

## **Economics of Resynchronization with Chemical Tests to Identify Nonpregnant Cows**

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### **INTRODUCTION**

A major goal of reproductive management programs used on dairy farms is to establish pregnancy as soon as possible after the end of the voluntary waiting period (**VWP**). Cows that become pregnant earlier produce more milk per day of lactation, produce more calves per lifetime, and are more profitable than cows that become pregnant later in lactation (Cabrera and Giordano, 2010). Coupling a resynchronization of ovulation program with practical and cost-effective methods to identify nonpregnant cows as early as possible after a previous insemination is critical to allow rapid re-insemination of nonpregnant cows. The economic value of any nonpregnant test, however, depends largely on the accuracy of the test, correctly identifying nonpregnant and pregnant cows, as well as the cost of the test (Galligan et al., 2009). Therefore, diagnostic tests should have high sensitivity (**Se**), high specificity (**Sp**), and provide the fewest possible questionable diagnoses (**Qd**).

Besides the traditional methods (rectal palpation and transrectal ultrasound) for non-pregnancy and pregnancy diagnosis in cattle, new methods have been developed and are now commercially available to producers. In this regard, chemical determination of pregnancy through the detection of placental pregnancy associated glycoproteins (**PAG**; Sasser et al., 1986; Zoli et al., 1992; Green et al., 2005) released into the maternal bloodstream are reliable methods to determine the pregnancy status of dairy cattle and can be incorporated into already established reproductive management schemes (Silva et al., 2007, 2009). The commercially available tests allow for determination of pregnancy in lactating dairy cows with good accuracy as early as 28 d after artificial insemination (**AI**; Silva et al., 2007; Romano and Larson, 2010). As a consequence, one of the major advantages of a chemical test is the identification of nonpregnant cows earlier after an insemination, which allows the implementation of aggressive resynchronization protocols that result in shorter interbreeding intervals (**IBI**).

Performing an early pregnancy test post-AI may be highly beneficial because shortening the IBI improves reproductive performance. However, like any other method for pregnancy diagnosis, chemical tests may have low accuracy if used too early after AI, and this should be taken into account. Another factor that affects the performance of an early test is the occurrence of pregnancy loss, which is detrimental to the overall reproductive performance of the herd. Naturally occurring pregnancy losses are more likely during the early stages of pregnancy and tend to decrease as gestation progresses (Santos et al., 2004). As a result, the earlier a pregnancy test is performed the greater the number of pregnancies that will be lost until the next pregnancy reconfirmation or calving. Therefore, there is a trade-off between the time gained with earlier pregnancy tests and the effect of pregnancy loss; because cows losing their pregnancy should be identified and resubmitted for AI as soon as possible after the loss.

Previous analyses have reported no major economic difference between the use of chemical test, transrectal ultrasound, or rectal palpation to detect pregnancy in dairy cattle (Galligan et al., 2009; Ferguson and Galligan, 2011). These previous studies have relied on cost/benefit and partial budgeting analysis in combination with some type of decision tree framework (Ferguson and Galligan, 2011; Galligan et al., 2009; Pitcher and Galligan, 1990; Oltenacu et al., 1990). Such approaches seem suitable because they can include the Se (and the positive predictive value of the test) and the Sp (and the negative predictive value of the test), as well as the probability of pregnancy loss within a solid economic framework. Nonetheless, to measure the overall impact of the pregnancy test we used a more comprehensive approach that allows following the whole herd dynamics through repetitive breeding cycles and complete lactations. Integrating the pregnancy testing method within the reproductive program, as opposed to its application as an isolated event, may better reflect its impact on the re-insemination process and generate results of greater

utility for producers (Ferguson and Galligan, 2011; Galligan et al., 2009).

The objectives of the present study were to:

1. Assess the economic value of decreasing the interval between inseminations when using a non-pregnancy test that allows earlier identification of nonpregnant cows; and
2. Assess the impact of pregnancy loss and inaccuracy of the test on the value of the method for earlier pregnancy diagnosis with chemical test.

Two experiments were performed using a model that simulates the reproductive, productive, and economic dynamics of commercial dairy herds. The major hypothesis was that the economic advantage of performing an earlier pregnancy test is reducing the interval between 2 successive AI. Moreover, the benefits of a shorter IBI will overcome potential additional costs and losses incurred due to inaccuracy of the earlier chemical test.

## MATERIALS AND METHODS

### Experiment 1

To assess the impact of different IBI on the economics and reproductive dynamics of a dairy operation, a 1,000-cow commercial dairy herd was simulated using the UW-DairyRepro\$ model (Giordano et al., 2011). The model calculates the status of all cows in each service and hence the herd dynamics, which respond to the parameters of a reproductive program. This process estimates sequentially, as finite Markov chains, the percentage of cows eligible for breeding after the end of the VWP, the proportion of cows serviced, the percentage of cows becoming pregnant, and the percentage of cows not becoming pregnant at each service. The first state in the Markov chain process is represented by the nonpregnant cows, which could move to the next state of being inseminated following transition probabilities determined by the reproductive program.

