WHAT IS NEW IN ESTROUS CYCLE SYNCHRONIZATION OF DAIRY HEIFERS?
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INTRODUCTION

Importance

Improvements in reproductive efficiency of dairy heifers lead to more optimal age at first calving, reduced rearing costs, and enhanced productive life (Gabler et al., 2000; Ettema and Santos, 2004). Most dairy operations in the U.S. use AI after observed estrus to manage reproduction in heifers (NAHMS, 2009). Nonetheless, advances in estrus-synchronization protocols have demonstrated that timed AI can be used as an alternative method to improve reproductive efficiency and profitability when the efficiency to detect estrus is not adequate (Ribeiro et al., 2012). Recent studies consistently have reported pregnancy per AI (P/AI) ranging from 53 to 60% in dairy heifers subjected to the 5-d timed AI program (Rabaglino et al., 2010a; Lima et al., 2013), which are comparable to those observed in heifers inseminated after detection of spontaneous estrus (Kuhn et al., 2006).

Recent Historical Perspective for Timed AI in Dairy Heifers in the US

Timed AI programs had a different history for dairy heifers than for lactating dairy cows in the US, with very low adoption rate at its outset. The first series of research projects that exploited the use of timed AI in dairy heifers failed to obtain acceptable P/AI. In fact, the P/AI in these studies ranged from 25.8 to 45.5%, which was consistently inferior to results obtained for AI after detected estrus (Schmitt et al., 1996; Pursley et al., 1997; Stevenson et al., 2000; Rivera et al., 2004; Rivera et al., 2005; Rivera et al; 2006). The first studies investigating timed AI for dairy heifers used the Ovsynch program or similar protocols that consisted of an interval of 7 d between the initial GnRH and PGF2α injections (Schmitt et al., 1996: Pursley et al., 1997; Stevenson et al., 2000) and reduced ovulation to the initial GnRH injection (Lima et al. 2011), which altogether compromises synchrony of follicle development and fertility in a 7-d program (Pursley et al., 1997). Approximately 50% of the dairy heifers have 2-wave and 50% have 3-wave cycles (Table 1). Therefore, when ovulation does not occur in response to the initial GnRH injection, it is possible that either a persistent follicle or lack of synchrony occurs when ovulation is induced at timed AI.

Subsequent studies reduced the interval between the initial GnRH injection and PGF2α to 6 d (Rivera., et al., 2004) and added a CIDR insert between the 2 hormonal injections to avoid occurrence of premature ovulation (Rivera et al., 2005). Pregnancy outcomes of these studies, however, were not comparable with AI performed after detected estrus. Improvements were obtained when the estrous cycle was presynchronized with a GnRH injection 7 d before the initiation of the 6-d program (Rivera et al. 2006).

The first stride toward the development of a timed AI program without presynchronization that resulted in acceptable fertility occurred when Rabaglino et al. (2010a) reduced the interval between GnRH and induced luteolysis to 5 d and included a controlled internal drug release (CIDR) progesterone insert between the initial GnRH and PGF2α injections. Induction of ovulation and timed AI occurred on d 8, 72 h after induced luteolysis. Those studies resulted in P/AI ranging from 53.1% to 59.5%, comparable with inseminations performed after detected estrus (56.3%; Kuhn et al., 2006). These studies
paved the way for the development of effective
timed AI programs for dairy heifers.

**SYNCHRONIZATION PROGRAMS**

Recently, several experiments were con-
ducted to improve or simplify the 5-d timed AI
program. A study investigated the effects of the
addition of a second PGF$_{2\alpha}$ 12 h after the first
treatment in the 5-d timed AI program for dairy
heifers detected no improvement in P/AI and
luteolysis (Rabaglino et al., 2010b). An interest-
ing finding from this study, however, was that
only 23% of the heifers had more than one cor-
pus luteum (CL) 5 d after the injection of GnRH
suggesting that the ovulatory response to the
initial GnRH injection was probably small and
perhaps not critical to improve fertility (Raba-
glino et al., 2010b). In addition, it is possible
that the 12-h interval between PGF$_{2\alpha}$ might have
not been sufficient to further enhance luteolysis
and P/AI.

