PEER REVIEWED

Evaluation of the Early Conception Factor (ECF™) Dip Stick Test in Dairy Cows Between Days 11 and 15 Post-Breeding

Luc DesCôteaux, *DMV, MSc, Dipl. ABVP (Dairy)*
Paul D. Carrière, *DMV, MSc, PhD*
Michel Bigras-Poulin, *DMV, MSc, PhD*
Department of Clinical Sciences (DesCôteaux), Centre de Recherche en Reproduction Animale (Carrière), and Department of Pathology and Microbiology (Bigras-Poulin)
Faculté de médecine vétérinaire
Université de Montréal
St-Hyacinthe, Québec (CANADA) J2S 7C6

Abstract

A field study was conducted between February and November 1995 using 139 Holstein cows to determine the accuracy and the usefulness of the ECF™ Dip Stick Test performed between days 11 and 15 post-breeding. Results of the ECF™ tests were compared to pregnancy diagnosis after 25 days post-breeding using ultrasonography and transrectal palpation. The apparent conception rate of the study population based on transrectal palpation was 38%. The ECF™ test sensitivity, specificity, positive and negative predictive values were 81%, 26%, 40% and 69%, respectively. The Kappa value (0.06; 95%CI –0.19 to 0.30) demonstrated no agreement between the ECF™ test and the final pregnancy status of dairy cows. These results would indicate that the ECF™ test is not a good predictor of pregnancy because the proportion of false positive results was high at 46% (64/139). Furthermore, the accuracy of the test to detect open cows is not acceptable with the high proportion of false negative tests of 31% (10/32). This finding would indicate that 19% (10/53) of the cows diagnosed pregnant rectally were misdiagnosed as non-pregnant by the ECF™ test. Finally, many factors such as the incubation time, the personal assessment of the test reaction and the source of light can also affect the ECF™ test reading. Based on the current technology, the authors would not recommend the use of the ECF™ test to help dairy producers reduce non-productive days.

Introduction

It is very costly when dairy cows are open for more than 90-100 days.[6,9] Closely following a regular herd health program to monitor reproductive performance, early detection of pregnancy status after insemination, and strict reproductive strategies involving intensive use of prostaglandins can significantly improve a herd's reproductive performance by decreasing the number of non-productive days.[3-5]

Over time, numerous methods for determination of pregnancy in cows have been evaluated. Oestrus observation, physical examination, biochemical tests and electronic instruments are some examples.[17] The main purpose of examining cows for pregnancy as early as possible after breeding is not to detect the pregnant cows, but to identify with confidence cows that are not pregnant in order to reinseminate them or to cull them.[7,17] For profitable lifetime production, the calving interval for dairy cows should be approximatively 12 to 13.5 months.

To date, the earliest, most practical and documented test to determine pregnancy status of dairy cows is the serum or milk progesterone assay.[12] However, the evaluation of an early pregnancy test is done without having a perfect gold standard of pregnancy. The current acceptable standard to confirm pregnancy is transrectal palpation performed at a later time after insemination.[1,7,8,11,12,14-16] This standard of pregnancy causes some problems in the evaluation of early preg-

nancy tests because early embryonic death, occuring between the first test and the subsequent confirmation of the pregnancy, results in an increase of apparent false positives. In a recent field study conducted on 472 cows to determine the usefulness of milk progesterone at 21 days post-breeding, Rajamenhendran *et al* reported a test specificity of 57.5% based on a cutoff level of less than 1 ng/ml.[12] At a conception rate of 53%, the predictive value of a negative result (< 1 ng/ml) was 94% and in accordance with other studies.[8,11] The low specifity of the test can be attributed, in part, to non-pregnant cows experiencing a later decline in progesterone due to estrous cycles longer than 21 days, and to a proportion of cows experiencing early embryonic losses.[7,8,12]

Recently, a new test called the ECF™ (Early Conception Factor) Dip Stick Test[a] has been developed. The ECF™ test detects an immunosuppressive glycoprotein with a molecular weight of 200,000-240,000 Daltons which can be isolated from the serum of pregnant cows 12 to 24 hours post-breeding.[14] The ECF protein can be detected in serum and milk from pregnant cows as soon as 24 hours after breeding.[10] A pilot study on the ECF™ test performed between 24 and 48 hours after breeding suggested that this assay could be useful to diagnose pregnancy in cattle.[14]

The objectives of the present study were to 1) evaluate the sensitivity, specificity and predictive values of the ECF™ test when performed between days 11 and 15 post-breeding, 2) evaluate the effect of incubation time on reading of the ECF™ test, and 3) evaluate the applicability of such a test in relation to different simulated herd conception rates in dairy cattle.

