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Additional Small Dose of Prostaglandin F2α at the Time of AI Fails to Improve Pregnancy Rates of Lactating Dairy Cows

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Abstract
In two experiments we tested the hypothesis that administering 10 mg of prostaglandin F$_2$α (PGF) to lactating dairy cows concurrent with timed artificial insemination would increase pregnancy outcome. In three herds with 2,135 inseminations, we failed to demonstrate a positive effect on pregnancy per AI. Although a trend was observed in experiment 1, with more cows in experiment 2, the PGF treatment failed to improve pregnancy outcomes.

Keywords
prostaglandin, timed AI, pregnancy

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Summary
In two experiments we tested the hypothesis that administering 10 mg of prostaglandin F$_{2\alpha}$ (PGF) to lactating dairy cows concurrent with timed artificial insemination would increase pregnancy outcome. In three herds with 2,135 inseminations, we failed to demonstrate a positive effect on pregnancy per AI. Although a trend was observed in experiment 1, with more cows in experiment 2, the PGF treatment failed to improve pregnancy outcomes.

Key words: prostaglandin, timed AI, pregnancy

Introduction
Treatment of domestic livestock with prostaglandin F$_{2\alpha}$ (PGF) administered intravenously, intramuscularly, or in the uterus concurrent with AI to improve conception has been a subject of recurring research since the early 1990s. Pregnancy outcomes have been improved in four studies, but not in three others. Studies in which positive pregnancy outcomes have been most consistent seem to have involved dairy cows inseminated at a timed AI near the peak of lactation in negative energy balance, in cows with less than acceptable reproductive performance (such as repeat breeders), and where stress factors negatively impact ovulation.

Apart from its primary luteolytic role to regress the corpus luteum and induce estrus, PGF has multiple actions on the female reproductive system in cattle and other farm animals. Treatment with PGF concurrent with AI could increase pregnancy rates by:
- increasing uterine contractility, thereby enhancing sperm transport;
- inducing LH release via a luteolysis-independent mechanism, thereby facilitating the ovulatory process;
- hastening luteal regression in cows that may otherwise have delayed or incomplete luteolysis at AI, thereby creating a low progesterone environment conducive for optimal gamete transport;
- inducing release of growth hormone (GH) and subsequent GH-induced insulin-like growth factor-1 secretion, known to increase fertility of lactating dairy cows during the pre- and peri-implantation periods resulting in improvements in conceptus development and a reduction in embryonic mortality;
• increasing the number of cows with a corpus luteum post-AI;
• increasing post-AI corpus luteum size and concentrations of progesterone; and (or)
• inducing secretion of oxytocin that may stimulate uterine contractions and support sperm transport because sperm transport has been improved by adding to semen or administering to females such compounds as PGF, oxytocin, estradiol, phenylephrine, or ergonovine.

Our hypothesis was that administering 10 mg of PGF (2 cc Lutalyse, dinoprost tromethamine sterile solution, Zoetis Animal Health, Whitehouse Station, NJ) concurrent with timed AI will increase pregnancy outcomes in lactating dairy cows. Our objectives were to assess ovarian characteristics and pregnancy outcomes in lactating dairy cows in response to administration of PGF.

**Experimental Procedures**

*Experiment 1*

We conducted this study with 287 lactating dairy cows in the Kansas State University Dairy Teaching and Research Center in a completely randomized design with 2 treatments. Cows diagnosed not pregnant (day 0) at 30 to 36 days after AI were body scored (1 = thin and 5 = fat), received GnRH at the open diagnosis, 25 mg of PGF on day 5 (second PGF on day 6) or on day 7, and GnRH at 56 h after the first or only PGF injection. Cows were stratified by parity (primiparous vs. multiparous) and assigned randomly to receive 10 mg PGF (n = 147) at timed AI (72 h after the first or only PGF injection) or served as untreated controls (n = 140).

Ovaries were scanned via transrectal ultrasonography 72 h before timed AI to characterize number and diameter of all ovarian follicles and re-examined 13 days after timed AI to determine incidence of ovulation, ovulation rate, and total volume of luteal tissue per CL. Blood samples were collected 3 days before timed AI (day -3), at timed AI (day 0), and on day 13 after timed AI to determine progesterone concentration. Pregnancy diagnosis was conducted at 30 to 36 days after AI.

Concentration of progesterone and volume of luteal tissue on day 13 were analyzed using procedure MIXED with the fixed effects of treatment, and with days in milk and BCS as covariates. Luteal regression was verified by changes in blood progesterone concentration between days -3 and 0. Differences in ovulation rate and progesterone were interpreted as direct effects of PGF treatment on functions of the preovulatory follicle(s) and subsequent forming CL.