Based on the potential poor ovulatory re-
sponse to the initial GnRH injection of 5-d timed
AI program and in effort to simplify the pro-
gram, Lima et al. (2011) designed a study to in-
vestigate the effect of omitting that injection on
ovarian responses and P/AI in dairy heifer. The
results of that study revealed no differences in
P/AI (No GnRH = 52.1% vs. GnRH = 54.5%); how-
ever, ovulation at onset of the synchroniza-
tion protocol and the proportion of heifers with
progesterone concentrations above 0.5 ng/mL at
AI was increased for heifers receiving GnRH
(Lima et al., 2011). These differences indicated
the possible benefits associated with follicle
turnover might have been offset by a less effec-
tive CL demise in heifers because of increased
presence of new CL (fewer than 5 d of age) and
its failure to lyse after PGF$_{2\alpha}$ treatment (Miy-
imoto et al., 2009). A follow-up study exploited
the hypothesis that a combination of GnRH at
the initiation of the 5-d timed AI and injections
of PGF$_{2\alpha}$ on d 5 and 6 of the protocol, could im-
prove synchrony of ovulation and fertility in
dairy heifers (Lima et al., 2013). Indeed, the
combination of GnRH with 2 injections of
PGF$_{2\alpha}$ increased the ovulatory response to the
initial GnRH injection (26.3% vs. 18.8%), the
proportion of heifers with progesterone concen-
trations < 0.5 ng/mL at AI (87.1% vs. 83.0), and

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the estrous cycle of heifers$^1$</th>
<th>Follicle wave</th>
<th>Emergence of 2$^{nd}$ wave</th>
<th>Estrous cycle length</th>
<th>Progesterone peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>2-wave (%)</td>
<td>2-wave Day of the cycle</td>
<td>3-wave Day of the cycle</td>
<td>ng/mL</td>
</tr>
<tr>
<td>Beef heifers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Savio et al. (1988)</td>
<td>16 (4)</td>
<td>10</td>
<td>20.5 ± 1.3</td>
<td>21.3 ± 1.5</td>
</tr>
<tr>
<td>Bong et al. (1993)</td>
<td>25 (3)</td>
<td>N/A</td>
<td>20.8 ± 0.9</td>
<td>21.3 ± 0.5</td>
</tr>
<tr>
<td>Dairy heifers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sirosi and Fortune (1988)</td>
<td>20 (2)</td>
<td>11</td>
<td>9.4 ± 0.5</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>Ginther et al. (1989)</td>
<td>82 (18)</td>
<td>9.6 ± 0.2</td>
<td>20.4 ± 0.3</td>
<td>22.8 ± 0.6</td>
</tr>
<tr>
<td>Knopt et al. (1989)</td>
<td>90 (9)</td>
<td>10 ± 0.4</td>
<td>21</td>
<td>23</td>
</tr>
<tr>
<td>Ko et al. (1991)</td>
<td>75 (9)</td>
<td>10 ± 0.4</td>
<td>19.5</td>
<td>23</td>
</tr>
<tr>
<td>Kulick et al. (2001)</td>
<td>57 (13)</td>
<td>10 ± 0.4</td>
<td>22 ± 0.2</td>
<td>7.3 ± 0.4</td>
</tr>
<tr>
<td>Sartori et al. (2004)</td>
<td>56 (15)</td>
<td>9.7 ± 0.2</td>
<td>20.7 ± 0.3</td>
<td>23.1 ± 0.7</td>
</tr>
<tr>
<td>Wolfenson et al. (2004)</td>
<td>70 (14)</td>
<td>9.0 ± 0.0</td>
<td>20.4 ± 0.3</td>
<td>22.8 ± 0.6</td>
</tr>
<tr>
<td>Overall</td>
<td>54 (87)</td>
<td>9.3 ± 0.3</td>
<td>20.6 ± 0.3</td>
<td>22.2 ± 0.3</td>
</tr>
</tbody>
</table>

$^1$ Lima (2013).
N/A = not applicable.
P/AI (61.7% vs. 52.9%), therefore, improving fertility in dairy heifers subjected to timed AI.

Another key physiological aspect explored in 5-d timed AI for dairy heifers was the effect of timing of the final GnRH to induce ovulation relative to AI (Lima et al., 2011). A study compared the effects of administering the second GnRH injection at 56 h after a single PGF$_{2\alpha}$ injection with administering the second GnRH concurrent with timed AI at 72 h after a single PGF$_{2\alpha}$ injection. This study revealed that heifers receiving GnRH at 72 h that did not display signs of estrus had increased P/AI (61.4% vs. 47.5%). Moreover, when heifers received the GnRH 72 h after PGF$_{2\alpha}$, more than 61% were in estrus at the time of AI. A spontaneous LH surge had probably already occurred in estrual heifers receiving GnRH concurrent with AI, 72 h after PGF$_{2\alpha}$. The fact that delaying the administration of GnRH to the time of AI improved P/AI of heifers not in estrus indicates that a longer proestrus was beneficial to their fertility (Lima et al., 2010).

