ABSTRACT

Nonreturn rates to professional technician service of 7240 first AI Holstein cows were calculated to evaluate differences between once daily and a.m.-p.m. AI. To determine whether management practices affected nonreturn rates, participating herd owners were surveyed for methods used for detection of estrus. Nonreturn rates for once daily and a.m.-p.m. AI were 64.6 and 65.6% for 60-d, 60.1 and 60.6% for 75-d, and 58.4 and 57.8% for 90-d nonreturn periods. Signs of estrus for AI and interval from detection of estrus to AI were related to nonreturn rates. Nonreturn rate was highest, 63.4%, when cows were in standing estrus. Nonreturn rates were lowest, 36%, when cows were bred after treatment with PGF2α without being detected in estrus or bred strictly on veterinary advice based on palpation. Nonreturn rates were similar for different times of the day when once daily AI was practiced. However, AI in the midmorning may have some advantages. The highest nonreturn rate for a 3-h period was 68.2% for 0800 and 1100 h; the lowest was 54.7% for 1300 to 1600 h. Movement before observation for estrus and an observation period >15 min improved nonreturn rates for once daily AI. Once daily AI can be used effectively with no difference from the traditional a.m.-p.m. system; results are best when AI is based on standing estrus and performed between 0800 and 1100 h.

(Key words: artificial insemination, nonreturn rates, reproduction, management)

INTRODUCTION

Timing of AI relative to the stage of estrus has been under investigation for approximately 50 yr (19). Numerous studies have confirmed the conclusions of Trimberger (17) and Trimberger and Davis (19) that conception rates are maximal when cows are submitted for AI from midestrus to the end of standing estrus (7, 9, 10, 18). This early work led to the a.m.-p.m. management guideline that permits the AI of most cows near their optimal time for fertilization. This guideline for AI states that cows in estrus during the a.m. should be submitted for AI that p.m. and that cows in estrus during the p.m. should be submitted for AI the next a.m.

Fertility did not differ between single and multiple AI during estrus (6, 19, 21). Using once daily AI schedules, others (3, 15, 16) reported higher conception rates when AI was performed before 1200 h. Paternity of calves was determined to assess timing of AI (1). Semen from three sires, of three breeds, was rotated at first observation of estrus and 12 and 24 h later (1). Three AI during estrus did not result in an exceptionally high conception rate, 70.1%, and time of AI with respect to ovulation was not important to conception. The AI of cows and heifers immediately following detection of estrus (a.m. or p.m.) or 12 h later did not adversely affect conception rates (6, 16, 20). However, a wide variety of conditions, such as single AI of beef cattle (13), dairy heifers synchronized with PGF2α (4), and the use of unfrozen semen in dairy cattle (3, 9, 10), resulted in near maximal conception rates.
High conception rates are dependent on correctly identified estrus in cows and heifers. Liberal interpretation of indicators of estrus reduces conception rates (12, 15). Major factors affecting intensity or expression of estrus include group size, number of females in estrus simultaneously, location (dirt vs. concrete flooring), housing facilities, environmental temperatures, proper training of observers, and frequency of observation periods (2, 5, 8, 11).

The main objective of this study was to establish nonreturn rates in a large sample of dairy cows submitted for AI once daily and following the a.m.-p.m. guideline with frozen and thawed semen. Additionally, because individual dairy producers using AI must interpret signs or symptoms of estrus before submitting cows for AI, a survey of methods and signs used in detection of estrus was conducted for herds participating in this field trial.

MATERIALS AND METHODS

This study involved 7240 first AI of Holstein cows from Pennsylvania dairy herds (n = 166) using Atlantic Breeders Cooperative professional technician service exclusively during 6 mo. Technicians within a service unit (<3 counties) were equally assigned to a particular AI program (once daily or a.m.-p.m.) for an initial 3-mo period and switched to the other program for the remaining 3 mo. This crossover design was utilized to minimize effects of herd and season on conception rates. Once daily AI was defined as a fixed 3-h period for AI. In the a.m.-p.m. system, cows first observed in estrus during the a.m. were submitted for AI that p.m., and cows first observed in estrus during the p.m. were submitted for AI the following a.m. A survey of management procedures used by each dairy producer for detection of estrus was conducted at initiation of the field trial (Figure 1). This survey contained questions concerning frequency and duration of observation periods for detection of estrus, interval between and location of observations, and whether devices and aids were used to assist in detection of estrus. Herd size and the 3-h period chosen for once daily AI were also recorded.