Materials and Methods

Herd selection, experimental protocol—Three dairy herds followed on a monthly herd health program (Ambulatory Clinic of the Faculty of Veterinary Medicine, Université de Montréal, Québec, Canada) were used in the study. These herds had a 305d mature equivalent (305ME) milk production average of 21,450 lb (9,750 kg). One hundred and thirty nine cows that were artificially inseminated between February 13, 1995 and November 14, 1995 were followed intensively according to the schedule presented in Figure 1. On a weekly basis, the participating farms were visited by a bovine practitioner to collect blood samples without anticoagulant from the coccygean vein of cows that had been artificially inseminated 11 to 15 days earlier. Within 4 hours after collection, blood samples were brought to the laboratory and centrifuged for 15 minutes at 3000 RPM. The serum was separated from the blood clot and kept at -4°F (-20°C) until it could be further analysed as a batch by the first author. Participating producers were asked to maintain a daily written log of any physical signs to determine estrus and breeding dates. Transrectal ultrasonographic (U/S) examinations were performed between days 25 and 40 post-insemination with a portable ultrasound scanner,[b] using a 5mHz rectal probe. The U/S examination was then followed by a transrectal palpation performed after day 40 post-insemination unless cows were rebred in the interim.

Definitions—Cows were assumed to be non-pregnant if they demonstrated signs of heat, were rebred and/or confirmed open by transrectal ultrasonography and palpation. On the other hand, cows were declared pregnant to a given artificial insemination if they were confirmed pregnant by the veterinarian with transrectal ultrasonography or palpation. The gestational age of the fetus was also assessed to confirm the date of artificial insemination. Hereafter, the transrectal pregnancy examinations refer to the ultrasonography and/or palpation results of the pregnancy outcome definitions described above.

Laboratory procedures and ECF™ Dip Stick Test—Each ECF™ Dip Stick kit contains a test cassette, a pipette, one humidity indicator, and a wash solution bottle per 20 tests. The ECF™ Dip Stick assay conducted on serum samples uses monoclonal and polyclonal antibodies incorporated into a nitrocellulose membrane with gold as the indicator to detect the immunosuppressive conception factor.[2] All tests were performed and evaluated at our laboratory by the first author according to manufacturer's specifications.[2] For quality control, the humidity indicator was verified before each test. According to the instructions, the test cassette had to be read after 30 minutes of incubation but before two (2) hours. The presence of two red lines on the cassette indicated conception, and the presence of one red line only in the control area indicated that the cow did not conceive. In order to address the second objective, the results of the first series of tests (n = 32)

	Artificial Insemination	Blood Sample Collection	Ultrasonographic Examination	Transrectal Re-examination
DAY	0 ----------	11 to 15 ----------	25 to 40 ----------	> 40 days

Figure 1. Schedule of events following artificial insemination of dairy cows in order to evaluate the ECF™ Dip Stick Test performed on serum samples taken between days 11 and 15 post-breeding.

were evaluated and recorded after 30, 60 and 90 minutes to verify if a difference existed after such a variation in incubation times. For the remainder of the study, the ECF™ tests were evaluated only after 90 minutes of incubation in order to compare results with another study that reported the same incubation time.[1]

As a measure of quality control, a series of 64 serum samples were analyzed blindly by the first author and by a qualified technician at another laboratory.[c] There was good agreement between the two laboratories for tests incubated for 90 minutes (kappa value = 0.69; 95% CI 0.47-0.90).

Statistical analysis—Statistical analysis was performed using SAS statistical software.[13] A contingency 2 by 2 table was created to compute the ECF™ test relative sensitivity, specificity, positive (PPV) and negative predictive values (NPV) according to the final outcome of pregnancy determined after 25 days post-insemination as the standard procedure (see definitions above). Kappa statistic was calculated and presented with a 95% confidence interval (95% CI) to verify the agreement corrected for chance between the results of the two laboratories. The kappa statistic was also used to compare the results of the ECF™ tests performed at different incubation times and to verify the agreement between the ECF™ test results and the standard test of pregnancy performed after 25 days post-breeding. Kappa values between 0.40 and 0.60 are considered to be fair, between 0.60 and 0.80 to be good and over 0.80 to have very good to excellent agreement.

