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## Dose and Frequency of $\text{PGF}_{2\alpha}$ Administration to Lactating Dairy Cows Exposed to Presynchronization and Either Five- or Seven-day Ovsynch Protocols: Ovulation, Luteolysis, and Pregnancy Rates

*J. S. Stevenson and J. A. Sauls*

### Summary

Lactating Holstein cows in one herd were milked three times daily and enrolled in a  $2 \times 2 \times 2$  factorial design with eight treatments before first postpartum artificial insemination (AI). These treatments were employed to test ovulatory, progesterone, and luteolytic outcomes to three main effects: (1) two GnRH- $\text{PGF}_{2\alpha}$  presynchronization programs (PG-3-G vs. Double Ovsynch); (2) 5- vs. 7-day Ovsynch-duration programs; and (3) two doses (25 mg on consecutive days) or one dose (50 mg) of  $\text{PGF}_{2\alpha}$  administered before timed AI. Results from this experiment demonstrate no differences in the presynchronization treatments of PG-3-G vs. Double Ovsynch; both are effective in initiating estrous cycles during warm-hot vs. cool-cold seasons. Although ovulatory responses were similar after the first GnRH administration, Double Ovsynch cows tended to have greater ovulation responses after the second GnRH administration. The single large, one-time administered 50-mg dose was effective in causing luteolysis in the 7-day program but slightly less effective in the 5-day program. Thus, when using the shorter 5-day program, the two 25-mg  $\text{PGF}_{2\alpha}$  doses administered 24 hours apart are recommended. Insufficient numbers of cows were treated to make conclusions about pregnancy outcomes in this one-herd study. Pregnancy rates, however, were reduced in 5-day Ovsynch program when the single large 50-mg dose of  $\text{PGF}_{2\alpha}$  was employed.

### Introduction

Potential limitations to a successful timed artificial insemination (AI) program for cows submitted for first service include cows that are not cycling (anovulatory), synchronization of the ovulatory follicle that should ovulate just after the timed AI, and regression of the corpus luteum or corpora lutea (if more than one exists when  $\text{PGF}_{2\alpha}$  is administered).

Two Ovsynch programs are used in the industry: 1) 5-day program: GnRH (G-1) — 5 days —  $\text{PGF}_{2\alpha}$  — 24 hours —  $\text{PGF}_{2\alpha}$  — 48 hours — GnRH (G-2) + timed AI; or

2) 7-day program: GnRH (G-1) — 7 days — PGF<sub>2α</sub> — 56 hours — GnRH (G-2) — 16 hours — timed AI.

Presynchronization programs before first postpartum AI used in the industry include combinations of PGF<sub>2α</sub> and GnRH (i.e., GnRH-PGF<sub>2α</sub> presynchronization options). These programs include: PG-3-G (PGF<sub>2α</sub> — 3 days — GnRH — 7 days — Ovsynch); G-6-G (PGF<sub>2α</sub> — 2 days — GnRH — 6 days — Ovsynch); or Double Ovsynch (GnRH — 7 days — PGF<sub>2α</sub> — 3 days — GnRH — 7 days — Ovsynch). These GnRH-PGF<sub>2α</sub> presynchronization programs are alternatives to the traditional PGF<sub>2α</sub> presynchronization programs (2 injections of PGF<sub>2α</sub> 14 days apart <sup>3</sup>/<sub>4</sub> 10, 11, 12, or 14 days — Ovsynch) because they often further improve pregnancy rates.

The advantages of these GnRH-PGF<sub>2α</sub> presynchronization programs include inducing ovulation in anovulatory cows, decreasing the percentage of cows with lower circulating progesterone concentrations (< 0.50 ng/mL) at G-1, increasing the percentage of cows with medium progesterone concentrations (0.50 < progesterone ≤ 3.0 ng/mL) at G-1, and increasing the proportion of cows with a corpus luteum (CL) at G-1. In addition, these GnRH-PGF<sub>2α</sub> presynchronization programs increase the percentage of cows with high progesterone (> 3.0 ng/mL) at the PGF<sub>2α</sub> treatment that immediately precedes timed AI and tend to increase average circulating progesterone at PGF<sub>2α</sub>.