The model allows defining reproductive programs that rely on a combination of estrous detection (**ED**) and synchronization. Cows detected in estrus are inseminated 22 d after the previous AI; whereas, cows not detected in estrus receive timed AI (**TAI**) at a predefined interval according to the synchronization protocol. Inseminated cows could then move to the pregnant state within the Markov chains based on transition probabilities of conception rate (**CR**) defined by the reproductive program. After each reproductive event, the proportion of cows

failing to conceive plus those not receiving AI in that service return to the nonpregnant state and are eligible for the next AI. This probabilistic process endowed by the Markov property that the next state depends solely on the current state and the transition probabilities, is used to construct pregnancy survival curves that reflect a program reproductive efficiency (Giordano et al., 2011). The model simultaneously calculates a future expected monetary value for cows becoming pregnant and for cows that remain open (based on milk income over feed cost, cost of non-reproductive culling and mortality, cost of reproductive culling, value of newborn, and cost of a reproductive program), which is used in conjunction with survival curves to estimate the NPV of a reproductive program (Giordano et al., 2011).

The baseline reproductive management program consisted of a combination of ED and TAI after synchronization of ovulation for first postpartum AI with the Presynch-Ovsynch protocol (Moreira et al., 2001) and the Ovsynch protocol (Pursley et al., 1995) for second and subsequent TAI services. Between the end of the VWP set at 50 days in milk (**DIM**) and the first TAI at 72 DIM, cows detected in estrus were inseminated. For second and subsequent AI services, AI after ED was also performed between TAI services. The proportion of cows inseminated after ED was sequentially increased from 30 to 80 % in 10 percentage point increments for all AI services with an expected CR of 35 % (Table 1). Under most circumstances, when ED is added before or between TAI services lower CR is observed for those cows inseminated to TAI because the population of cows reaching that point is different than when no ED is added (Chebel et al., 2010; Keskin, 2011). Therefore, for each 10 percentage point increment in ED before first service TAI, the initial CR of 40 % after TAI services decreased by 2 percentage points (Table 1). Additionally, the CR of second and subsequent TAI services decreased by 2 percentage points when the proportion of cows receiving AI after ED was between 60 and 80 % (Table 1).

Once the baseline reproductive programs were defined, the IBI for cows receiving a TAI service was incremented by 7 d intervals from 28 to 56 d to reflect IBI observed in commercial dairy operations when using resynchronization of ovulation for second and subsequent AI services. In all cases, the Ovsynch protocol for resynchronization was initiated before the pregnancy diagnosis, which was performed at the time of the PGF<sub>2α</sub> injection of the Ovsynch protocol. Conception rate for all resynchronized TAI services was similar regardless of the different IBI. Economic parameters (i.e., milk price, cull cow cost, heifer

replacement cost, female calf cost, feed cost) used as input in the model were compiled from the Wisconsin Calculated Milk Cost of Production reported by the Center for Dairy Profitability at the University of Wisconsin-Madison for the months of April, 2010 to April, 2011. Additional input parameters (i.e., milk production curves, culling, mortality rate) were compiled from a commercial 1,000-cow dairy farm in Wisconsin.

## Experiment 2

Following up on Experiment 1, the UW-DairyRepro\$ model was modified to study different pregnancy tests at different time points after AI. Some of the reproductive programs described in Experiment 1 (Table 1) were used to test plausible and realistic scenarios of pregnancy diagnostic tests defined in Table 2. Two sets of programs were simulated to assess the impact of a week earlier pregnancy diagnosis with a chemical test. In all cases the basic programs were similar to those described for Experiment 1 combining ED for AI services with the Presynch-Ovsynch and Ovsynch protocols for TAI. One of the scenarios consisted of 2 programs: the early testing program used a chemical test at 32 d

after a previous AI; whereas the late testing program used rectal palpation at 39 d after AI. For both programs, on the day of pregnancy diagnosis, nonpregnant cows received the PGF<sub>2α</sub> injection of the Ovsynch protocol and their next TAI at 35 and 42 d after the previous AI, respectively.

Another comparison consisted of an early testing program using a chemical test at 25 d after a previous AI; whereas the late testing program used transrectal ultrasound at 32 d after AI. For both programs, on the day of the pregnancy diagnosis, nonpregnant cows received the PGF<sub>2α</sub> injection of the Ovsynch protocol and their next TAI at 28 and 35 d after the previous AI, respectively. Even though the current commercially available chemical test does not allow a pregnancy test to be performed 25 d after AI, the program with such timing for the pregnancy test was included to demonstrate the potential of a very early pregnancy diagnosis. Based on current research we speculate that this type of chemical test will be available in the market in the near future.

**Table 1.** Expected reproductive performance of programs used for simulation in Experiment 1.

Program	Interbreeding Interval (range, d)	<sup>2</sup> ED before 1 <sup>st</sup> TAI <sup>4</sup> (%)	First AI <sup>1</sup>		Second and subsequent AI		
			<sup>3</sup> CR ED before 1 <sup>st</sup> TAI (%)	CR TAI (%)	ED before TAI (%)	CR TAI (%)	CR TAI (%)
Presynch-Ovsynch & Ovsynch	28 – 56	30	35	40	30	35	30
Presynch-Ovsynch & Ovsynch	28 – 56	40	35	38	40	35	30
Presynch-Ovsynch & Ovsynch	28 – 56	50	35	36	50	35	30
Presynch-Ovsynch & Ovsynch	28 – 56	60	35	34	60	35	28
Presynch-Ovsynch & Ovsynch	28 – 56	70	35	32	70	35	28
Presynch-Ovsynch & Ovsynch	28 – 56	80	35	30	80	35	28

<sup>1</sup>AI = artificial insemination.

<sup>2</sup>Percentage of cows AI after estrous detection before first TAI.

<sup>3</sup>Conception rate of cows AI after estrous detection.

<sup>4</sup>TAI = timed AI.