*Experiment 2*

We conducted this study with 782 cows in one dairy operation and 1,066 cows in a second dairy. Cows were milked thrice daily. Cows with odd-numbered ear tags received no treatment (control) and even-numbered ear tag cows receiving (i.m.) 10 mg PGF concurrent with timed AI as in experiment 1. Cows were treated after first and repeat inseminations. Initial pregnancy diagnoses occurred between days 32 and 35 and were verified between days 63 and 68 after timed AI. Body condition scores were assessed biweekly so they were assigned 3 days before or 4 days after timed AI and treatment.
Outcome variables included pregnancy rates per timed AI and intervening embryo loss. Nuisance variables include lactation number, days in milk at timed AI, body condition score at timed AI, herd, and season. Binomial outcomes were modeled using logistic regression in procedure GLIMMIX in SAS. The model included the fixed effects of treatment (PGF vs. control), lactation number (1 vs. 2+), treatment × lactation number, with days in milk and BCS as covariates. Herd was treated as a random variable, with treatment differences tested by the treatment within herd variance.

**Results and Discussion**

**Experiment 1**

Percentage of cows in which luteolysis had occurred (progesterone ≤ 0.5) before treatment at timed AI did not differ between treated and control cows (93 vs. 89%). Incidence of single ovulation after timed AI exceeded 95% and did not differ between treatments (Table 1). In contrast, treatment with PGF reduced \( P < 0.05 \) the proportion of cows with double ovulation compared with controls. Concentrations of progesterone at AI and volume of the luteal tissue 13 days after timed AI did not differ between treatments (Table 1). Pregnancy per AI at days 32 and 80 tended to be 10 and 18% greater for cows treated with PGF, but did not differ between treatments (Table 1).

**Experiment 2**

Test-day daily milk yield (92 vs. 86 lb), days in milk at treatment (114 vs. 100), and percentage of first-lactation cows enrolled in the study (38 vs. 34%) for herds 1 and 2 were similar. Pregnancy per AI at 32 to 35 days and at 63 to 68 days did not differ between treatments. Pregnancy loss also did not differ between treatments.

Despite a recent report that 10 mg of PGF increased pregnancy outcomes in 1 herd of dairy cows in Canada, we could not corroborate those results. The fact that PGF reduced double ovulation and tended to increase pregnancy outcome in experiment 1 is evidence for a potential biologic effect. Inconsistency in the pregnancy outcome, however, is consistent with other studies. In a study in which PGF at the time of AI increased pregnancy outcome, it also increased luteal tissue and progesterone concentration 13 days after treatment. Results in experiment 1 failed to verify those findings.

In conclusion, results of our study fail to demonstrate a pregnancy outcome benefit of treating cows with a small dose of PGF at AI.
Table 1. Ovarian responses and pregnancy outcomes after treatment of lactating dairy cows with 10 mg PGF$_2\alpha$ at AI (experiment 1)

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment $^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Cows, no</td>
<td>147</td>
</tr>
<tr>
<td>Single ovulation, %</td>
<td>95.6</td>
</tr>
<tr>
<td>Multiple ovulation, %</td>
<td>24.2</td>
</tr>
<tr>
<td>Progesterone, $^2$ ng/mL</td>
<td>5.8 ± 0.3</td>
</tr>
<tr>
<td>Volume of CL, $^3$ cm$^3$</td>
<td>9.1 ± 0.5</td>
</tr>
<tr>
<td>Pregnancy per AI at d 32, %</td>
<td>29.2</td>
</tr>
<tr>
<td>Pregnancy per AI at d 80, %</td>
<td>24.9</td>
</tr>
</tbody>
</table>

$^*_{Differs}$ from control ($P < 0.05$).

$^1_{Cows}$ were treated with 10 mg PGF$_2\alpha$ at AI.

$^2_{Day}$ 13 after treatment.

Table 2. Herd characteristics and effects of 10 mg PGF$_2\alpha$ at timed AI on pregnancy outcomes (experiment 2)

<table>
<thead>
<tr>
<th>Item</th>
<th>Herd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>No. cows treated</td>
<td>1,066</td>
</tr>
<tr>
<td>Pregnancy per AI at 32-35 d</td>
<td></td>
</tr>
<tr>
<td>Control, %</td>
<td>33.9 (549)</td>
</tr>
<tr>
<td>PGF$_2\alpha$ treatment,$^1$ %</td>
<td>30.2 (517)</td>
</tr>
<tr>
<td>Pregnancy per AI at 63-68 d</td>
<td></td>
</tr>
<tr>
<td>Control, %</td>
<td>32.2 (549)</td>
</tr>
<tr>
<td>PGF$_2\alpha$ treatment,$^1$ %</td>
<td>27.7 (516)</td>
</tr>
<tr>
<td>Pregnancy loss</td>
<td></td>
</tr>
<tr>
<td>Control, %</td>
<td>4.8 (186)</td>
</tr>
<tr>
<td>PGF$_2\alpha$ treatment,$^1$ %</td>
<td>7.7 (155)</td>
</tr>
</tbody>
</table>

$^1_{Cows}$ were treated with 10 mg PGF$_2\alpha$ at AI.