Early studies in beef and dairy cows indicated that an improvement in pregnancy outcome seemed to occur when a shorter 5-day Ovsynch program was compared with a 7-day program. The problem with these published studies was the confounding of program duration (5 vs. 7 days) and use of one vs. two doses of PGF<sub>2α</sub> (6 to 24 h apart) to induce luteolysis. In none of the experiments were the two doses of PGF<sub>2α</sub> tested in both the 5- and 7-day programs. Therefore, a test of the two doses of PGF<sub>2α</sub> must occur in both programs to interpret the data correctly. If the 5-day program is not superior in pregnancy outcome, then the difference may simply be the result of improved complete luteolysis in cows treated with two doses of PGF<sub>2α</sub>, regardless of program duration.

Applying a second standard dose or a single larger dose of PGF<sub>2α</sub> seems to maximize complete luteolysis before timed AI. Corpora lutea less than 10 days old are resistant to complete regression after a single standard dose of PGF<sub>2α</sub>. Administering PGF<sub>2α</sub> as a single large dose on day 7 or as two standard doses on days 5 and 6 after G-1 (5-day Ovsynch) usually results in 70 to 84% of cows with progesterone < 0.3 ng/mL on the day of the timed AI. Progesterone concentrations at or near baseline at the final GnRH treatment influence the characteristics of GnRH-induced LH release and subsequent pregnancy risk. Increased dose or frequency of PGF<sub>2α</sub> at the end of Ovsynch program has enhanced luteolysis, reduced progesterone concentrations at timed AI, and in some cases slightly increased pregnancy risk compared with a standard single dose. Recent studies demonstrated increased complete luteolysis when 7-day Ovsynch programs included a second standard dose of PGF<sub>2α</sub> compared with a single standard dose.

Therefore, we proposed to address three questions. (1) Does the additional preGnRH (PreG; Figure 1) injection of Double Ovsynch improve synchronization characteristics compared with the PG-3-G presynch program? (2) Will one large dose (50 mg) of PGF<sub>2α</sub> produce complete luteolysis similar to two standard 25-mg doses given 24 h

apart? (3) Is the 5-day Ovsynch program superior to the 7-day program when similar dose and frequency of PGF<sub>2α</sub> injections are administered in both programs?

## Experimental Procedures

We enrolled 407 lactating dairy cows in a  $2 \times 2 \times 2$  factorial design consisting of 8 treatments for 20 months (September 2015 through April 2017). This approach was employed to test ovulatory, progesterone, and luteolytic outcomes to three main effects (Figure 1): (1) two GnRH-PGF<sub>2α</sub> presynchronization programs (PG-3-G vs. Double Ovsynch); (2) 5- vs. 7-day Ovsynch-duration programs; and (3) two doses (25 mg on consecutive days) or one dose (50 mg) of PGF<sub>2α</sub> administered before timed AI (Figure 1). Double Ovsynch includes the additional PreGnRH injection (PreG; high-lighted box in Figure 1) that is not part of the PG-3-G presynch program.

Blood samples were collected before G-1, before PGF<sub>2α</sub>, and at 24, 48, and 72 h after PGF<sub>2α</sub> (first or only PGF<sub>2α</sub> injection) to determine concentration ranges of progesterone and assess complete luteolysis (progesterone < 0.5 ng/mL at 48 to 72 h after the first or only dose of PGF<sub>2α</sub>). Transrectal ovarian scans by ultrasonography were used to assess ovulation after G-1 and G-2. Although pregnancy risk was assessed, this experiment was not designed to test pregnancy risk but focused on: (1) ovulation responses to G-1 and G-2; and (2) proportion of cows with complete luteolysis, as illustrated in Figure 1.

