not accurate for use in dairy cattle as a reproductive management tool.

Previous studies characterizing the ECF test have suggested an accuracy for detecting the nonpregnant cow of 94.5% within 24 to 48 h after breeding to 100% later in gestation (24). In contrast, a study by Whisnant et al. (26) reported that the ECF test agreed with pregnancy confirmation (ultrasound/palpation) only 55% of the time between 9 and 15 days post-breeding, and that 24% of cows with ECF negative results were in fact later confirmed to be pregnant (i.e., ECF false negatives). Where accounts of true negative results as a proportion of negative ECF tests were 100% in this study (as in Trial III on Day 9 post-breeding), it should be noted that only two ECF tests provided a negative diagnosis on this day and both were from cows confirmed to be nonpregnant (2/2 = 100%). However, 20 cows were in fact confirmed nonpregnant and therefore the ECF test missed (false positives) 18 of the 20 nonpregnant cows (90%) on Day 9 post-breeding in Trial III. Throughout most of the ECF testing we noted a high proportion of false positive determinations of this magnitude. Positive ECF findings must be treated as nondeterminable results that require retesting since the ECF test is marketed for the detection and confirmation of nonpregnancy.

Diagnostic hormonal tests for nonpregnancy in cattle have been developed previously with the most notable assays for early detection being the milk P_4 and serum PSPB assays (7, 8, 26). A trial performed by Wimpy et al. (27) using milk P_4 on Day 21 post-breeding found that milk P_4 was 92% accurate for detecting the nonpregnant cow, while only 76% accurate for confirming pregnancy. The serum PSPB assay is even more specific in that the detection of PSPB post-breeding is dependent on the presence of an implanting conceptus and is 86 to 95% effective in the detection of pregnancy between Days 30 and 35 post-AI, and 100% effective in detection of nonpregnant cows after Day 70 post-AI (7, 8). In heifers embryonic mortality may account for 46 to 75% of pregnancy failures after AI (4, 6, 17). One explanation for a high proportion of false positives with the ECF test may be the detection of EPFs as a result of conception and early embryonic development followed by embryonic loss, resulting in a diagnosis of nonpregnancy by ultrasonography and/or palpation later post-breeding. Nonetheless, repeated ECF diagnostic testing should have been able to detect a change in EPF presence after embryonic loss since studies using the RIT for monitoring EPF presence after embryonic and/or fetal removal suggest that EPFs disappear within 8 to 24 h (5, 13, 14). However, only 5% of the animals that tested positive for ECF 24 to 72 h after breeding in Trial II, and were later confirmed nonpregnant by ultrasound and palpation, ever reverted to a negative ECF test on Days 7 to 9; and none of the cows in Trial III diagnosed by the ECF test as pregnant at 24 to 72 h post-breeding were nonpregnant by ECF (i.e., a negative ECF test) on Days 15, 21 or 30 post-breeding. Serum P_4 has been used previously as an indicator of late embryonic mortality that occurs after Day 14 post-breeding by the presence of extended inter-estrus intervals and sustained P_4 levels after Day 24 (15). In Trial III where frequent P_4 sampling was conducted, only 15% of confirmed nonpregnant cows exhibited prolonged luteal activity past 25 d post-breeding. This suggests a low proportion of late embryonic mortality as determined by P_4 analysis, though this cannot be confirmed definitively. Nevertheless, the failure of the ECF test to identify nonpregnant cows indicates a lack of specificity of the test for the absence of EPF which further undermines its use.

Specificity of the ECF diagnostic test also appeared to be affected by the use of serum versus milk and fresh versus frozen serum. As much as a 20 to 33.4% disagreement in ECF predicted pregnancy rates, and as high as 26.6% difference in agreement with actual pregnancy rates, was noted in comparing fresh versus frozen serum ECF results on Days 1 to 3 and 7 to 9, respectively. Therefore, these data indicate that additional variability in the ECF dipstick test can be expected when using fresh versus frozen serum samples. Moreover, substantial differences in diagnostic results in the use of milk versus serum were also observed. In Trial III on Days 9 and 15, which have been recommended by the manufacturer of the ECF test as optimal days for testing, the ECF predicted pregnancy rate differed by 15 and 37.5% between serum and milk, respectively, while on Day 30 a 90% difference in pregnancy rate was observed. These findings indicated that the two tests disagreed 17.5 to 34.4% of the time depending on the day of the test. Ultimately, these data call into question the reliability of the ECF test for use in different physiological fluids (serum and milk) where one can expect conflicting results in > 30.0% of animals tested.

While this first generation of ECF test kits does not appear suitable for commercial application in the livestock industry, it is anticipated that further development of this and other technologies may yield an acceptable assay in the future for the detection of EPFs. The ability to detect conception or conception failure within days after breeding or any time before Day 21 post-breeding would represent a significant economic benefit to the livestock producer. This could occur

through application of EPF testing programs after breeding in conjunction with PG 7 to 9 d later for accelerating the synchronization of estrus after a conception failure (identified by EPF assays), thus decreasing the number of days a cow remains nonpregnant. However, such an assay must be repeatable, specific, sensitive and cost-effective. Undoubtedly, this will require additional advancements in our understanding of the functional chemistry of EPFs in vivo, and the application of novel hormonal assay technologies for both qualitative and quantitative EPF detection.

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