The time of day that cows were initially observed in estrus and the hour of AI were recorded for each AI. A maximum of three reasons for submitting a cow for AI could be recorded. Cows were submitted for AI based on the following indicators of estrus: 1) standing estrus, 2) mounting activity, 3) clear mucus discharge from reproductive tract, 4) fully red or missing Kamar® (Steamboat Springs, CO), 5) partially red Kamar®, 6) PGF2α administration (72 to 80 h prior) without observed standing estrus, 7) nervousness and excessive vocalization, 8) redness and edema of external genitalia or rubbed tailhead, 9) veterinary recommendation based on ovarian palpation per rectum, and 10) other subtle symptoms or aids used for detection of estrus. Fertility was estimated from 60- to 90-d percentage of nonreturn to first AI. An indicator variable was constructed for each nonreturn interval. When a cow did not return for AI within the 60-, 75-, or 90-d interval, the variable was assigned a value of 1. A value of 0 was assigned when the cow was submitted for AI within the particular AI interval.

Independence of AI program and nonreturn interval was tested using nonreturn percentages generated from the indicator variable and a chi-square statistic. The 75-d nonreturn interval was analyzed by ANOVA (14) in a model containing herd, sire, AI program, indicators of estrus, intervals to AI, and interactions with AI program. Effects were tested by their interactions with herd. Significant effects were further compared with Tukey’s pairwise contrasts. Probabilities of nonreturn were estimated simultaneously for indicators of estrus and intervals from estrus to AI using a stepwise logistic regression. Herd differences in 75-d nonreturn between AI programs were analyzed by general linear models relative to management procedures acquired by survey (14).

RESULTS AND DISCUSSION

Nonreturn rates for once daily and a.m.-p.m. AI programs are summarized in Table 1. Of most importance is the lack of difference (P > .05) in nonreturn rates for once daily and a.m.-p.m. AI programs at all intervals of return to estrus measured (60 to 90 d). This finding agrees with results of Gwazdauskas et al. (5), who, using one university experimental dairy herd, reported that conception rates did not differ for cows and heifers submitted to AI shortly after detection of estrus or 12 h later.
Results were also similar for dairy heifers synchronized with PGF$_2\alpha$ and submitted for AI following the a.m.-p.m. guideline or a.m. only (4, 6). Foote (3) reported that, with unfrozen semen, the best time for AI of dairy cattle is midmorning. Figure 2 shows 75-d nonreturn rates for once daily AI by 3-h period of AI (P > .05). Timing of once daily AI did not affect nonreturn rates when management practices associated with detection of estrus were con-

1. Does this farm have a routine estrus detection program?
   Yes  No
2. If a program exists, do observations occur at a prescribed time?
   Yes  No
3. If there is no program, under what conditions does estrus detection occur?
   Use of aids or devices  Casual observation
   Observed in holding area  Other
4. How many times per day are cows observed for estrus?
   Once  Twice  More than twice
5. What is the time interval between observation periods?
   12 h  <12 h  >12 h
6. How long are estrus observation periods?
   <15 min 15 min  >15 min
7. Are cows moved just prior to estrus observation period?
   Yes  No
8. Where are cows located when observed for estrus?
   Free stalls  Dirt lots  Holding pens
   Pastures  Milking parlor  Feed lots
   Other
9. Has the person who is observing cows for estrus been trained to observe secondary signs of estrus?
   Yes  No
10. Which of the following estrus detection aids or devices are used?
    — Calendar to record and anticipate day of estrus
    — Kamar® (Steamboat Springs, CO) heat mount device
    — Chalk, crayon, or paint smeared on tail head
    — Teaser animal, such as hormonally treated female, cystic cow, or surgically altered bull
    — Milk progesterone test to confirm estrus
    — Other
11. Circle the 3-h time period chosen for once daily AI.
    a.m. 0600 to 0900 0700 to 1000 0800 to 1100
         0900 to 1200 1000 to 1300 1100 to 1400
         1200 to 1500
    p.m. 1300 to 1600 1400 to 1700 1500 to 1800
12. What is the number of cows in the milking herd?

Figure 1. Questionnaire of management practices associated with a program for estrus detection.
TABLE 1. Nonreturn rates for once daily and a.m.-p.m. AI.