## Results and Discussion

### *Ovarian Cyclicity*

On the basis of progesterone concentrations, the proportion of cows that had initiated estrous cycles at the start of the Ovsynch program did not differ between PG-3-G and Double Ovsynch (Table 1). More cows tended ( $P = 0.09$ ) to return to having estrous cycles before the onset of ovulation synchronization programs during the cool-cold season compared with the warm-hot season (88.4 vs. 81.9%).

### *Ovulation Response to GnRH-1*

Neither single nor multiple ovulation responses to G-1 differed between PG-3-G and Double Ovsynch treatments (Table 1). Season had differing effects on multiple ovulation in cows treated with PG-3-G and Double Ovsynch. During the warm-hot season, multiple ovulation occurred more frequently in PG-3-G than Double Ovsynch cows (17.4 vs. 6.4%), whereas during the cool-cold season, the reverse was detected (8.6 vs. 13.6%, respectively).

### *Luteolysis*

Decreasing concentrations of progesterone (luteolysis or regression of the corpus luteum or corpora lutea) after the PGF<sub>2α</sub> treatments just preceding timed AI are summarized in Table 2. At 24 hours after the first or only PGF<sub>2α</sub> dose, the proportion of cows with progesterone concentration < 1 ng/mL was greater ( $P < 0.05$ ) in Double Ovsynch than PG-3-G treatments; greater ( $P < 0.05$ ) in 7- vs. 5-day Ovsynch cows, and greater in cows treated with the  $1 \times 50$  mg than  $2 \times 25$  mg PGF<sub>2α</sub> dose (no interactions). At 48 and 72 hours, more ( $P < 0.01$ ) cows had progesterone < 1 ng/mL in the 7- vs. 5-day Ovsynch cows. At 72 hours, more ( $P < 0.01$ ) cows had progesterone < 0.5 ng/mL in the 7- vs. 5-day Ovsynch cows and more ( $P < 0.01$ ) cows receiving

the  $2 \times 25$  mg dose than the  $1 \times 50$  mg dose. An interaction, however, was detected at 48 and 72 hours between the Ovsynch duration and the  $\text{PGF}_{2\alpha}$  dose-frequency (Figure 2). Although mean progesterone was  $< 0.05$  ng/mL at both time points, the single  $1 \times 50$  mg was slightly less effective than the  $2 \times 25$  mg dose in reducing concentrations of progesterone in the 5- vs. 7-day Ovsynch-treated cows. Thus, the single large dose was equally effective as the  $2 \times 25$ -mg dose in the 7-day Ovsynch program, but less so when applied to cows in the shorter 5-day program.

### *Ovulation Response to GnRH-2*

Single ovulation after G-2 differed slightly between presynch treatments (Table 3). Cows presynched with Double Ovsynch tended ( $P = 0.085$ ) to have greater ovulatory responses than PG-3-G cows. Duration of Ovsynch (5 vs. 7 days) had no effect on ovulation response to G-2. Single ovulatory response to G-2 tended ( $P = 0.073$ ) to be greater during the cooler-cold season than during the warmer-hot season (94.2 vs. 88.3%, respectively).

In contrast, multiple ovulation tended ( $P = 0.108$ ) to be greater in PG-3-G than Double Ovsynch cows and greater ( $P = 0.004$ ) in 5- vs. 7-day cows. An interaction ( $P = 0.011$ ), however, was detected between presynchronization treatments and Ovsynch duration (Table 3). Multiple ovulation was greater in PG-3-G cows in the 5-day compared with the 7-day program, whereas no differences in multiple ovulation occurred between program durations in Double Ovsynch cows. Season had no effect on the frequency of multiple ovulation.

