Effects of a Prostaglandin \( F_{2\alpha} \) Synchronization Program in Lactating Dairy Cattle

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ABSTRACT
A comparison was made between two different methods of reproductive management in the dairy cow. One protocol administered prostaglandin to open cows weekly; the other administered prostaglandin to open cows with a corpus luteum identified by rectal palpation. Survival analysis was used to analyze the data. Cows receiving weekly doses of prostaglandin had a 30% higher pregnancy rate (number of pregnancies per time) than the cows receiving prostaglandin based on rectal palpation of a corpus luteum. The average number of days to first insemination was shorter in cows given prostaglandin weekly.

(Key words: dairy cattle, reproduction, synchronization, prostaglandin)

INTRODUCTION
Reproductive efficiency is an important element of cost effective milk production. Prolonged calving intervals can be costly (2, 13); traditionally, ranges of $2.25 to 4.68/d for cows not pregnant beyond 85 d postpartum have been used to quantify reproductive inefficiency (7). A more recent approach is the determination of optimal days open and days to first insemination as a function of parity based on cumulative milk and calf production (21).

In herds utilizing artificial insemination, prolonged open periods are often due to poor estrus detection. Several studies have estimated that up to 40% of estrous periods may be missed in normally cycling cattle (11, 22).

Prostaglandins (PG), such as PGF\(_{2\alpha}\) and its analogs, can be used to regulate estrus in cows (16, 17, 18). Cows with a corpus luteum (CL) that are given PG can be expected to be in estrus by 120 h. The administration of PG may improve estrus detection because the dairy farmer knows which cows to watch and when to expect the onset of estrous behavior. When PG is used to synchronize a number of cows, the increase in group estrous activity may also result in better estrus detection by the producer. The use of PG has no adverse effect on the fertility of treated cows (3, 6, 9, 14, 16).

The success of estrus induction with PG depends on the presence of a functional CL. Traditionally, rectal palpation (RP) of the reproductive tract is used to identify CL and to identify cows eligible for PG treatment. However, the ability of a palpator to identify a CL and, therefore, to select a cow eligible for PG treatment may not be reliable. A review article by Ott et al. (15) summarized the findings of multiple studies comparing RP with progesterone concentrations. Overall, those authors (15) reported a 77% agreement between the diagnosis of a CL by an experienced palpator and the progesterone concentration.
Ott et al. (15) concluded that RP may be inadequate for identifying cows for PG estrous induction.

In a study published by Kelton et al. (10), circulating concentration of progesterone quantified by radioimmunoassay (RIA) was used as the basis to identify the presence of a functional CL. The RP results were compared with this standard. In a population in which 77.4% of the cows had functional CL identified by the RIA, the positive predictive value of RP was 85.6%, and the negative predictive value was 46.9% (sensitivity 82.6%, specificity 52.6%) (10). Therefore, RP identification of a CL was 85% accurate, and a RP diagnosis of “no CL” was false as many times as it was true. The study by Kelton et al. (10) supports the idea that a major factor decreasing the success of synchronization of estrus programs relying on RP of a mature CL to select cows for PG treatment would be failure to identify all cows eligible for PG treatment.

Given the problems associated with RP, programs based on the administration of PG to all open cows at set intervals (SI) may conceivably be as effective as programs that identify CL by RP. Prostaglandin given at SI to all open cows may result in acceptable estrus detection and conception without reliance on RP. Improved reproductive efficiency was reported in one uncontrolled field study (8) in which all cows in the herd were injected with weekly PG; days open decreased from 129 to 106 d during the trial (8). The objective of the present study was to compare the reproductive effects of a program based on administration of PG at SI with those of a program utilizing RP to select cows for PG treatment.

**MATERIALS AND METHODS**

Three hundred and forty-five cows from four commercial dairy farms served by the University of Pennsylvania field service were used in a randomized block design study. Cows were blocked by lactation number (primiparous vs. multiparous) and season of parturition. All cows received a preinsemination examination. The cows were randomly allocated using a random number table to the SI or RP group. The study began January 17, 1989 and ended July 1, 1990.

Routine RP of the cow’s reproductive tract were scheduled every 2 wk, except in the case of one farm where routine RP were scheduled weekly.