<table>
<thead>
<tr>
<th>AI Program</th>
<th>Cows (no.)</th>
<th>60 d (%)</th>
<th>75 d (%)</th>
<th>90 d (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once daily</td>
<td>3659</td>
<td>64.6</td>
<td>60.1</td>
<td>58.4</td>
</tr>
<tr>
<td>a.m.-p.m.</td>
<td>3581</td>
<td>65.6</td>
<td>60.6</td>
<td>57.8</td>
</tr>
</tbody>
</table>

Sedation and analyzed across all 3-h periods. However, nonreturn rates tended to be higher before 1200 h; actual nonreturn rates were highest for cows bred between 0800 and 1100 h or midnight.

When the herd and the service sire were held constant, indicators of estrus and interval from detection of estrus to AI affected 75-d nonreturn rates (Table 2). The AI program had no effect on 75-d nonreturn rates. Although several of the interactions with herd (error terms) were significant, indicating variation among herds, main effects tested by them were still significant.

Cows submitted for AI based on standing estrus or secondary indicators of estrus had similar nonreturn rates (63.4 vs. 59.5%). Stevenson et al. (15) observed that cows detected for AI because of a fully triggered Kamar@ device had lower nonreturn rates than did cows detected standing or by secondary indicators. However, a fully triggered Kamar® device was a better indicator of estrus than veterinary recommendation based on presence of ovarian structures or the AI of cows given PGF2α without observation of standing estrus (P < .01). This finding was not surprising, because Reimers et al. (12) had similar conception rates. Hurnik et al. (8) reported that 79% of all mounting cows and 90% of cows exhibiting standing estrus were in true estrus. Cows presented for AI because of a fully triggered Kamar® device had lower nonreturn rates than did cows detected standing or by secondary indicators. However, a fully triggered Kamar® device was a better indicator of estrus than veterinary recommendation based on presence of ovarian structures or the AI of cows given PGF2α without observation of standing estrus (P < .01). This finding was not surprising, because Reimers et al. (12)

TABLE 3. Influence of indicator of estrus used for submission to AI on 75-d nonreturn rate.

<table>
<thead>
<tr>
<th>Indicator of estrus</th>
<th>AI (no.)</th>
<th>75-d Nonreturn rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing estrus</td>
<td>4622</td>
<td>63.4*</td>
</tr>
<tr>
<td>Secondary indicators²</td>
<td>1057</td>
<td>59.5*</td>
</tr>
<tr>
<td>Positive Kamar® device</td>
<td>575</td>
<td>50.8*</td>
</tr>
<tr>
<td>PGF2α without observed standing estrus</td>
<td>126</td>
<td>35.8*</td>
</tr>
<tr>
<td>Veterinary recommendation³</td>
<td>73</td>
<td>35.6*</td>
</tr>
</tbody>
</table>

*a,b,cMeans with different superscripts differ (P < .01).

1Total of 746 AI with no estrus sign recorded.

2Mounting activity, clear mucus discharge, nervousness and excessive vocalization, redness and edema of genitalia or tail head, and partially red Kamar® (Steamboat Springs, CO).

3Based on ovarian palpation per rectum.

TABLE 2. Analysis of variance for 75-d nonreturn rates.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd</td>
<td>165</td>
<td>.84**</td>
</tr>
<tr>
<td>Service sire</td>
<td>243</td>
<td>.39**</td>
</tr>
<tr>
<td>AI Program¹</td>
<td>1</td>
<td>.07</td>
</tr>
<tr>
<td>Indicator²</td>
<td>5</td>
<td>9.11**</td>
</tr>
<tr>
<td>Interval³</td>
<td>5</td>
<td>1.15**</td>
</tr>
<tr>
<td>AI Program x indicator</td>
<td>5</td>
<td>.15</td>
</tr>
<tr>
<td>AI Program x interval</td>
<td>5</td>
<td>.27</td>
</tr>
<tr>
<td>Herd x AI program</td>
<td>164</td>
<td>.33**</td>
</tr>
<tr>
<td>Herd x indicator</td>
<td>437</td>
<td>.19</td>
</tr>
<tr>
<td>Herd x interval</td>
<td>618</td>
<td>.23*</td>
</tr>
<tr>
<td>Herd x AI program x indicator</td>
<td>229</td>
<td>.25*</td>
</tr>
<tr>
<td>Herd x AI program x interval</td>
<td>334</td>
<td>.26*</td>
</tr>
<tr>
<td>Residual</td>
<td>4977</td>
<td>20</td>
</tr>
</tbody>
</table>

1Once daily and a.m.-p.m. AI.
²Interval from detection of estrus to AI.
³Indicators of estrus.

*P < .05.
**P < .001.