Duration of proestrus and use of 5-d program to improve synchrony in dairy heifers also was reported in recent study that compared a modified 5-d timed AI program (the second GnRH injection and insemination performed concurrently at 53 to 60 h after the PGF$_{2\alpha}$ injection) with a 7-d timed AI program with timed AI occurring 53 to 60 h after PGF$_{2\alpha}$ (Lopes Jr. et al., 2013). The P/AI for the modified 5-d timed AI with a shorter proestrus was only 44.8%, but was still greater than the P/AI for the 7-d timed AI program (35.7%). These results indicate the shorter proestrus might be detrimental to P/AI and use of a 7-d program may have more detrimental effects on fertility of dairy heifers.

A program used for beef cattle in the recent year named “Show-Me-Synch” was used in dairy heifers leading to high pregnancy outcomes (Mallory et al., 2013). These programs consisted of a CIDR inserted from d 0 to 14 followed by an injection of PGF$_{2\alpha}$ 16 d after CIDR insert removal (d 30) and an injection of GnRH concurrent with timed AI at 66 h after the PGF$_{2\alpha}$ injection (d 32.5). When conventional semen was used, the P/AI for the “Show-Me-Synch” dairy heifers was 68.0%.

Another recent study investigated the effects of progesterone concentration on LH release and ovulation in response to GnRH in dairy heifers (Lima et al., 2013). The results revealed that heifers with high concentrations of progesterone at GnRH injection had reduced LH release and a lesser ovulatory response to GnRH (19.0 vs. 48.4 %) compared with heifers having low progesterone at GnRH injection. These results indicate that removal of endogenous source of progesterone at the initiation of the 5-d timed might lead to greater ovulatory response to the initial GnRH and potentially greater P/AI in dairy heifers.

**ECONOMIC COMPARISONS OF REPRODUCTIVE PROGRAMS USING TIMED AI AND DETECTION OF ESTRUS**

An economic analysis of reproductive programs for dairy heifers with timed AI and detection of estrus was performed using a simulation to calculate P/AI, average time to pregnancy, total costs per AI, and cost per pregnancy for 4 reproductive management strategies. These strategies were used during a breeding period of 84 d allowing for approximately 4 estrous cycles (Ribeiro et al., 2012). The 4 breeding programs used were: (1) 100% timed AI; (2) 100% detection of estrus; (3) timed AI for first AI and detection of estrus for the remaining AI; and (4) timed AI for first AI followed by insemination upon detected estrus or resynchronized AI after a nonpregnant diagnosis. Pregnancy per AI for those inseminated in estrus was assumed to be 60% for the first AI and 54% for the remaining inseminations (Norman et al., 2010). For timed AI, nonpregnant heifers were reinseminated every 40 d for up to 3 AI, and P/AI were 59, 55, and 51% for the first, second and third breedings, respectively (Lima et al., 2011, 2013). For detection of estrus, costs for labor were calculated for daily tail chalking and observation of heifers. Costs per pregnancy included costs incurred to implement the breeding program and feed costs associated with the interval from beginning of breeding to pregnancy. It was assumed that each extra day to pregnancy after 400 d of age would
increase feed costs by $2, which represented the additional feed cost if calving was delayed because of pregnancy after 400 d of age. Therefore, nonpregnant heifers at the end of the 84-d simulation period had an additional cost of $168 for feed. Sensitivity analyses were performed for 4 estrus-detection rates of 50, 60, 70, and 80%.

The results of the simulation revealed that incorporation of timed AI for first service reduced the cost per pregnancy compared with detection of estrus, although the benefits were less as estrus-detection rates increased (Table 2). Likewise, programs with 100% timed AI were less expensive than programs with exclusive use of insemination at detected estrus if detection of estrus was < 70%. When additional timed AI was incorporated into the breeding program to resynchronize nonpregnant heifers that had not been detected in estrus, it further benefited programs with marginal estrus-detection rates of 50 and 60%. For better estrus-detection rates of 70 and 80%, the benefits were minor or nonexistent. Incorporation of detection of estrus after 1 timed AI was superior to timed AI alone only when estrus-detection rate was 60% or more. Most of the changes in costs per pregnancy resulted from feed costs associated with heifers becoming pregnant later in the breeding period.