**Definition of Treatment Groups**

**SI.** The PG synchronization program consisted of weekly injections of PGF<sub>2α</sub> (Lutylase, Upjohn, Kalamazoo, MI) given every Monday, starting the first Monday following 50 d postpartum. Cows were inseminated if they were observed in estrus or retreated with PG the following Monday if they were not. Any cow not seen in estrus following three weekly injections of PG was examined at the regular herd check, treated if necessary, and then returned to the weekly schedule of PG injections. All inseminated cows were examined for pregnancy by RP at 35 to 45 d postinsemination. Open cows were kept on the weekly PG treatment until observed in estrus and then were reinseminated.

**RP.** These cows received the traditional reproductive program in current use on the four farms. On these farms, all cows more than 50 d postpartum were palpated by a veterinarian unless they had been seen in estrus and inseminated. If a CL was identified on RP, cows were given PGF<sub>2α</sub>. Cows were inseminated on observed estrus. Cows not exhibiting estrus were palpated at the next scheduled veterinary visit and received PG if a CL was present. All inseminated cows were examined for pregnancy by RP at 35 to 45 d postinsemination. Open cows returned to the RP protocol.

**Analysis**

This study evaluated the relationship between treatment group and days open. Days open is defined as the time between parturition and conception. The comparison was performed using survival analysis, a regression technique applicable to event history studies that use time as an outcome variable (1, 12). The advantages of using survival analysis to analyze reproductive data are that data from cows that conceive or fail to conceive can be analyzed simultaneously. Potential biases in analysis of reproductive data can occur if the data are analyzed for only cows that conceive, excluding the cows that fail to conceive or are culled (1).

In survival analysis, cows not experiencing the event of interest or cows withdrawn from
the study before experiencing the event of interest contribute “censored” data. In this study, cows that were culled before they became pregnant and cows that were still not pregnant by the end of the study were considered to be censored. For those cows, days open values were calculated as the days from parturition to the last insemination date. Cows not receiving at least one insemination were not included in the analysis. The reproductive data used for analysis of cows that lost pregnancies after being diagnosed pregnant were based on the initial confirmed pregnancy. Seventeen RP and 15 SI group cows were culled without insemination and were not considered in the analysis. Two RP and 1 SI group cow had not been inseminated by the end of the study and were not included in the analysis. The data were analyzed using EGRET statistical software (19).

In this study, the variable of interest was treatment group. Cows were classified as exposed to the SI or to the RP group. The analysis was adjusted for lactation number (primiparous vs. multiparous cows) and season of the year. Seasonal categories were March to May, June to August, September to November, and December to February. A Cox proportional hazards regression model of the following form was used to model the data:

\[ h_{ij}(t) = h_{0j}(t) \exp\{ \text{treatment} + \text{lactation} + \text{season} \}. \]

Because pregnancy rates (number of pregnancies per time) varied between herds, the analysis was stratified by herd. The hazard function, \( h_{ij}(t) \), was the probability of observation \( i \) in herd \( j \) of a cow becoming pregnant at \( t \) days after parturition. The baseline hazard function, \( h_{0j}(t) \), was the unknown underlying baseline probability of pregnancy in herd \( j \) when all covariates were equal to 0. The hazard for any covariate pattern (i.e., treatment, lactation, or season) was proportional to the baseline hazards of the four herds.

A hazard ratio was estimated for each independent variable in the model. The hazard ratios were obtained by exponentiating the coefficients (β) (raising \( e^{2.718} \) to the β power). The hazard ratio estimates the rate of pregnancy for different levels compared with a base level and is analogous to relative risk (1). For example, the hazard ratio for exposure to the SI group estimates the rate of pregnancy (number of pregnancies per time) in cows in that group compared with the RP group. A hazard ratio of 1 indicates that the SI and RP group cows had the same pregnancy rate.

Parameters for the model were estimated using a partial likelihood method. All second-order interactions were evaluated and were nonsignificant. The likelihood ratio test was used for statistical significance (1). Statistical significance was \( P < .05 \) (two-sided test).

A graph (Figure 1) was drawn to show visually the difference described by the hazard ratio. Kaplan Meier (KM) estimators for the RP group cows were exponentially raised by the hazard ratio based on the assumption of proportional hazards (4, 5).

