Successful Timed AI Programs:
Using Timed AI to Improve Reproductive Efficiency
in High Producing Dairy Cattle

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Introduction

Managers of high producing dairy herds have known for some time that optimizing reproductive efficiency is an essential issue in maintaining profitability of a dairy herd. A recent survey by the University of Wisconsin Center for Dairy Profitability asked a random sample of 2,700 dairy producers in Wisconsin to rank the importance of different dairy issues to the future profitability of their businesses. Herd health and reproduction were ranked as the top priority for future farm profitability out of a 52 possible items.

Many of the costs associated with reproduction are readily apparent from a quick glance at the balance sheet, such as semen costs, drug costs, and veterinary fees. Most dairy producers also realize that there are major opportunity costs associated with culling a high producing dairy cow because she did not become pregnant. Other opportunity costs, such as decreased milk production due to excessive days open and decreased genetic progress in replacement heifers are also major costs on a dairy operation although they are difficult to precisely quantify.

What determines reproductive efficiency on a dairy operation? There are a multitude of management, physiologic, nutritional, genetic, and disease issues that can dramatically alter reproduction. In this presentation I will focus on improving reproductive efficiency in lactating dairy cows and will primarily use data from high producing dairy cattle in the United States.

In dairy operations that use artificial insemination (AI), reproductive efficiency depends on how efficiently cows are detected in estrus and serviced (service rate) and fertility to each service (pregnancy rate per AI [PR/AI]). Calculation of these 2 key rates can help in evaluating current reproductive programs and in designing and monitoring the success of new reproductive programs. In dairy operations with high producing cows, there are a variety of management and physiological factors that are having a serious impact on these 2 rates and this will be discussed in some detail in the subsequent section. Efficient timed AI programs can increase service rate with little or no effect on pregnancy rate per AI.

In my experience, the best way to design a reproductive management program is to start with when and how the first timed AI will be performed (Section I below). The second aspect is determining what will be done before the first timed AI. This will be
discussed in the paper on identification and treatment of anovular cows and will not be
discussed in this paper. The third critical aspect is to determine how and when
pregnancy diagnosis will be performed and how cows will be treated after pregnancy
diagnosis (Section II below). I will then discuss a few general aspects that I feel are
critical to consider in implementing a timed AI program.

I. First Timed AI

For the purposes of this review I will assume that all breedings in a herd are
being done by timed AI. I know that this will not be the case in most herds but it will
make the discussion easier to follow. I will then discuss putting in effective heat
detection programs in the subsequent paper on identification and treatment of anovular
cows and in the general discussion below (Section III). Even if you do not currently
have a program that uses only timed AI, I find it easier to start with designing a program
that could use all timed AI and then adding in heat detection "bonuses" on top of this
program.

The first 2 decisions to be made are: What days in milk (DIM) will I start my
timed AI program? AND How will I monitor if I have effectively implemented the
program? In Figure 1 is shown the pattern that will be found in a herd that does a
reasonable job of heat detection without a timed AI program. It is clear that many of the
cows are being detected in heat and bred before 100 DIM. The problem is that there
are a good number of cows that are getting well past 100 DIM without being bred. In
my analysis of herds, most of the reproductive problems are going to be with the cows
that get more than 100 DIM without receiving their first AI.

Figure 2 again shows the distribution of days to first AI for a herd that has an
effective timed AI program combined with heat detection. To be effective this program
needs to be implemented at least every other week and once a week is even better.
The program that is implemented should assure that all eligible cows are bred for the
first time before 100 DIM. So in my mind the answers to the first 2 questions could be:

What DIM will I start my timed AI program? Any cows not bred by 75 DIM will
start a timed AI program. This can be implemented by generating a list of cows for
treatments on a given day of the week after this time. This will assure no cows more
than 100 DIM without AI.

How will I monitor if I have effectively implemented the program? Dairy
Comp 305 and other dairy management software programs have ways to evaluate the
distribution of DIM at first AI. The DIM at first AI is not a useful value. You need a
figure with every cow shown as an individual point to help you monitor the effectiveness
of your program.
Now let’s discuss the specifics of the Ovsynch protocol. We, in general, recommend using a 100 µg dose of GnRH at the first GnRH injection (2 cc). Most protocols currently use 7 days between the first GnRH injection and the PGF2a injection. There are some studies that suggest that 6 days would also be adequate but they have not yet been replicated sufficiently in high producing herds to assure that change from 7 days to 6 days will work well in all herds. I would currently recommend that you stay with 7 days. This is also easier since it will happen on the same day of the week. The time from PGF2a to the second GnRH injection and the time to AI has been the subject of substantial research.