**Table 2.** Baseline and range for parameters for two hypothetical sets of scenarios using an early chemical pregnancy tests compared with rectal palpation or transrectal ultrasound.

	32 vs. 39 d pregnancy test <sup>1</sup>			25 vs. 32 d pregnancy test <sup>2</sup>		
	Baseline	Minimum	Maximum	Baseline	Minimum	Maximum
Sensitivity (%)	98	94	99	97	94	99
Specificity (%)	98	94	99	97	94	99
Pregnancy loss (%) <sup>3</sup>	5.25	0	10	5.25	0	10
Questionable diagnosis (%)	3.3	0	10	8.5	0	10
Estrous detection rate (%)	50	30	80	50	30	80
Cost chemical pregnancy test <sup>4</sup> (\$/test)	2.4	0.5	5.0	2.4	0.5	5.0

<sup>1</sup>Early test performed using chemical blood test at 32 d resulted in an interbreeding interval of 35 d; whereas late test performed by rectal palpation at 39 d resulted in an interbreeding interval of 42 d.

<sup>2</sup>Early test performed using chemical blood test at 25 d resulted in an interbreeding interval of 28 d; whereas late test performed by transrectal ultrasound at 32 d resulted in an interbreeding interval of 35 d.

<sup>3</sup>During the 7 d period between early and late pregnancy tests (32 vs. 39 d and 25 vs. 32 d) based on Vasconcelos et al. (1997).

<sup>4</sup>First pregnancy test after AI.

Additional parameters were introduced to study the economic value of an early first pregnancy diagnosis with a chemical test. The parameters included were Se, Sp, Qd, cost of the chemical test, and expected pregnancy loss. Sensitivity and Sp were defined using the test to which the chemical test was being compared to as a gold standard. In addition, pregnancy loss was defined as the total pregnancy loss occurring during the 7 d period between the early chemical test and late transrectal ultrasound or rectal palpation pregnancy diagnosis.

**Sensitivity:** This value was multiplied by the CR in each breeding period, because a test with lower than 100 % Se will fail to detect a proportion of pregnant cows (false negative). These cows misdiagnosed as nonpregnant will lose their pregnancy when submitted to the TAI program. The model accounts internally for this value loss.

**Specificity:** This value was multiplied by the proportion of expected nonpregnant cows in each breeding period, because a test with lower than 100 % Sp will fail to detect a proportion of nonpregnant cows. These cows are misdiagnosed as pregnant (false positive). Therefore, the complement of the Sp (100 % - Sp) was used to account for the proportion of false pregnant cows. Because cows misdiagnosed as pregnant can only be re-inseminated if detected in estrus or when enrolled into the synchronization program after a negative pregnancy reconfirmation, these cows will have a delayed re-insemination. Pregnancy reconfirmation was set at 28 d after the first pregnancy diagnosis. The model accounts internally for the value loss of cows with delayed re-insemination.

**Questionable diagnosis:** When the results of any pregnancy test are inconclusive, cows are given a Qd and retested after a certain number of days. For cows with a Qd retesting (re-check) was assumed to be performed 1 wk later, hence cows that were actually nonpregnant will miss 1 wk until they are re-enrolled into a synchronization protocol. The total proportion of Qd was first split into a questionable pregnant diagnosis (71.43 %) and questionable nonpregnant diagnosis (28.57 %) according to Romano and Larson (2010). The model accounts internally for this loss value. The model also accounts for the cost of additional re-checks for cows with previous Qd assuming same costs as the first diagnosis test.

**Pregnancy loss:** This value was added to the population of false pregnant cows in each breeding period because these cows, although correctly diagnosed as pregnant, will lose their pregnancy. Consequently their next breeding will not occur until they are either detected in estrus or are found nonpregnant 28 d later at pregnancy reconfirmation. Because the impact of pregnancy loss on the herd dynamics is similar to that of decreased Sp, 1 percentage point of increased pregnancy loss was equivalent to 1 percentage point of decreased Sp.

**Cost of the chemical test:** This value was added to the value of hormones, labor, and AI to the first and subsequent services including ED and TAI within the model structure.

Each parameter was divided into 4 levels from minimum to maximum (Table 2) and were used as inputs in the modified UW-DairyRepro\$ model. The model was run for all possible combinations of

parameter values ( $4^6 = 4096$  scenarios for each comparison). Results were then summarized in datasets and analyzed by regression and breakeven analyses.