Results and Discussion

Results of the ECF™ test incubated for 90 minutes compared to transrectal pregnancy examinations are presented in Table 1. The apparent conception rate of the study population based on transrectal examinations was 38% (53/139). At this prevalence, the ECF™ test relative sensitivity, specificity, positive and negative predictive values were 81%, 26%, 40% and 69%, respectively. The Kappa value (0.06; 95%CI –0.19-0.30) demonstrated no agreement between the ECF™ test results and the final pregnancy status of the cows. The low specificity (i.e. the ability of the ECF™ test to correctly identify open cows after 25 days post-breeding) of 26% and the low predictive value of a positive ECF™ test (i.e. the proportion of ECF™ test positive cows that were confirmed pregnant after 25 days post-breeding) of 40% lead to a high proportion of false positive results. These false positive results could be due in part to early embryonic death (EED) occuring between the time of the ECF™ test (days 11 to 15 post-breeding) and the final confirmation of pregnancy (between days 25 and 60). Since no perfect gold standard exists to evaluate the embryo viability between 11 and 25 days post-breeding, it is difficult to evaluate real EED. However, if we use the false positive results to estimate the apparent EED[7] (EED = 1 – PPV) and assume that all false positives detected by the ECF™ test were deemed EED, then under our experimental farm's situation, the ECF™ test would indicate that there was an apparent EED of 60% (64/107). Such a high incidence of EED for mature dairy cows has not been published. In the 3 herds in this study, we have previously reported an apparent EED incidence of 6 ± 5% between days 26 and 40 post-breeding.[7] Based on previous research, various authors report that the incidence of EED ranges from 5-20%.[7] Therefore, if the true EED of our study population after 11 to 15 days post-breeding is in the range of 20%, we can speculate that the high rate of false positive cows on the ECF™ test might be due to secretion of the early conception factor antigen in the serum of non-pregnant cows, or to other cross reaction factors that would explain the source of discrepency. Our results indicate that the ECF™ test is not accurate as a predictor of pregnancy because of a high proportion of false positive results (64/139 or 46%). Furthermore, in agreement with Adams and Jardon,[1] the low predictive value of a negative ECF™ test (i.e. the proportion of cows with a negative ECF™ test that were confirmed non-pregnant after 25 days post-breeding) at 69% is not acceptable for use in the field by bovine practitioners because of a high proportion of wrongly interpreted negative tests of 31% (10/32). This finding also indicates that 19% (10/53) of the transrectally diagnosed pregnant cows were misdiagnosed as non-pregnant by the ECF™ test. This finding is also in agreement with Adams and Jardon, with the exception that they reported a higher proportion (49%) of misdiagnosis on transrectally diagnosed pregnant cows.[1] The discrepency between these two results is probably due to differences in the evaluation of the ECF™

Table 1. Results of the ECF™ test, incubated for 90 minutes, performed between 11-15 days post-breeding are compared to transrectal pregnancy examinations performed after 25 days post-insemination.

	Pregnant (+)	Non-Pregnant (-)	Total
ECF positive (+)	43	64	107
ECF negative (-)	10	22	32
Total	53	86	139

Prevalence or apparent conception rate : 38% (53/139)
Relative sensitivity and specificity of 81% and 26%, respectively.
Predictive values of positive and negative tests of 40% and 69%, respectively.

dip stick reaction or to other unknown factors (ie. freezing of samples, herd nutrition and environment, etc.) which might affect the quality of the cow's serum. In the present study, 77% of the ECF™ dip stick reactions were called positive compared to 47% in the study by Adams and Jardon,[1] despite the fact that both studies had similar conception rates (38% and 34% respectively). In the present study, the light source was good and a positive test was declared as soon as a second very faint red line was apparent on the test cassette. The difficulty in distinguishing a positive from a negative test result could explain the differences between studies.