### *Pregnancy Outcomes*

No overall differences were detected in pregnancy rate between PG-3-G and Double Ovsynch treatments (Table 3) or between the 5- vs. 7-day program, although numerically pregnancy rates were greater for PG-3-G and 7-day program cows. Because of the differences in the effectiveness of luteolysis (decreasing progesterone after  $\text{PGF}_{2\alpha}$  during the timed AI week), an interaction between Ovsynch duration and  $\text{PGF}_{2\alpha}$  dose-frequency was detected (Table 3). Cows receiving the  $1 \times 50$  mg dose in the 7-day program had greater ( $P < 0.05$ ) pregnancy rates than cows receiving the  $1 \times 50$  mg dose in the 5-day program.

Results from this experiment demonstrate no significant differences in the presynchronization responses to PG-3-G vs. Double Ovsynch treatments. Both are effective in initiating estrous cycles during all seasons. Although ovulatory responses were similar after G-1, Double Ovsynch cows tended to have greater ovulation responses after G-2. The single large, one-time administered 50-mg dose was effective in causing luteolysis in the 7-day but less so in the 5-day cows. Thus, when using the shorter 5-day program, the two 25-mg doses administered 24 hours apart are recommended. Insufficient numbers of cows were treated to make conclusions about pregnancy outcomes in this one-herd study. Pregnancy rates, however, were reduced in 5-day compared with the 7-day Ovsynch program when the single large 50-mg dose of  $\text{PGF}_{2\alpha}$  was employed. As expected, pregnancy rate was greater ( $P = 0.056$ ) during the cooler-cold months compared with warmer-hot season (40.2 vs. 30.0%).

**Table 1. Ovarian cyclicity assessed at 7 days after two presynchronization treatments and ovulatory responses response to GnRH-1 (G-1)**

| Item                         | Presynchronization <sup>1</sup> |        |         |
|------------------------------|---------------------------------|--------|---------|
|                              | DO                              | PG-3-G | P-value |
| Cyclicity before G-1, %      | 88.8                            | 90.5   | 0.566   |
| Single ovulation to G-1, %   | 68.4                            | 63.2   | 0.267   |
| Multiple ovulation to G-1, % | 7.8                             | 8.4    | 0.845   |

<sup>1</sup>Double Ovsynch (DO) = GnRH — 7 days — PGF<sub>2α</sub> — 3 days — GnRH; PG-3-G = PGF<sub>2α</sub> — 3 days — GnRH.

**Table 2. Percentage of cows with progesterone concentrations < 1 ng/mL at 24, 48, and 72 hours after the first or only dose of PGF<sub>2α</sub> or < 0.5 ng/mL at 72 hours**

| Hours after the first or only PGF <sub>2α</sub> treatment | Presynchronization           |                                  | Ovsynch, days                  |                                 | PGF <sub>2α</sub> dose, <sup>4</sup> mg |                     |
|-----------------------------------------------------------|------------------------------|----------------------------------|--------------------------------|---------------------------------|-----------------------------------------|---------------------|
|                                                           | DO <sup>1</sup><br>(n = 164) | PG-3-G <sup>2</sup><br>(n = 163) | Five <sup>3</sup><br>(n = 163) | Seven <sup>3</sup><br>(n = 164) | 2 × 25<br>(n = 164)                     | 1 × 50<br>(n = 163) |
| 24 (< 1 ng/mL)                                            | 57.3 <sup>a</sup>            | 36.0 <sup>b</sup>                | 46.0 <sup>a</sup>              | 57.3 <sup>b</sup>               | 40.5 <sup>c</sup>                       | 62.8 <sup>d</sup>   |
| 48 <sup>5</sup> (< 1 ng/mL)                               | 88.4 <sup>a</sup>            | 90.2 <sup>a</sup>                | 84.0 <sup>c</sup>              | 95.5 <sup>d</sup>               | 92.0 <sup>a</sup>                       | 86.6 <sup>a</sup>   |
| 72 <sup>5</sup> (< 1 ng/mL)                               | 95.7 <sup>a</sup>            | 94.5 <sup>a</sup>                | 90.8 <sup>c</sup>              | 99.4 <sup>d</sup>               | 98.2 <sup>a</sup>                       | 92.7 <sup>b</sup>   |
| 72 <sup>5</sup> (< 0.5 ng/mL)                             | 95.9 <sup>a</sup>            | 94.1 <sup>a</sup>                | 90.6 <sup>c</sup>              | 99.4 <sup>d</sup>               | 98.2 <sup>a</sup>                       | 92.0 <sup>b</sup>   |