## RESULTS

### Experiment 1

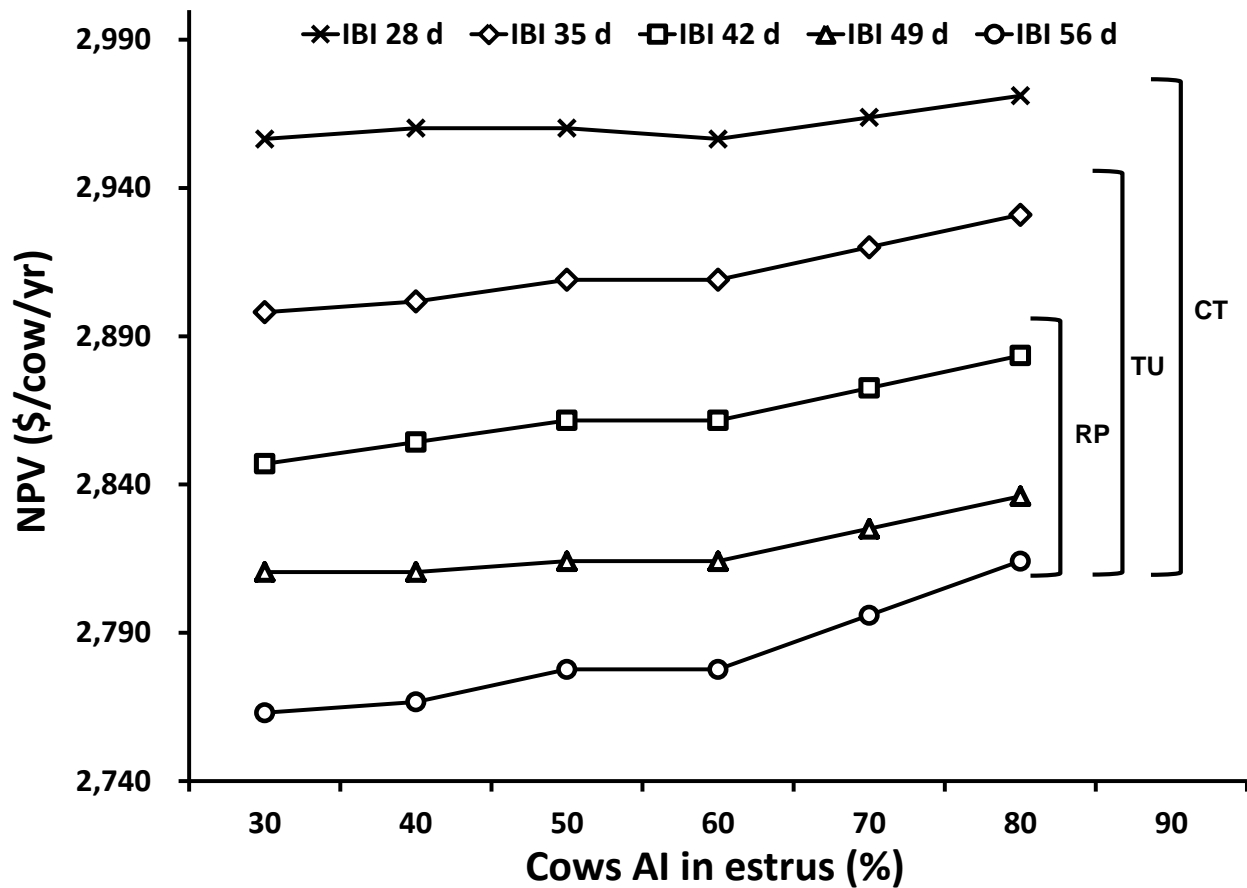
The net present value (NPV) for all reproductive programs simulated is presented in Figure 1. Programs with shorter IBI had greater NPV at all levels of ED. The NPV also showed a positive trend with increasing percentage of cows inseminated after ED from 30 to 80 %. A greater impact of adding cows receiving AI after ED was observed for programs with longer IBI as opposed to those programs with shorter IBI; which presented smaller

gains when increasing the percentage of cows receiving AI after ED.

### Experiment 2

#### *Reproductive survival curves for programs using different pregnancy testing methods*

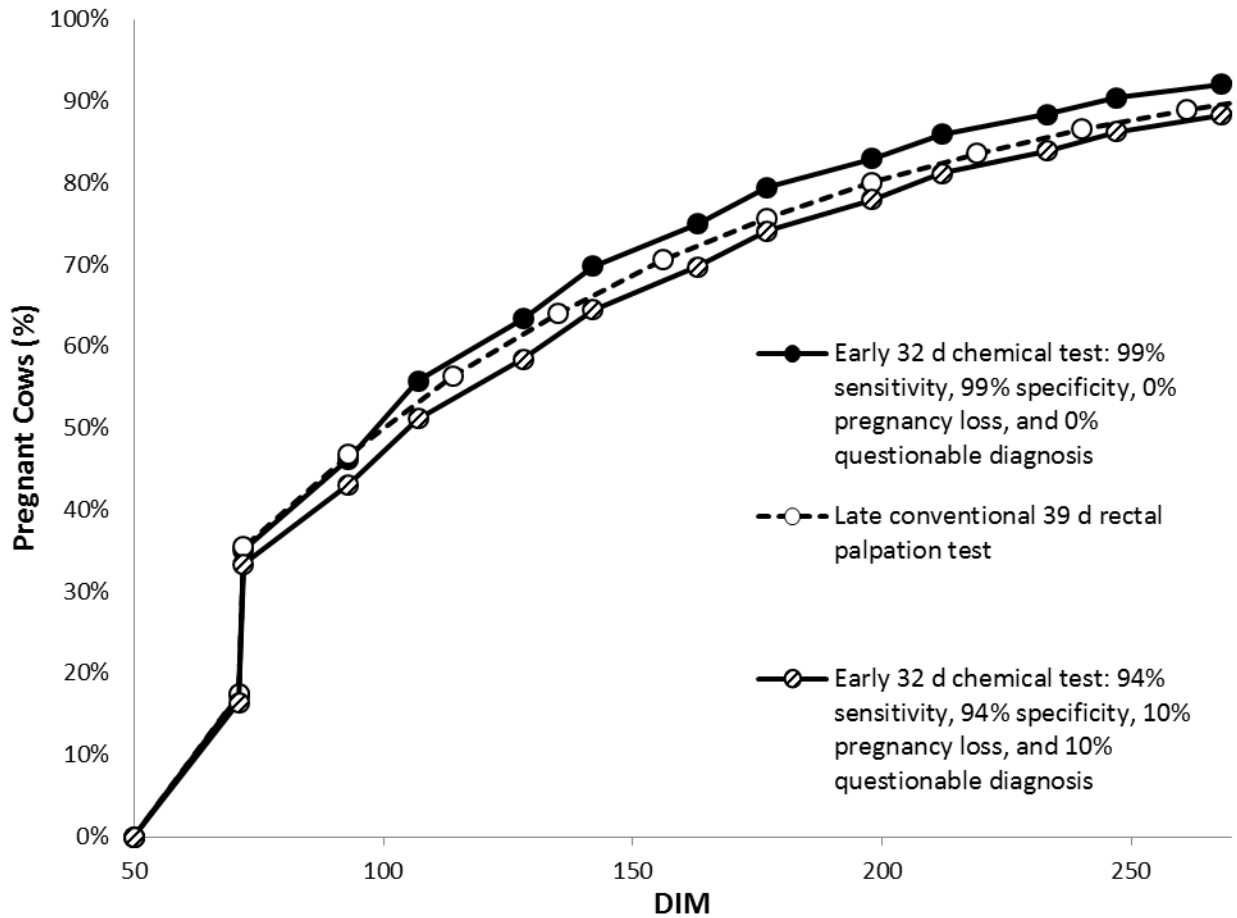
Economic results responded to the rate at which cows became pregnant and the total number of pregnancies generated with a reproductive program that used transrectal ultrasound, rectal palpation, or a chemical test a week earlier. Important characteristics of the test such as Se, Sp, and Qd as well as the expected pregnancy loss greatly influenced the survival curves for pregnancy as a response to the first pregnancy diagnosis after AI. By the end of the



