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# Synchronization of estrus and ovulation in dairy heifers using norgestomet, GnRH, and PGF2 $\alpha$

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## SYNCHRONIZATION OF ESTRUS AND OVULATION IN DAIRY HEIFERS USING NORGESTOMET, GnRH, AND PGF<sub>2α</sub><sup>1</sup>

*J. S. Stevenson, K. E. Thompson,  
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### Summary

Two experiments were performed using the same treatments. All heifers received two injections of PGF<sub>2α</sub> 14 days apart. Controls then were inseminated after detected estrus. Heifers assigned to the two treatments also received 6 mg of norgestomet for 8 days beginning 7 days before the second of two PGF<sub>2α</sub> injections. The heifers in the last treatment also received GnRH 48 hr after the second PGF<sub>2α</sub> injection to induce ovulation in any heifer not observed in estrus before a fixed-time insemination at 72 hr after PGF<sub>2α</sub>. In Experiment 1, any control heifer or heifer in the two treatments not detected in estrus by 72 hr after PGF<sub>2α</sub> received a fixed-time insemination at 72 hr. Heifers receiving GnRH tended to have fewer standing events and a shorter duration of estrus. Fixed-time inseminations reduced conception compared to those after detected estrus. In Experiment 2, when inseminations were performed only after detected estrus, all measures of fertility were unaffected by treatments. These results indicated that addition of norgestomet and(or) GnRH did not improve measures of estrus synchronization or fertility of dairy heifers.

(Key Words: Estrus Synchronization, Standing Estrus, Hormones, Heifers.)

### Introduction

The objectives of estrus-synchronization programs are to control precisely the onset of estrus and facilitate the use of A.I.-breeding and

fixed-time inseminations. Because a progestin implant removed within 48 hr after the corpus luteum is regressed by PGF<sub>2α</sub> generally improves the onset and synchrony of estrus, fixed-time inseminations at 48 to 54 hr after progestin withdrawal have produced acceptable conception rates in beef heifers. Injection of GnRH causes the release of luteinizing hormone (LH) from the pituitary gland and induces ovulation of a preovulatory-size follicle. Follicles ovulate between 24 and 32 hr after GnRH injection. Therefore, the objective of two experiments reported herein was to determine whether treating heifers with PGF<sub>2α</sub> plus the addition of norgestomet and(or) GnRH would improve estrus-detection rates and subsequent measures of fertility.

### Procedures

#### Experiment 1

Pubertal Holstein replacement heifers at the Kansas State University Dairy Teaching and Research Center were used in three replications during January (n = 11), February (n = 9), and August (n = 11) of 1996. Each heifer was fitted with a HeatWatch® rump-mounted device (DDx, Inc., Denver, CO) to measure estrual activity during an estrus-synchronization program consisting of two treatments and a control (Figure 1). Control heifers (2×PGF<sub>2α</sub>) received two injections of PGF<sub>2α</sub> (Lutalyse®, Pharmacia & Upjohn, Kalamazoo, MI) 14 days apart before inseminations were made after detected estrus. Any heifer not inseminated by 72 hr after PGF<sub>2α</sub>

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received one fixed-time insemination at 72 hr. A second group of heifers also received two injections of  $\text{PGF}_{2\alpha}$  14 days apart plus one ear implant containing 6 mg of norgestomet (Syncro-Mate-B implant; Rhone-Merieux, Inc., Athens GA) 7 days before the second  $\text{PGF}_{2\alpha}$  injection ( $2 \times \text{PGF}_{2\alpha} + \text{N}$ ); the implant was removed 24 hr after  $\text{PGF}_{2\alpha}$ . Inseminations were made after detected estrus. Any heifer not inseminated by 72 hr after  $\text{PGF}_{2\alpha}$  received one fixed-time insemination at 72 hr. A third group of heifers also received two injections of  $\text{PGF}_{2\alpha}$  14 days apart plus one ear implant containing 6 mg of norgestomet (Syncro-Mate-B implant; Rhone-Merieux, Inc., Athens GA) 7 days before the second  $\text{PGF}_{2\alpha}$  injection; the implant was removed 24 hr after  $\text{PGF}_{2\alpha}$ . In addition, 100  $\mu\text{g}$  of GnRH (Cystorelin®, Rhone-Merieux, Inc., Athens, GA) was administered 48 hr after the second injection of  $\text{PGF}_{2\alpha}$  ( $2 \times \text{PGF}_{2\alpha} + \text{N} + \text{G}$ ). Inseminations were made after detected estrus or remaining heifers received one fixed-time insemination 18 hr after GnRH or 72 hr after  $\text{PGF}_{2\alpha}$ . Inseminations were made by one technician using semen from one sire. Pregnancy was diagnosed by palpation of the uterus and its contents between 38 and 52 days after insemination.

From 10 control heifers and from five heifers in each of the two treatments, daily blood samples were collected beginning at the first injection of  $\text{PGF}_{2\alpha}$  and continuing until 48 hr after the second  $\text{PGF}_{2\alpha}$  injection. Concentrations of estradiol-17 and progesterone were measured in blood serum with specific validated radioimmunoassays. Hormonal concentrations were plotted for each heifer and normalized to the peak in estradiol-17 that occurred near the onset of estrus after the second  $\text{PGF}_{2\alpha}$  injection.

The estrus-detection rate was calculated as the proportion of heifers detected in estrus by visual observation (twice daily) or by the HeatWatch device during the first 96 hr after the second  $\text{PGF}_{2\alpha}$  injection. Conception rate was the proportion of heifers detected and inseminated that became pregnant. Pregnancy rate was the proportion of heifers that became pregnant of the total treated. Calving rate was the proportion of heifers that calved of the total treated. The

interval from  $\text{PGF}_{2\alpha}$  to the onset of estrus, duration and number of standing events per heifer in estrus, and duration of standing heat were calculated using information collected by the HeatWatch system.