In Figure 3 is shown the results from an experiment that we did that test the fertility at various times of AI during the Ovsynch protocol. As can be seen the 16 h time is slightly better than other times. Some herds have attempted to utilize these results by moving the time of the second GnRH injection to the afternoon instead of the morning and then breeding the next day. This means that the time from PGF2a to second GnRH is slightly longer than 2 days (~ 56 hours) and the time from second GnRH to AI is about 16 hours. However, many herds have had trouble with locking cows up at more than one time per day. Therefore, they have either bred at the same time as the second GnRH (48 hours after PGF2a) or the next day. Both these times give acceptable fertility. The only worry is that cows are not bred too late because fertility drops off rapidly if cows are bred at more than 24 hours after the second GnRH injection (Figure 3).

A recent study (see Jeff Stevenson, January 2004 Hoard’s Dairyman) has evaluated whether there is an even better time to do the second GnRH injection and timed AI in herds that only lock up cows once per day. In this recent study from Kansas
State University, cows received the Presynch protocol as shown in Figure 4. There were 3 treatment groups. The first group received the second GnRH and timed AI at 48 hours after PGF2α and had 23% pregnancy rate per AI (PR/AI). The second group received second GnRH at 48 hours after PGF2α and then received time AI 24 hours later and had 29% PR/AI. The third group received second GnRH and timed AI at 72 hours (3 days) after PGF2α injection and had 35% PR/AI. Thus, the optimal time of AI and second GnRH injection appears to be 3 days after the PGF2α injection if you are using a Presynch protocol. It is not clear if similar results will happen with just the Ovsynch protocol.

**Figure 3.** The experiment design and results from an experiment that evaluated the pregnancy rate per AI (PR/AI) after timed insemination at various times after the Ovsynch protocol.

**Figure 4.** Effect of time of AI and second GnRH injection after the Presynch protocol on pregnancy rate per AI (PR/AI). As can be seen Group 3 had the second GnRH and AI at 3 days after PGF2α and had the highest PR/AI of 35%.
Thus, all herds should consider using a well designed timed AI program even if effective heat detection programs are currently being utilized. This program should be implemented so that no cows are at more than 100 DIM without getting their first AI. Each herd should use a calendar and computer-generated lists to assure that all treatments are done on the correct day to assure optimal results. The implementation of this program correctly should be monitored by using a program that shows the distribution of all first breedings in a herd. It should not be assumed that all injections are given correctly but compliance (injections given to correct cow at correct time in correct way) needs to be continuously monitored in the herd to assure an effective timed AI program at first breeding.

II. Reproductive Management after First Timed AI

Obviously at the current level of fertility in high-producing dairy cows, it is essential to have an effective program to assure timely second, third and later inseminations in those cows that do not get pregnant to the first AI. However, any non-pregnant cows must be detected non-pregnant (open), resynchronized or caught in heat, and rebred. Catching these non-pregnant cows requires a good pregnancy diagnosis program. The next 2 sections outline various pregnancy diagnosis procedures and then give an example of how these can be used to improve reproduction on a commercial dairy farm.

A. Methods for Pregnancy Diagnosis

The major reason that pregnancy diagnosis allows an improvement in reproductive efficiency is that cows that did not become pregnant to the previous AI can be rebred in a more timely fashion. Therefore it should be called an open check rather than pregnancy check. It is critical that the cows that are not pregnant be detected and rebred. There are 4 general methods that I will discuss concerning pregnancy diagnosis.

1. **Heat Detection** - This is obviously the most common method and many times the most overlooked method for diagnosing pregnancy. An intensive heat detection program can be designed at 18-25 days after AI. This could be done with visual observation combined with chalking of the tail head, tail paint, K-mars, Bovine Beacons, HeatWatch, pedometers, or any other heat detection aids. Intensive heat detection should be an essential part of any heat detection program.

   One difficulty in using heat detection for pregnancy diagnosis is that about 5% of cows will show standing heat during pregnancy. Recently an analysis was performed of the Dutch Cattle Syndicate database that includes about 350,000 inseminations. This analysis showed that about 4% of calves were born after a gestation length of less than 262 days and after at least 2 inseminations (Pool and Van der Lende, unpublished
observations). It is very likely that these calvings were from a previous insemination. In addition, 0.16% were probably from 2 inseminations previous to the last one. A study by Williamson et al., 1972 showed that 7.3% of pregnant cows were detected in estrus during the first 21 days after insemination. A recent study evaluated estrus during the first 6 weeks of pregnancy and found that 3.1% of pregnant cows showed standing estrus. In addition, 10.8% of pregnant cows showed multiple secondary signs of estrus (mucous, mounting etc.). Obviously some caution should be exercised if the individual suspects that a cow may be pregnant. Nevertheless, I have seen no clear reports that accidental insemination of pregnant cows with semen that contains antibiotics will cause pregnancy loss. Thus, the first method that should be used to identify and breed non-pregnant cows is an effective heat detection program.