The validity of the Cox proportional hazards model depends on satisfying the assumptions of the model. The first assumption is that censoring is independent of prognosis or the occurrence of the event (1, 12). The second assumption, known as the proportional hazards assumption, is that the effects of the explanatory variables are independent of time (1, 12).

To test whether censoring was independent of prognosis, the model was estimated twice, altering the censored observations each time (1). The model was first estimated by changing the data such that the censored observations had the event when censoring occurred (1). The model was next estimated by setting all of the censoring times equal to 320 d, which was the longest time observed in the data set (1). The assumption of independence between prognosis and censoring was satisfied if the parameter estimates from the normal analysis and two extreme analyses were similar (1). The proportional hazards assumption was checked by stratifying on each independent variable and graphing the survival curves using KM estimators. The proportional hazards assumption was satisfied if the curves were parallel (1, 12).

The average days to first insemination was analyzed by the Wilcoxon rank sum test (20). Acceptance level of a Type 1 error was set at .05 for a one-sided test. A chi-square analysis was used to compare first service conception rates, culling rates, and the number of pregnancies in each group. Type 1 error was .05.

A partial budget was used to evaluate the costs of the two treatment groups.
TABLE 1. Cox proportional hazard model.

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
<th>SE</th>
<th>Wald</th>
<th>P value</th>
<th>LRCS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactation</td>
<td>-0.2802</td>
<td>0.139</td>
<td>0.043</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.1533</td>
<td>0.395</td>
<td>0.698</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.761</td>
<td>0.184</td>
<td>0.338</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-0.6165E-01</td>
<td>0.166</td>
<td>0.711</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>2.654</td>
<td>0.129</td>
<td>0.040</td>
<td></td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

1 The coefficients for the categorical variables represent the loge of the ratio of hazard functions for different levels compared with the base level (1).

2 Wald's statistic.

3 Likelihood ratio chi-square statistic (1) for group.

4 Lactation = 1, 1 = lactation >1.

5 = December to February, 1 = March to May, 2 = June to August, and 3 = September to November.

6 Rectal palpation group, 1 = set interval prostaglandin treatment group.

RESULTS

There were 154 RP group cows and 156 SI group cows used in the analysis of the data. Twenty-four of the SI group cows and 28 of the RP group cows contributed censored data.

Treatment group (SI or RP) and lactation number (1 or >1) were significant variables in the final regression model (Table 1). Controlling for other covariates, the hazard ratio for the SI group was 1.3 (Table 2). This value indicates that cows in the SI group had a 30% higher pregnancy rate than cows in the RP group, controlling for lactation number and season of parturition.

Estimating the model twice and substituting extreme values for censored observations did not affect the parameter estimates, which indicates that the assumption of independence between censoring and prognosis was not violated. Use of graphical methods indicated that the assumption of proportional hazards was satisfied.

Cumulative pregnancy rates using KM estimators were plotted for the SI and RP groups (Figure 1). Kaplan Meier estimators for the SI group were obtained by exponentially raising the RP group KM estimators by the hazard.

Figure 1. Cumulative pregnancy rates ( Pregnancies per time) for rectal palpation (---) and set interval prostaglandin treatment group (---).

TABLE 2. Hazard ratios and 95% confidence intervals.

<table>
<thead>
<tr>
<th>Term</th>
<th>Hazard ratio</th>
<th>95% Confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactation</td>
<td>0.7556</td>
<td>0.5759 to 0.9914</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.166</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.193</td>
<td>0.8316 to 2.527</td>
</tr>
<tr>
<td>3</td>
<td>0.9402</td>
<td>0.6786 to 1.303</td>
</tr>
<tr>
<td>Group</td>
<td>1.304</td>
<td>1.012 to 1.680</td>
</tr>
</tbody>
</table>

1 Hazard ratios are obtained by exponentiating the coefficients (b) (raising e(2.718) to the b power). The hazard ratio estimates the rate of pregnancy for different levels compared with a base level (1).

2 Lactation = 1, 1 = lactation >1.

3 = December to February, 1 = March to May, 2 = June to August, and 3 = September to November.

4 Rectal palpation group, 1 = set interval prostaglandin treatment group.
ratio of 1.3 (4, 5). Using this technique, we determined the median days open for the SI group to be 97 d compared with the RP group of 110 d (Figure 1).