The ECF™ test readings that were performed at 30 minutes of incubation at room temperature were in good agreement with those read at 60 and 90 minutes of incubation (kappa = 0.69; 95% CI 0.44-0.94 and kappa = 0.62; CI 0.36-0.89, respectively). However, the readings after 60 minutes of incubation were in excellent agreement with those read at 90 minutes of incubation (kappa = 0.919; 95% CI 0.76-1.00). There was a trend toward more positive reactions with increased incubation time. In fact, the percentage of ECF™ tests with positive results increased from 50% at 30 minutes to 66% and 69%, respectively, after 60 and 90 minutes of incubation. The overall consequence of increasing the incubation time was an increase in false positive results, which in turn decreased the specificity of the test. For example, when the incubation time increased from 30 to 60 minutes, the specificity of the test decreased from 80% to 50%. In turn, for the same situation, the sensitivity of the ECF™ test was improved and increased from 58% to 75%. These results suggest that readings of the ECF™ cassettes should not be done before 60 minutes of incubation and should be performed at a precise time so that results from different studies can be compared.

Finally, when considering the relative sensitivity and specificity results of the ECF™ test from the present study, one can calculate the positive and negative predictive values of the test if used in different conception rate scenarios (Table 2). This simulation shows that using such a test could be detrimental if used in herds with good conception rates (> 45%) as it would decrease the predictive value of a negative ECF™ test to less than 60%. As a consequence, an increased rate of iatrogenic EED would result from the use of prostaglandins on pregnant cows that would have been falsely called negative by the ECF™ test. Threlfall *et al* were the first to conclude that the ECF™ test could be used commercially to save valuable breeding time in cows found non-pregnant by the test, but their results have not been corroborated by others.[16]

Conclusions

The results of this study suggest that the ECF™ Dip Stick Test performed on serum is not an accurate indicator of non-conception when used between days 11 and 15 post-insemination in dairy cattle. Based on the current technology, the authors would not recommend the use of the ECF™ test in an attempt to reduce non productive days.

Acknowledgments

This study was supported by the Fonds du Centenaire de la Faculté de Médecine Vétérinaire and the CAFIR (Comité d'Attribution des Fonds Internes de Recherche) from l'Université de Montréal. The authors would like to thank Biovet Inc. and in particular Dr René Lallier and Mr Pierre Desmarais, Agr. for their help in the quality assurance comparison and for some ECF™ test donations, Dre Roxanne Rémillard and Cornelis van den Wijngaard for their technical help and finally, the three dairy producers for their willingness to participate in our project.

Footnotes

[a]Concepto Diagnostics™, P.O. Box 6275, Knoxville, TN 37914, USA
[b]Echo 900, Alliance Medical Inc., Montreal, Quebec, CANADA
[c]Biovet Inc., St-Hyacinthe, Québec, CANADA

References

1. Adams CS, Jardon PW: Evaluation of the early conception factor test in cows 3-7 days post-breeding. Proc Am Assoc Bov Prac 32 : 240-241, 1999.
2. Concepto Diagnostics: Early conception factor assay kit for cattle, ECF Dip Stick Test, product insert. Concepto diagnostics, Knoxville, TN. 1999.
3. DesCôteaux L, Bateman KG, Moffatt D, Shantz R, Walton JS:

Table 2. Positive (PPV) and negative (NPV) predictive values of the ECF™ Dip Stick test calculated based on a test sensitivity of 81%, a test specificity of 26% and different simulated conception rates (CR).