In summary, combination of timed AI program for first insemination with detection of estrus and additional timed AI for nonpregnant heifers reduced the cost a reproductive program. In contrast, when estrus-detection rates were ≥ 70%, benefits of using timed AI were negligible (Ribeiro et al., 2012).

MANAGEMENT CONSIDERATIONS FOR IMPLEMENTING TIMED AI IN DAIRY HEIFIERS

The acceptable P/AI achieved for programs such as the 5-d timed AI, which consists of an initial GnRH injection and a CIDR insert on d 0 for 5 d, PGF$_{2\alpha}$ on d 5 and 6, and timed AI on d 8 concurrent with the final GnRH injection, are a feasible option for inseminating dairy heifers to ensure that AI takes place at a pre-determined day or age of the heifer. Simulations have shown that incorporation of timed AI in breeding programs is economically advantageous when compared with AI based on detected estrus when the latter is < 70%. In fact, when detection of estrus in heifers is < 70%, timed AI can be economically justifiable as the sole breeding method or as part of the breeding

eliminating the hassles with daily heat detection. In addition, timed AI can facilitate creating homogeneous groups of pregnant heifers that can be housed and moved together according to farm needs. Although implementation of timed AI programs seems a simple task, some managerial aspects must be considered before the implementation of successful program can occur. First, each farm must to evaluate if personnel have the ability to manage the program and inseminate larger groups of heifers at one time without compromising fertility. For large dairy farms, it is important to consider if sufficient numbers of personnel exist to handle the heifer AI program and physical room in the maternity pen to handle a relatively large group of heifers calving in short period of time. As for any timed AI program applied to lactating dairy cows, a successful program compliant-dependent. Appropriate handling of all lists, injections, and CIDR insertions and removals is critical to separate a program that works from those programs that fail to produce satisfactory results. The combination of timed AI and detection of estrus produced the smallest cost per pregnancy, but the benefits are less attractive when estrus-detection efficiency is 70% or greater. For farms with estrus-detection rates of 70% or greater, use of timed AI is less justifiable and an option only to facilitate management when the tasks of daily detection of estrus and breeding become time-limiting and otherwise difficult.

CONCLUSIONS

The take home message is that programs to synchronize estrus, ovulation, or both, yielding P/AI of 55 to 60% such as the 5-d timed AI, which consists of an initial GnRH injection and a CIDR insert on d 0 for 5 d, PGF$_{2\alpha}$ on d 5 and 6, and timed AI on d 8 concurrent with the final GnRH injection, are a feasible option for inseminating dairy heifers to ensure that AI takes place at a pre-determined day or age of the heifer. Simulations have shown that incorporation of timed AI in breeding programs is economically advantageous when compared with AI based on detected estrus when the latter is < 70%. In fact, when detection of estrus in heifers is < 70%, timed AI can be economically justifiable as the sole breeding method or as part of the breeding
Table 2. Reproductive efficiency and costs of 4 breeding programs for dairy heifers according to estrus-detection rate

<table>
<thead>
<tr>
<th>Estrous-detection rate, %</th>
<th>Breeding program(^1)</th>
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<tbody>
<tr>
<td></td>
<td>TAI only</td>
</tr>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Pregnant, %</td>
<td></td>
</tr>
<tr>
<td>Day 20</td>
<td>59.0</td>
</tr>
<tr>
<td>Day 40</td>
<td>81.6</td>
</tr>
<tr>
<td>Day 84</td>
<td>91.0</td>
</tr>
<tr>
<td>Age at pregnancy, d</td>
<td>418.2</td>
</tr>
<tr>
<td>Labor days</td>
<td>12</td>
</tr>
<tr>
<td>Labor hours per AI</td>
<td>0.18</td>
</tr>
<tr>
<td>Cost per AI, US $</td>
<td>20.8</td>
</tr>
<tr>
<td>AI per pregnancy, no.</td>
<td>1.75</td>
</tr>
<tr>
<td>Feed cost per pregnancy, US $</td>
<td>53.1</td>
</tr>
<tr>
<td>Total cost per pregnancy, US $</td>
<td>89.5</td>
</tr>
</tbody>
</table>

\(^1\)TAI = timed artificial insemination; TAI + DE = timed artificial insemination for first AI followed by detection of estrus for remaining AI; TAI + DE between TAI = timed AI for first AI followed by detection of estrus and resynchronization of nonpregnant heifers for timed AI after a nonpregnant diagnosis.
program concurrent with AI at estrus. For dairy farms with excellent estrus-detection rates, timed AI can still be incorporated as an option to facilitate management if daily detection of estrus is time-limiting.

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