Descriptive statistics for the treatment groups are presented in Table 3. The average number of days to first insemination decreased (P < .05) for the SI group. First service conception rates, percentage of inseminated cows that became pregnant, and culling rates were not different between groups.

A partial budget (Table 4) was used to evaluate the costs of the two treatment groups. Costs included in the analysis were RP fees ($2.00 per RP) and drug costs of PG ($3.00 per dose). The SI group costs were $3.73 more per cow than the costs for the RP group.

**DISCUSSION**

This study indicates that weekly use of PG can result in an efficient reproductive program. Cows in the SI group had shorter days to first insemination and higher pregnancy rates, resulting in fewer days open than cows receiving a traditional veterinary reproductive protocol that relied on RP to select for PG treatment. There were no differences between groups in first service conception rates, number of cows that became pregnant, or culling rates.

Part of the improved reproductive performance of the SI group was due to the decrease in days to first insemination compared with the RP group. Administering PG on a weekly basis enhanced earlier insemination in the SI group because cows lacking a CL at the time of the first injection were all treated 1 wk later. In contrast, cows requiring RP of a CL for PG administration received consideration for PG treatment based on the frequency of RP in that particular herd. Therefore, a cow without a CL might not have received a follow-up examination when she had a CL. In addition, in the RP group, false negatives likely occurred; that is, cows with functional CL were misdiagnosed and not given PG. Because the SI protocol treated all cows, no cow with a CL was missed.

The partial budget analysis for the SI and RP group considered RP fees and drug costs for PG. The potential labor-saving costs for the dairy farmer (because the SI cows did not have to be presented to the veterinarian for RP) were not considered. The SI group cost $3.73 more per cow than the RP group but saved 13 median days open per cow compared with the RP group. The decision to use the SI PG program in a herd would be made by considering the economic returns of an improved pregnancy rate in the herd compared with the extra drug costs incurred for the program.

The SI protocol resulted in the administration of PG to cows without functional CL. However, results from this study suggest that

### TABLE 3. Descriptive statistics for the treatment groups.

<table>
<thead>
<tr>
<th>Term</th>
<th>SI Group</th>
<th>RP Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average days to first insemination</td>
<td>72.5*</td>
<td>78.3</td>
</tr>
<tr>
<td>First service conception rate</td>
<td>46.9</td>
<td>42</td>
</tr>
<tr>
<td>Percentage of inseminated cows pregnant</td>
<td>85</td>
<td>82</td>
</tr>
<tr>
<td>Percentage of cows culled</td>
<td>16.8</td>
<td>14.5</td>
</tr>
</tbody>
</table>

*1Set interval prostaglandin treatment group.
2Rectal palpation group.
3Lutalyse, Upjohn, Kalamazoo, MI.
4Includes one prebreeding palpation, all anestrus palpations, and pregnancy palpations.

<table>
<thead>
<tr>
<th>Term</th>
<th>SI Group</th>
<th>RP Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cows</td>
<td>156</td>
<td>154</td>
</tr>
<tr>
<td>Doses of PGF2α2</td>
<td>555</td>
<td>169</td>
</tr>
<tr>
<td>Cost of PGF2α2 at $3.00 per dose</td>
<td>1665</td>
<td>507</td>
</tr>
<tr>
<td>Number of rectal palpations4</td>
<td>547</td>
<td>835</td>
</tr>
<tr>
<td>Cost of RP at $2.00 per RP</td>
<td>1094</td>
<td>1670</td>
</tr>
<tr>
<td>Total costs, $</td>
<td>2759</td>
<td>2177</td>
</tr>
<tr>
<td>Cost difference between groups, $</td>
<td>582</td>
<td></td>
</tr>
<tr>
<td>Cost difference per cow, $</td>
<td>3.73</td>
<td></td>
</tr>
<tr>
<td>Median days open saved per cow</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

1Set interval prostaglandin treatment group.
2Rectal palpation group.
3Lutalyse, Upjohn, Kalamazoo, MI.
4Includes one prebreeding palpation, all anestrus palpations, and pregnancy palpations.
this error is less costly than failure to give PG to a cow with a CL. The benefits of an SI PG administration program may be less pronounced in herds with excellent estrus detection rates.

CONCLUSIONS

The routine weekly use of PG beginning 50 d postpartum resulted in improved reproductive performance compared with a traditional reproductive program in this population of cows.

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