TABLE 4. Influence of interval from detection of estrus to AI on 75-d nonreturn rate.

<table>
<thead>
<tr>
<th>Interval from detection of estrus to AI (no.)</th>
<th>75-d Nonreturn rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 6</td>
<td>1126</td>
</tr>
<tr>
<td>6 to 12</td>
<td>2352</td>
</tr>
<tr>
<td>12 to 18</td>
<td>2455</td>
</tr>
<tr>
<td>18 to 24</td>
<td>962</td>
</tr>
<tr>
<td>24 to 30</td>
<td>99</td>
</tr>
</tbody>
</table>

*a,b,cMeans with different superscripts differ (P < .01).

¹Total of 205 AI with no detection or AI time recorded.
observed that 10.6% of the cows with fully triggered Kamar® devices presented for AI were not in estrus, as determined by high progesterone concentrations in milk, and a 7.1% lower conception rate resulted from the AI of these cows.

Cows that were submitted for AI at 80 h following PGF2α administration had a nonreturn rate of 35.8%. Similar results were reported by Gwazdauskas et al. (6) for cows that were submitted for AI after PGF2α administration without observed estrus. Gwazdauskas et al. (6) reported that cows treated with PGF2α and detected in estrus achieved a conception rate of 49.5 versus 20.7% for cows that were not observed in estrus. Cows submitted for AI as recommended by veterinary palpation of ovarian structures resulted in a 35.6% nonreturn rate (Table 3). Although nonreturn rates (P < .01) were higher for cows observed in standing estrus or displaying secondary signs than for cows identified as having a positive Kamar® device or after veterinary recommendation or 80 h after PGF2α administration without estrus, approximately one of three incidences of cows submitted for AI resulted in nonreturn to AI in the latter groups. Stevenson et al. (15) suggested that liberal interpretations of indicators of estrus would increase pregnancy rates even with lower conception rates because of the low submission rates or efficiency of detection of estrus in most US dairy herds.

Nonreturn rates based on when cows were first observed in estrus ranged from 49.6 to 60.7% (Table 4). Intervals extending beyond 12 h from detection of estrus to AI reduced nonreturn rates (P < .01). Similar results have been reported to others (6, 9), who suggested that reduced fertility with AI 12 to 24 h after the end of estrus is probably related to lower viability of the ovum when sperm reaches the
oviducts, a result of the time required for sustained phase of sperm transport to site of fertilization.

A stepwise logistic regression on 75-d nonreturn rates from both AI programs for indicators of estrus and intervals from detection of estrus to AI was used to determine probability of nonreturn to AI; other variables were held constant in the model. Indicators of estrus that were related \( (P < .01) \) to probability of conception or nonreturn were standing estrus (66.7%), AI at 80 h following PGF2α administration without detection of estrus (41.9%), and veterinary prediction of ovulation based on ovarian palpation per rectum (40.1%). Additionally, two intervals from detection of estrus to AI were related negatively to probability of conception, 12 to 18 h (57.7%) and 18 to 24 h (53.8%). From previous reports (6, 7, 9, 10, 17, 18, 19), near optimal nonreturn rates would be expected for cows detected in standing estrus and submitted for AI 12 to 18 h after detection of estrus.

Two management practices were identified as affecting nonreturn rates obtained with once daily AI \( (P < .05) \). Movement of cows immediately before observing for estrus and an observation period >15 min improved nonreturn rates with once daily AI. A majority of dairy producers (71.4%) moved cows immediately prior to observation for signs of estrus; however, only 18.5% indicated that their observation period lasted >15 min.

CONCLUSIONS

When observations for estrus are frequent (every 2 to 4 h), cows should not be submitted for AI the first few hours after detection (7, 17). Under routine field conditions of less frequent observation for estrus, as reported by this study, in which only 36.9% of the dairy producers used observation periods more than twice daily, cows can be submitted for AI shortly after detection of estrus with nearly optimal results with respect to timing. Observation periods are usually associated with milking; thus, AI in midmorning, as suggested by Foote (3), when most cows have been in estrus between 12 to 18 h, probably yields the highest probability of conception. Movement of cows immediately prior to observation of estrus and an observation period >15 min are two management practices that improved nonreturn rates with once daily AI. Thus, once daily AI is an efficient system if performed during midmorning, especially when cows can be moved prior to observation periods >15 min.

ACKNOWLEDGMENTS

The authors are grateful to the professional AI technicians and herd owners who participated in this field trial.

REFERENCES