2. Rectal Palpation - Palpation of the uterus and ovaries through the rectum of the cow has been used for pregnancy diagnosis for many years. Generally, this can be accurately performed at 40 days or more after breeding. Some individuals can also accurately diagnose pregnancy at a slightly earlier time than 40 days.

Most studies have shown an economic benefit for reducing the time to diagnosis of pregnancy. Oltenacu et al., 1990 showed that earlier pregnancy diagnosis was always more profitable in their economic analysis. For example, pregnancy diagnosis by rectal palpation at 50 days returned $2.50 per cow; whereas, diagnosis at 65 days only returned $0.10 per cow. Earlier, accurate pregnancy diagnosis could also yield more per cow.

Thompson et al., 1995 evaluated the reproductive records of 15 U.S. dairy herds for 713 cows that did not calve within 294 days of first-service insemination. They found that if pregnancy diagnosis was done between 30 and 65 days after AI that each day later than 30 days resulted in an increase of 1.09 days in the interval until parturition. Thus, earlier pregnancy diagnosis is extremely critical for cows for reducing the days open in cows that are expected to have long calving intervals.

3. Trans-rectal Ultrasound - Many individuals now use trans-rectal ultrasound for diagnosis of pregnancy. The advantage to this technique is that it is extremely accurate and can be used at earlier time in pregnancy than rectal palpation. Generally pregnancy will be evaluated by trans-rectal ultrasound at about 30 days after breeding. It can also be accurate at slightly earlier times (25 days) but is less accurate and requires more experience when earlier times are used. For a thorough review of the use of ultrasound in bovine reproduction see the recent review (Rajamahendran et al., 1994). On difficulty that needs to be considered is that any cow that is diagnosed pregnant at about 28 days of pregnancy has about a 20% chance of losing that pregnancy. Most of the pregnancy loss will occur between 30 and 60 days after AI. Therefore, any program that uses ultrasound MUST have a second pregnancy diagnosis at a later time, possibly around 60 days after AI. If this is not implemented effectively, the use of ultrasound may actually have a detrimental effect on reproductive management because of the early pregnancy loss that will occur in some cows.
There are also devices called Doppler Ultrasound Devices that have been advertised to evaluate pregnancy. These devices detect pregnancy by measuring the quantity of blood flow in the uterine artery. They are attractive because they are much less expensive than the real-time B-mode ultrasound devices discussed above ($2,000 versus $17,000). Cameron and Malmo, 1993 compared the use of a commercial Doppler ultrasound to rectal palpation. The Doppler ultrasound was accurate in detecting a pregnancy in 93% of pregnant cows but was only 76% accurate in detecting cows that are not pregnant. It should be remembered that the main reason for pregnancy diagnosis is to accurately diagnosis open cows. The authors of this study concluded that "This level of accuracy was found to be insufficient to recommend the probe be used by farmers for the diagnosis of pregnancy"

4. Chemical Assays for Pregnancy. The most common chemical assays for pregnancy are the milk progesterone assays. These assays are not widely used at this time but are available commercially from a number of different distributors. It is important to realize that these tests are only accurate in the designation of some of the non-pregnant cows. Some non-pregnant cows will still have high progesterone concentrations due to the continued presence of a corpus luteum. A number of studies have evaluated different days post-AI to use the assays but the best day for performance of the milk progesterone assay is still controversial. In one large study (Pennington et al., 1985) it was found that on day 21 after breeding the milk progesterone assay was 97.2% accurate (383/394) in correctly identifying cows as non-pregnant (low progesterone concentration) and was 77.4% accurate (411/531) in correctly identifying cows as pregnant (high progesterone concentration). Thus, a low progesterone concentration was definitely indicative of a non-pregnant cow; however, a high progesterone concentration was only about 80% correct in designating pregnant cows.

Another chemical assays for pregnancy has recently become available commercially (pregnancy-specific protein-B). This assay appears to be accurate in diagnosing pregnancy if used correctly. The practical implementation of this assay into a reproductive management program for dairy cattle will require further research.