<sup>a-b</sup>Means differ ( $P < 0.05$ ) within main effect category.

<sup>c-d</sup> Means differ ( $P < 0.01$ ) within main effect category.

<sup>1</sup>Double Ovsynch (DO) = (DO) = GnRH — 7 days — PGF<sub>2α</sub> — 3 days — GnRH;

<sup>2</sup>PG-3-G = PGF<sub>2α</sub> — 3 days — GnRH.

<sup>3</sup>Five = GnRH — 5 days — PGF<sub>2α</sub>; Seven = GnRH — 7 days — PGF<sub>2α</sub>; both were initiated 7 d after the end of the presynchronization treatments.

<sup>4</sup>Two, 25-mg doses 24 h apart or one, 50-mg dose.

<sup>5</sup>Interaction ( $P < 0.05$ ) between Ovsynch duration and PGF<sub>2α</sub> dose.

**Table 3. Ovulatory responses to GnRH-2 (G-2) in response to the presynchronization (Pre; PG-3-G or Double Ovsynch) and 5- or 7-day Ovsynch (Ovs) programs**

| Item                  | DO <sup>1</sup>   |                    | PG-3-G <sup>2</sup> |                    | P-value |       |           |
|-----------------------|-------------------|--------------------|---------------------|--------------------|---------|-------|-----------|
|                       | Five <sup>3</sup> | Seven <sup>3</sup> | Five <sup>3</sup>   | Seven <sup>3</sup> | Pre     | Ovs   | Pre × Ovs |
| Single ovulation, %   | 96.0              | 95.4               | 90.6                | 93.0               | 0.085   | 0.822 | 0.569     |
| Multiple ovulation, % | 16.0              | 14.7               | 32.4                | 11.0               | 0.108   | 0.004 | 0.011     |

<sup>1</sup>Double Ovsynch (DO) = (DO) = GnRH — 7 days — PGF<sub>2α</sub> — 3 days ¾ GnRH;

<sup>2</sup>PG-3-G = PGF<sub>2α</sub> — 3 days — GnRH.

<sup>3</sup>Five = GnRH — 5 days — PGF<sub>2α</sub>; Seven = GnRH — 7 days — PGF<sub>2α</sub>; both were initiated 7 d after the end of the presynchronization treatments.



**Table 4. Pregnancy rate for lactating cows treated with two presynchronization treatments (Double Ovsynch [DO] vs. PG-3-G), two Ovsynch durations (5- vs. 7-days), and two PGF<sub>2α</sub> (2 × 25-mg vs. 1 × 50-mg doses)**

| Presynch <sup>1</sup> | 5 day <sup>2</sup>       |                         | 7 day <sup>2</sup>       |                         | DO vs.<br>PG-3-G |
|-----------------------|--------------------------|-------------------------|--------------------------|-------------------------|------------------|
|                       | 2 × 25 mg <sup>3</sup>   | 1 × 50 mg <sup>3</sup>  | 2 × 25 mg <sup>3</sup>   | 1 × 50 mg <sup>3</sup>  |                  |
| DO                    | 34.5 (55)                | 35.1 (57)               | 27.8 (54)                | 36.8 (57)               | 33.6 (223)       |
| PG-3-G                | 40.3 (57)                | 24.1 (54)               | 40.7 (59)                | 49.1 (57)               | 38.8 (227)       |
| Total <sup>4</sup>    | 37.5 <sup>ab</sup> (112) | 29.7 <sup>a</sup> (111) | 34.5 <sup>ab</sup> (113) | 43.0 <sup>b</sup> (114) |                  |
| 5- vs. 7-day          | 33.6 (223)               |                         | 38.8 (227)               |                         |                  |

<sup>a,b</sup> Mean percentages with different superscript letters differ ( $P < 0.05$ ).