CR	25%	30%	35%	40%	45%	50%	55%	60%
PPV	27%	32%	37%	42%	47%	52%	57%	62%
NPV	80%	75%	71%	67%	61%	56%	52%	48%

Evaluation of a dairy breeding program utilizing coloured tail tapes and scheduled prostaglandin administration in a Holstein herd in Ontario. Proc Can Vet Med Assoc/CPMVQ: 425, 1994.
4. Ferguson JD, Galligan DT: Prostaglandin synchronization in dairy herds-Part 1. Comp Cont Edu Pract Vet 15: 646-655, 1993.
5. Ferguson JD, Galligan DT: Prostaglandin synchronization in dairy herds-Part 2 Comp Cont Edu Pract Vet 15: 1127-1130, 1993.
6. Ferry JW: Do we need postpartum fertility exams as part of a reproductive herd health program? Proc Am Assoc Bov Prac: 235-238, 1992.
7. Filteau V, DesCôteaux L: Accuracy of a portable ultrasound scanner for early pregnancy diagnosis in the cow. A retrospective study. M V Quebec 28: 81-85, 1998.
8. Gowan EW, Etches RJ, Bryden C, King GJ: Factors affecting accuracy of pregnancy diagnosis in cattle. J Dairy Sci 65: 1294-1302, 1982.
9. Harness JK, Ott JM, Butt MT, Bower KA, Henry RT: Response of reproductive control programme to computer enhancement. Proc World Congress on Diseases of Cattle: 272-276, 1986.
10. Jordan T: President, Concepto Diagnostics. Personal communication, 1994.
11. Pennington JA, Schultz LH, Hoffman WF: Comparison of pregnancy diagnosis by milk progesterone on day 21 and day 24 postbreeding : field study in dairy cattle. J Dairy Sci 68: 2740-2745, 1985.
12. Rajamahendran R, Burton B, Shelford J: A field study on the usefulness of milk progesterone determination to confirm estrus and pregnancy of dairy cows in the Fraser Valley area of British Columbia. Can Vet J 34: 349-352, 1993
13. SAS® System for Windows, version 6.12, SAS Institute Inc., Cary, NC, 27513, USA.
14. Threlfall WR, Walter R: Early conception assay application to dairy cow pregnancy determination. Presented at the Soc for Theriogenology, 1993.
15. Threlfall WR: Immunosuppressive early pregnancy factor (ISEPF) determination for pregnancy diagnosis in dairy cows. Theriogenology 41: 317, 1994.
16. Threlfall WR, Bilderbeck GM: Early conception factor dipstick test for the non-conception determination in cattle. Proc Am Assoc Bov Prac 32: 239, 1999.
17. Youngquist RS: Pregnancy diagnosis, in Youngquist RS (ed) *Current Therapy in Large Animal Theriogenology*. Philadelphia, WB Saunders Co, 1997, pp 295-303.

Abstract

Disposal and Disease Rates in 340 British Dairy Herds

D.A. Whitaker, J.M. Kelly, S. Smith

Veterinary Record (2000) 146, 363-367

Data derived from 340 dairy herds, mainly in southern England, between April 1998 and March 1999, showed that the average total culling rate was 22·1 percent, with 5·6 percent for infertility, 3·6 percent for mastitis, 1·7 percent for lameness, 2·0 percent for poor milk yield, 3·7 percent for age and 5·5 percent for miscellaneous reasons which included death. The average annual rate of assisted calvings was 8·7 percent, of injury 0·9 percent, digestive disease 1·3 percent, ketosis 0·4 percent, hypomagnesaemia 0·7 percent, hypocalcaemia 5·3 percent, mastitis 36·6 percent, and lameness 23·7 percent. There was a significant association ($P<0·001$) between higher rates of mastitis in cows housed in straw yards as opposed to cubicles and also between higher rates of lameness in cows housed in cubicles as opposed to yards ($P<0·015$). However, there were farms with low rates of mastitis in cows kept in straw yards and low rates of lameness in cows kept in cubicles. Larger herds tended to have more problems with lameness and higher bulk milk somatic cell counts (BMSCC). There was a positive association between BMSCC and mastitis rate.

Milk Metabolic Profiles in Dairy Cows and Fertility

N.B. Cook

Cattle Practice (1999) 7(3): 249-254

Two techniques for the evaluation of milk ketone bodies were evaluated and compared with plasma betahydroxybutyrate (BHB) concentrations. Milk BHB was determined via an enzymatic method and milk acetone was measured by the technique of Flow Injection Analysis (FIA). Milk acetone had a slightly lower sensitivity than milk BHB, but specificity was higher, giving a predictive value of a positive test of 0.8 compared to 0.66 for milk BHB. Milk acetone was more closely related to plasma BHB, making analysis of acetone by FIA the preferred milk ketone test in this study.

All cows calving on three farms were milk sampled twice in early lactation and milk acetone and urea were analysed by FIA. The overall incidence of ketotic cows; defined as having at least one milk acetone concentration greater than or equal to 0.4 mmol/l before 60 days postpartum, was low at 7.6 %, which may have been due to the method of feeding utilised. A seasonal peak in incidence occurred late in the grazing season, despite access to a TMR, suggesting that high yielding cows at grass from August are particularly at risk of developing ketosis.

Ketotic cows suffered significantly longer calving to first service and calving to conception intervals on only one of the three farms studied. This farm also suffered the highest incidence of ketosis. Fertility parameters were not significantly different in 22 ketotic cows matched for parity and calving date with 42 control cows. The incidence of vaginal discharge after 15 days was however significantly increased ($p<0.05$).

Milk acetone testing in early lactation could not be used to identify individual cows at risk of subsequent poor fertility.