**Figure 1.** Impact of interbreeding interval (IBI) on the net present value (NPV; \$/cow/yr) for timed AI (TAI) for a reproductive management program combining estrous detection with the Presynch-Ovsynch protocol for first TAI and the Ovsynch protocol for resynchronization of ovulation for second and subsequent TAI. The IBI varied between 28 to 56 d. For each of the IBI, the percentage of cows receiving AI after detection of estrus before first TAI and between resynchronized TAI ranged from 30 to 80 % in 10 percentage point increments. Brackets indicate the set of interbreeding intervals using Ovsynch for resynchronization of ovulation and TAI that can be achieved when using rectal palpation (RP), transrectal ultrasound (TU), and chemical tests (CT) for pregnancy diagnosis. The program with 28 d IBI is not possible with the current commercially available CT, but may be in the near future.

breeding period, the proportion of expected pregnant cows was 89.0 % when using rectal palpation 39 d after AI (Figure 2). By contrast, the total proportion of pregnant cows was 92.1 and 88.3 % when using a chemical test for pregnancy diagnosis 32 d after AI under hypothetical extreme conditions of Se, Sp, Qd, and pregnancy loss (Figure 2). In the first case, 92.1 % of the cows were pregnant by the end of the breeding period when Se and Sp were 99 %; Qd, 0 %; and pregnancy loss, 0 %. Conversely, only 88.3 % of the cows were pregnant by the end of the

breeding period when Se and Sp were 94 %; Qd, 10 %; and pregnancy loss, 10 %. Curves in Figure 2 depict the shorter IBI for the earlier pregnancy test and the pregnancies achieved at different DIM. Similar results were observed for the chemical test at 25 d compared with transrectal ultrasound 32 d after AI (not shown). The value of the pregnancy tests within the studied reproductive programs was determined by multiplying these curves by the expected monetary values at the reproductive events.



**Figure 2.** Pregnancy survival curves expected when performing a late conventional rectal palpation pregnancy test 39 d after AI and when performing an early chemical blood test 32 d after AI under two extreme situations considering sensitivity, specificity, pregnancy loss, and proportion of questionable diagnosis. Estrous detection rate was set at 50 %.

**General results: Relative impact of test accuracy, cost, and pregnancy loss**

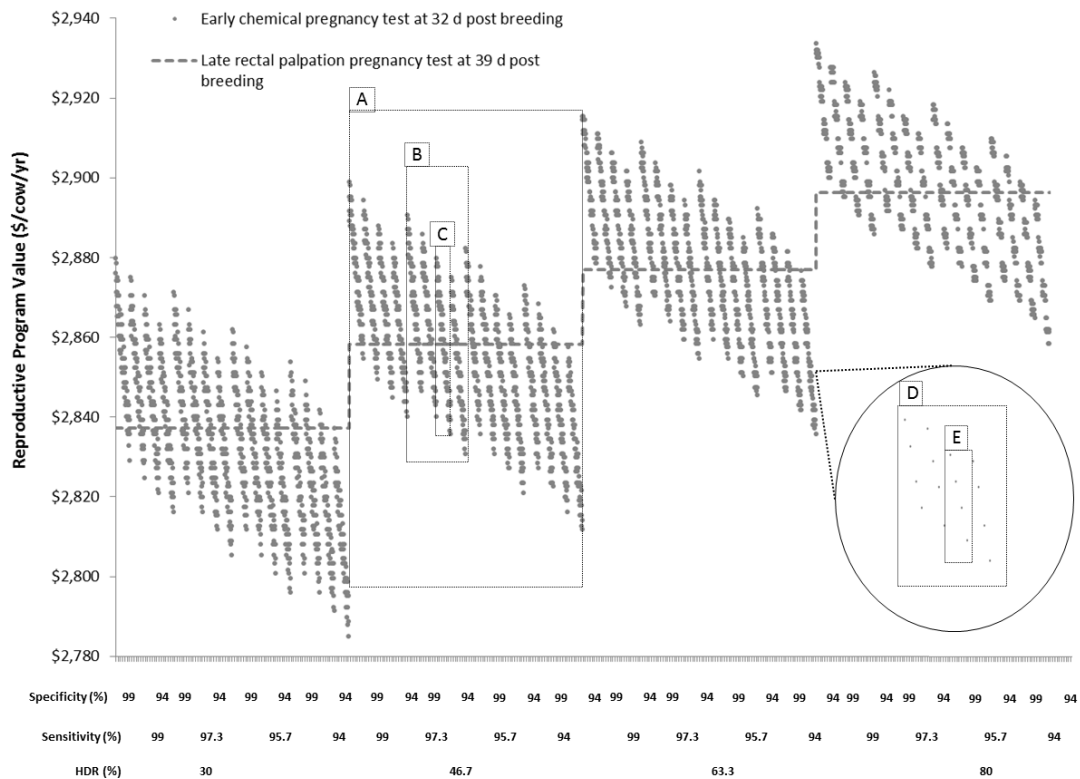
For the comparison of programs using a pregnancy diagnosis of chemical test at 32 d vs. rectal palpation at 39 d after AI, the value of the earlier chemical test was largely impacted (in order) by the