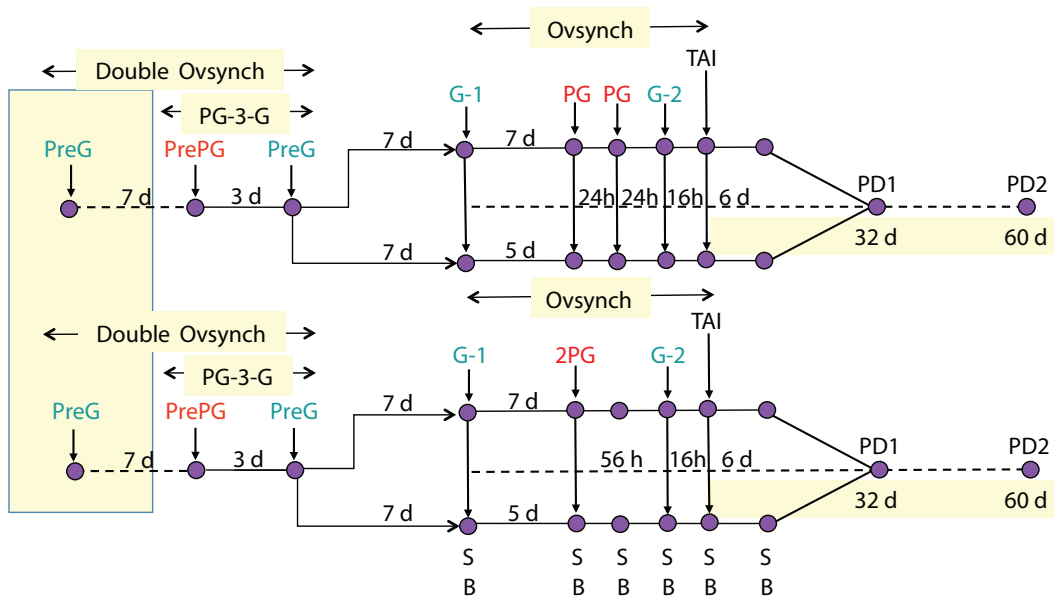
<sup>1</sup>DO = GnRH — 7 days — PGF<sub>2α</sub> — 3 days — GnRH; PG-3-G = PGF<sub>2α</sub> — 3 days — GnRH;

PG-3-G = PGF<sub>2α</sub> — 3 days — GnRH.