The commercially available test for early pregnancy factor does not appear to be accurate in scientific studies and therefore should not be utilized at this time. In humans an accurate pregnancy test has been developed that recognizes the presence of human chorionic gonadotropin. This protein is not secreted during pregnancy in cows and cannot be used for diagnosing pregnancy.

B. An Example of Using Pregnancy Diagnosis to Improve Service Rate

A dairy herd with all cows in a free stall barn was putting substantial labor into heat detection every day and still days open was over 178 days. The overall service rate was about 45% and it was similar for service rate to first AI or later AIs. This herd made the decision to implement the Ovsynch program for first AI. All cows at 75 days
or more in milk were synchronized with Ovsynch and received timed AI after the second GnRH injection. They decided to synchronize the cows every week. Any cows detected in estrus after the voluntary waiting period were bred. Therefore, all cows were bred for the first time between 60 and 92 days in milk. This increased the service rate at first AI to greater than 100%.

There was still a problem with getting cows bred for a second time if they did not become pregnant to the first AI. The producer decided to use 4 procedures to improve diagnosis and rebreeding of open cows. First, daily tail chalk would be used for pregnancy diagnosis. Second, the AI technician that was normally breeding cows on this farm was hired to be in charge of all tail chalking, hormonal injections, and AI. This person also handled the reproductive records. This allowed people that were devoting labor to daily heat detection to spend time on other jobs. Third, pregnancy diagnosis was moved to once per week, on Tuesday morning using rectal palpation instead of longer and less consistent intervals that had occurred prior to this time. Fourth, a resynch program was put in place with the first injection of GnRH given to all cows that are one week before the next pregnancy diagnosis and greater than 30 days since last AI.

These methods will increase some costs on the farm. There are increased costs for the Ovsynch and Resynch programs. There are also costs for the daily tail chalk and hormonal injections done by the AI technician, for veterinary visits, and for increased AI technician fees (more AIs). This procedure produced a very consistent program for dealing with reproduction on the dairy and reduced days open (from 185 to 161 days). Only one person was involved with the reproduction and there was an improvement in the tight labor situation on the farm. In farms that have used this type of program the service rate has increased, the number of pregnancies per year has increased, and the consistency of the reproductive program from month to month has greatly improved.

Figure 5 shows the results from a recent study that evaluated the optimal time to start the Ovsynch protocol during a Resynch program (Fricke et al.; Journal of Dairy Science 86:3941; December 2003). The first GnRH was given at Day 19, 26 or 33 after the first timed AI. It appears that the timing of this GnRH injection does not have an effect (either positive or negative) on the % pregnant to the first timed AI. However, giving the GnRH injection on Day 33 appears to result in slightly better fertility than giving the injection at either Day 19 (23%) or Day 26 (34%). On thing that should be noted is that the pregnancy diagnosis for the Day 33 group was done at 34 days after AI as compared to 27 days after AI for the other 2 groups. There is 5-8% pregnancy loss during this critical 1 week time period and so the difference in pregnancy rate between the group would be expected to increase if the same time of pregnancy diagnosis were utilized.

Figure 6 shows a calendar that shows how these programs can be implemented in a herd. Using this program no cows would go more than 42 days between each AI.
Some cows would obviously be bred more frequently based on effectiveness of estrus detection program.

<table>
<thead>
<tr>
<th>Time of GnRH After 1st AI n</th>
<th>% Pregnant During Resynch Procedure n</th>
<th>% Pregnant Preg</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 19</td>
<td>235</td>
<td>33% 120 23%a 27d</td>
<td></td>
</tr>
<tr>
<td>Day 26</td>
<td>240</td>
<td>30% 121 34%b 27d</td>
<td></td>
</tr>
<tr>
<td>Day 33</td>
<td>236</td>
<td>29% 143 38%b 34d</td>
<td></td>
</tr>
</tbody>
</table>

a, b -Different (P<0.05) in column

**Figure 5.** Effects of different times of GnRH in a Resynch program (data from Fricke et al.; Journal of Dairy Science 86:3941; December 2003).

**Resynch Schedule**

<table>
<thead>
<tr>
<th>SUN</th>
<th>MON</th>
<th>TUE</th>
<th>WED</th>
<th>THU</th>
<th>FRI</th>
<th>SAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>GnRH</td>
<td>PGF</td>
<td>GnRH+AI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7d</td>
<td>14d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Estrus Detection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28d</td>
<td>35d</td>
<td>42days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGF</td>
<td>GnRH+AI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If not pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 6.** A calendar showing how an Ovsynch and Resynch program can be implemented to assure that no cows go more than 42 days between each AI.