- 1) Se,
- 2) Sp,
- 3) Pregnancy loss,
- 4) Proportion of Qd,
- 5) The chemical test cost, and
- 6) The ED rate (**HDR**) as shown in Figure 3.

This value was calculated as the difference between the value of the earlier chemical test (dots) and the value of the conventional later test (dashed line). Therefore, positive values are those where the dots are above the dashed line. A similar situation was observed when comparing an earlier chemical test 25 d after AI vs. transrectal ultrasound performed 32 d after AI (not shown).

Because each of the 6 factors was divided into 4 sections from minimum to maximum (Table 2), it is

possible to assess general trends for the interaction between the main factors by looking at sections in Figure 3. The HDR splits the figure into 4 distinguishable sections indicating HDR from low (30 %) to high (80 %). Inside each HDR block, there are 4 distinguishable sections indicating Se from high (99 %) to low (94 %; Figure 3, Section A). By far, the Se of the earlier chemical test is the most important factor determining its value judging by the large difference between the first and fourth block within Section A. When Se is high, most dots are above the horizontal dashed line (positive values). Conversely, when Se is low (fourth block) only a few dots are above the horizontal line with most of them appearing below the dashed line. Within each Se block, there are 4 distinguishable blocks indicating Sp from high (99 %) to low (94 %; Figure 3, Section B). Specificity of the earlier chemical test is the second factor of importance in determining its value, which can be assessed easily by observing the difference between the upper and lower group of dots in each Sp block. Similarly, within the Sp blocks are 4 blocks that show the impact of pregnancy loss from low (0 %) to high (10 %; Figure 3, Section C). Higher values are observed with lower pregnancy loss.



**Figure 3.** The value of early pregnancy chemical test 32 d after AI compared with a late rectal palpation test 39 d after AI according to 4 levels of 6 different factors: 1) Estrous detection rate (30 to 80 %), 2) Sensitivity (99 to 94 %, Section A), 3) specificity (99 to 94 %, Section B), 4) pregnancy loss (0 to 10 %, Section C), 5) questionable diagnosis (0 to 10 %, Section D), and 6) cost of early pregnancy chemical test (\$0.50 to \$5.00, Section E). Dots above the dashed line represent situations in which positive values for chemical test were found when compared to the later rectal palpation test.

**Table 3.** Regression coefficients and quantitative impact of the 6 factors evaluated on the value of the early chemical test compared with rectal palpation or transrectal ultrasound.

	32 vs. 39 d pregnancy test <sup>1</sup>			25 vs. 32 d pregnancy test <sup>2</sup>		
	Regression Coefficient	Quantitative Impact (\$/+1 % or +\$0.10)	Relative Impact to Sensitivity <sup>3</sup>	Regression Coefficient	Quantitative Impact (\$/+1 % or +\$0.10)	Relative Impact to Sensitivity <sup>3</sup>
Constant	-795.39			-637.71		
Sensitivity (%)	534.48	+5.34	---	450.33	+4.50	---
Specificity (%)	305.43	+3.05	1.75	253.35	+2.53	1.78
Pregnancy loss (%)	-305.51	-3.05	-1.75	-253.51	-2.54	-1.78
Questionable diagnosis (%)	-39.04	-0.39	-13.69	-33.73	-0.34	-13.35
Estrous detection rate (%)	9.72	0.097	55.0	-22.01	-0.22	-20.46
Cost chemical pregnancy test (\$)	-1.75	-0.175	-305.75	-1.92	-0.019	-235.10

<sup>1</sup>Early test performed using chemical blood test at 32 d resulted in an interbreeding interval of 35 d; whereas late test performed by rectal palpation at 39 d resulted in an interbreeding interval of 42 d.

<sup>2</sup>Early test performed using chemical blood test at 25 d resulted in an interbreeding interval of 28 d; whereas late test performed by transrectal ultrasound at 32 d resulted in an interbreeding interval of 35 d.

<sup>3</sup>Quantitative impact of factor analyzed divided by quantitative impact of sensitivity.