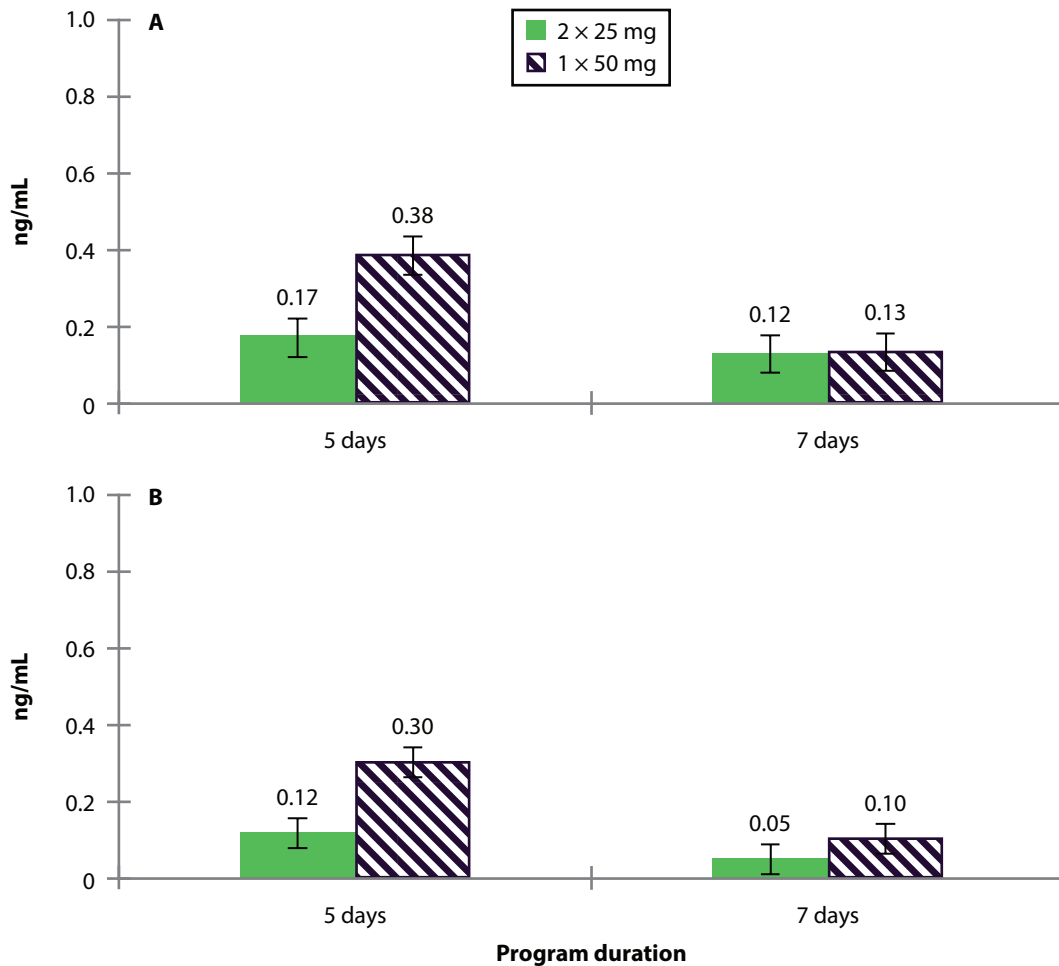
<sup>2</sup>Five = GnRH — 5 days — PGF<sub>2α</sub>; Seven = GnRH — 7 days — PGF<sub>2α</sub>; both were initiated 7 d after the end of the presynchronization treatments.

<sup>3</sup>Treatment with PGF<sub>2α</sub> (2 × 25-mg vs. 1 × 50-mg doses).

<sup>4</sup>Interaction of Ovsynch duration vs. PGF<sub>2α</sub> dose ( $P = 0.043$ ).



**Figure 1. Illustration of the 2 × 2 × 2 factorial design of 8 treatments. Main effects included presynchronization (Double Ovsynch vs. PG-3-G), Ovsynch duration (5 vs. 7 d), and PGF<sub>2α</sub> dose-frequency (2 × 25 vs. 1 × 50-mg doses) and schedule for blood collection and transrectal ultrasonograms. B = blood sample, S = transrectal ultrasonogram of ovaries; PrePG or PG = 25 mg PGF<sub>2α</sub>, 2PG = 50 mg PGF<sub>2α</sub>, PreG, G-1 or G-2 = 100 μg gonadotropin-releasing hormone (GnRH), PD = pregnancy diagnosis.**



**Figure 2. Progesterone concentrations at 48 (A) and 72 (B) hours after the first or only injection of  $\text{PGF}_{2\alpha}$  showing the lesser effectiveness of the  $1 \times 50$  mg dose to reduce progesterone concentrations in the 5-day but not the 7-day Ovsynch program.**