A detailed zoom inside each pregnancy loss level shows the impact of the Qd and the cost of the earlier chemical test (Figure 3, Section D). By comparing the 4 horizontal blocks in the detailed zoom, the relative impact of the factor Qd varying from low (0 %) to high (10 %) can be assessed. By comparing blocks of 4 vertical dots, it is possible to assess the relative impact of the cost of the chemical test from low (\$0.50) to high (\$5.00; Figure 3, Section E). Compared with other factors, the cost of the chemical test is the least important factor impacting the value of the earlier chemical test.

#### **Regression analysis to quantify the impact of the earlier chemical test**

Results of 4,096 scenarios (4 levels per each factor and 6 factors) for the comparison of earlier chemical test 32 d after AI (Figure 3) and 4,096 scenarios of earlier chemical test 25 d after TAI were analyzed by multiple regressions to determine the quantitative impact of each factor on the value of the chemical test (Table 3). The coefficients are used to approximate the value of the chemical test based on the 6 factors considered.

The value of the earlier chemical test under ideal hypothetical conditions of high test accuracy (implying 99 % Se, 99 % Sp, and non-existence of Qd) assuming no pregnancy loss for a 50 % HDR and an earlier chemical test cost of \$2.40 would be \$36.78 and \$43.33 for the earlier chemical tests at

32 and 25 d, compared with conventional tests at 39 d rectal palpation and 32 d transrectal ultrasound, respectively. However, the early chemical test is not that accurate and pregnancy loss will occur anyway after the test is performed. Considering a more realistic situation of 98 % Se, 98 % Sp, and 3.3 % of Qd for a chemical test at 32 d (35 d IBI), and 5.25 % pregnancy loss in 7 d (between 32 and 39 d postpartum; Table 2) the value of the earlier chemical test was \$11.06/cow/yr. For the more aggressive scenario with chemical test and 25 d vs. transrectal ultrasound at 32 d after AI, considering 97 % Se, 97 % Sp, 8.5 % Qd, 5.25 % pregnancy loss in 7 d (between 25 and 32 d postpartum; Table 2) the value of the earlier chemical test was \$13.08/cow/yr.

#### **Breakeven analysis**

A breakeven analysis (point where the economic benefit becomes zero) was performed using regression coefficients from Table 3 and baseline chemical test characteristics from Table 2. For the chemical test 32 d vs. rectal palpation 39 d after AI, an economic breakeven would occur if the Se is 95.9 %, the Sp is 94.2 %, or the pregnancy loss is 8.9 % when all other parameters are as specified in Table 2. For the chemical test 25 d vs. transrectal ultrasound at 32 d after AI, an economic breakeven could be reached if the Se is 94.3 %, the Sp is 92.0 %, and the pregnancy loss is 10.5 % when all other parameters are as specified in Table 2.



## DISCUSSION

### Experiment 1

As expected, the results of this experiment clearly demonstrated that reducing the time interval between 2 successive TAI services resulted in greater economic returns. Because the CR of all AI services remained unchanged for the different IBI simulated, it was not surprising that shorter IBI periods resulted in greater NPV. A shorter IBI for TAI services affects the herd dynamics by generating greater pregnancies in a shorter period of time after the end of the VWP. Therefore, any method for non-pregnancy diagnosis that allows the implementation of reproductive management programs that reduce the time interval between 2 successive TAI services without affecting the fertility of cows will improve the profitability of the farm. Thus, use of chemical tests available in the market today might be beneficial, at least when compared to rectal palpation, because they can be applied earlier after AI.

Another important observation was that increasing the percentage of cows receiving AI after detection of estrus always increased the NPV for all programs. This occurred because the CR of AI services after ED for second and subsequent AI services was 35 %, which represents an improvement of either 5 or 7 percentage points when compared to TAI. In addition, as the percentage of cows receiving AI after ED increases the NPV of programs continued to improve, because more cows have a 22 d IBI as opposed to the longer IBI for cows receiving TAI. Similar observations were reported by Giordano et al. (2011) when adding ED to a 100 % TAI D32 Resynch program with 30 % CR across all resynchronized AI services. The greater benefit of increasing the percentage of cows receiving AI after ED in programs with longer IBI (steeper increase in NPV line) clearly reflected and supported this notion. The greatest benefits were observed when a significant percentage of cows received AI at estrus as opposed to receiving TAI at a longer IBI.

For simplification of simulation and interpretation of results, a major assumption of this analysis was that whatever test was being used, it had 100 % accuracy to detect nonpregnant and pregnant cows, had the same cost of application, and there was no pregnancy loss. Because this may not reflect the results observed in commercial operations, the impact of inaccurate diagnosis, variable costs, and pregnancy loss are addressed with Experiment 2.

### Experiment 2

Results of Experiment 2 are consistent with those recently found by Ferguson and Galligan (2011) and others (Galligan et al., 2009; Oltenacu et al., 1990; Pitcher and Galligan, 1990). The order and direction of the impact of changing the values for the factors evaluated in this experiment for the chemical test (Se, Sp, Qd, chemical test cost, and pregnancy loss) are highly consistent with previous studies. Regarding the herd's HDR, we found both similar and somewhat differing results. When contrasting our results with those of previous reports it is important to emphasize that the framework used in the present study was substantially different. Most other studies (Galligan et al., 2009; Oltenacu et al., 1990; Pitcher and Galligan, 1990) used some type of decision tree approach in which the accuracy of the pregnancy test was either rewarded or punished according to probabilistic outcomes. Conversely, in the present study a comprehensive approach that considered the impact of the chemical test on whole herd dynamics was used. Moreover, while in previous studies the impact of using different pregnancy tests was assessed at the same number of days after AI, we also included in the analysis the added value of earlier testing possible when new technologies become available.

As in the previous studies, Se of the chemical test was the most important test characteristic and had the greatest impact on its relative value. Nonetheless, the impact of Se on the total value was 1.8 times greater than Sp. By contrast, Ferguson and Galligan (2011) reported a 4 times greater magnitude for Se than Sp. Oltenacu et al. (1990) reported that the error rate for an earlier pregnancy test needs to be  $\leq 3$  % to have a positive value. Ferguson and Galligan (2011) reported that the test value would become negative if the Se went below 90 %. By contrast, results of the present study indicate that to have a positive value for the chemical test, Se should be approximately  $> 96$  % when the chemical test is used at 32 d and  $> 94$  % when used 25 d after AI. Certainly, some of the differences between studies are due to the different frameworks used for analysis. One important difference is that we defined Se as a relative value of the pregnancy test used for the later diagnosis test, which was considered the gold standard having 100 % accuracy. Because in reality no test has 100 % accuracy, the actual value of the chemical test in the present study may have been slightly underestimated.

A test with low Se results in misdiagnosis of truly pregnant cows as nonpregnant, as a result, when these cows continue into the synchronization protocol iatrogenic pregnancy loss will occur after they receive a PGF<sub>2α</sub> injection. Certainly induced pregnancy losses are expensive; however, they may not be as expensive as previously reported. Our model accounts internally for the value of these pregnancy losses (Giordano et al., 2011) whereas in the decision tree approach this is an external input. We speculate that such value may have been previously overestimated. For example, Galligan et al. (2009) used \$300; whereas in a more recent report Ferguson and Galligan (2011) used \$46, recognizing that a lower value should be used as these cows are re-inseminated within 10 d of diagnosis.

A test with low Sp leads to the misclassification of cows as pregnant when they are truly nonpregnant. Consequently, instead of completing the resynchronization protocol and receiving their next TAI, these cows with a false pregnancy diagnosis will not be re-inseminated until they are either detected in estrus or after diagnosed not pregnant at the next pregnancy reconfirmation. In our approach, another group of cows that followed the same dynamics were those experiencing pregnancy loss. However, these cows are correctly classified as pregnant by the test and subsequently lose their pregnancy, which results in delayed rebreeding until they either receive AI after ED or are diagnosed not pregnant at the next pregnancy reconfirmation. The value lost by cows experiencing pregnancy loss was then similar to that of cows affected by the low Sp of the test being used.

In agreement with Ferguson and Galligan (2011), Qd had a significantly lower negative impact than Se and Sp in the present study (Table 3). This is due, at least in part, because cows with a Qd were assumed to be re-examined within a week and if nonpregnant promptly re-assigned to the TAI program. Based on these observations, Qd is much preferable than a misdiagnosis.

The impact of HDR was the second to the last factor influencing the value of the chemical test in contrast to the observations of Ferguson and Galligan (2011) and Galligan et al. (2009) who reported that HDR had a greater influence on the value of a pregnancy test. This difference could be attributed to the fact that in the present study only highly aggressive reproductive programs combining TAI with ED (more likely to implement a chemical test) were included. For this type of program, the impact of changing the HDR may not be as dramatic as for

programs using 100 % ED for insemination of cows, because cows not detected in estrus receive a TAI. Overall, the effect of increasing HDR on the final NPV of a program was positive under all circumstances (Figure 1). However, it was interesting that for the comparison between the programs with an IBI of 28 vs. 35 d, increasing the HDR decreased the specific value of chemical test; whereas an opposite trend was observed when comparing the programs with an IBI of 35 vs. 42 d. In the latter case, the difference in the expected value of the chemical test was greater at higher HDR. This likely occurred because for longer IBI (35 vs. 42 d compared to 28 vs. 35 d) the impact of HDR increases.

Finally, consistent with Ferguson and Galligan (2011), the chemical test cost was not an important factor to determine the overall value of the test. In our analysis, the value of the conventional (transrectal ultrasound or rectal palpation) tests was fixed at \$2 per cow; whereas the chemical test cost was allowed to change. Then, for each \$0.10 increase in the chemical test cost there was a decrease of \$0.18 to \$0.19 on the chemical test value.

Even though a comprehensive approach that took into account the herd reproductive dynamics was used for this economic analysis of different pregnancy testing methods, other factors of importance in the value of a pregnancy test were beyond the scope of this study. For example, the involvement of a veterinarian in the reproductive management program may provide valuable information beyond a simple pregnancy diagnosis. Additional information, such as ovarian status, abnormalities of the reproductive tract, or abnormalities of the pelvic and abdominal cavity may have some added value not taken into account by our analyses, but should also be accounted for during the decision-making process.

## CONCLUSIONS

The major impact of using a chemical test for pregnancy testing in lactating dairy cows was the potential of shortening the IBI with a consequent increase in herd profitability. However, when the potential inaccuracy of the chemical test was included in the analysis, smaller economic differences were observed when comparing hypothetical scenarios performing the chemical test vs. rectal palpation or transrectal ultrasound 7 d later. The results of the present study also indicate that rather than focusing on the value of the pregnancy test alone, the impact of using different testing

methods on the outcome of the reproductive program applied at the farm should be considered. Although results presented here would not apply to every farm and reproductive management program, these are important to demonstrate a solid framework that could be perfected to perform economic assessments of using different testing methods as reproductive management programs for dairy cattle continue to evolve.

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