## Experiment 2

An experiment using the same treatments was conducted on two dairy farms (Big Sky and Valley View dairy farms) located in southern New Mexico during February, March, and May of 1996. Dairy heifer replacements ( $n = 287$ ) were treated as in Figure 1. A total of six sires was used between the two dairy farms. All inseminations were performed after detected estrus, and in one herd, a second insemination was given if the heifer was in estrus at the next heat check period. Clean-up bulls were used after three unsuccessful inseminations. Pregnancy was diagnosed by calving date, assuming that a normal distribution of gestation for Holstein heifers was  $280 \pm 14$  days (266 to 294 days). Any heifer not calving during this period after the treatment insemination became pregnant to a repeat insemination or to the clean-up bull. Estrus-detection, conception, pregnancy, and calving rates were calculated as in Experiment 1.

## Results and Discussion

### Experiment 1

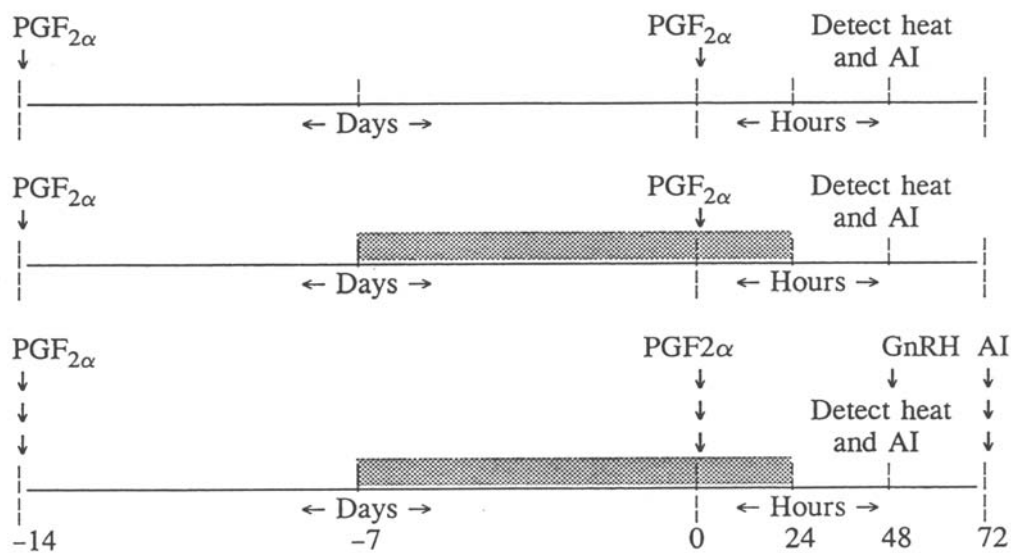
Results of the first experiment are summarized in Table 1. Interval to the onset of estrus was in the 55- to 67-hr range and not different among treatments; however, the estrus-detection rate tended to be greatest in the heifers given norgestomet without the subsequent GnRH treatment. The number of standing events during estrus was reduced ( $P < .05$ ) when GnRH was given at 48 hr after  $\text{PGF}_{2\alpha}$ . Similarly, the duration of estrus tended to be less for that same treatment, whereas duration of individual standing events was not different. Conception, pregnancy, and calving rates were similar, but each measure tended to be less in the last treatment with GnRH. Heifers that received the fixed-time insemination had lower ( $P < .01$ ) conception rates than those inseminated after a detected estrus (21.3 vs 82.5%).

Profiles of estradiol-17 $\beta$  and progesterone in blood serum were not different between the controls and those additionally treated with norgestomet (Figure 2).

These results indicate that administration of GnRH following the removal of the norgestomet implant may have suppressed estrual behaviors such as standing activity and duration of estrus, despite similar peak concentrations of estradiol-17 $\beta$  near the onset of estrus.

## Experiment 2

Results of the second experiment are summarized in Table 2. In this experiment, inseminations were performed only after detected estrus. Es&us-detection, conception, pregnancy, and calving rates were unaffected by treatments. Herd and sire effects were detected for conception and pregnancy rates. Interval to inseminations after PGF $_{2\alpha}$  was 1 day later in one herd than in the other herd. In 22.8% of the heifers, double inseminations were made at estrus, and a tendency ( $P=.13$ ) for improved conception was detected (73.8 vs 60.9%). These results indicate that the addition of norgestomet and(or) GnRH did not improve either estrus synchrony or any measure of fertility.



**Figure 1. Treatment Protocol for Experiments 1 and 2.** Inseminations were performed after detected estrus or at 72 hr after the second PGF $_{2\alpha}$  injection in the absence of estrus in Experiment 1. Inseminations were made only after detected estrus in Experiment 2. ■ = 6-mg norgestomet implant.

**Table 1. Reproductive Performance of Kansas Holstein Heifers**

| Item                                     | Treatment           |                        |                          |
|------------------------------------------|---------------------|------------------------|--------------------------|
|                                          | 2×PGF <sub>2α</sub> | 2×PGF <sub>2α</sub> +N | 2×PGF <sub>2α</sub> +N+G |
| No. of heifers                           | 14                  | 9                      | 8                        |
| Estrus-detection rate <sup>1</sup> , %   | 53.4                | 87.9                   | 47.7                     |
| PGF <sub>2α</sub> to onset of estrus, hr | 55.0                | 66.6                   | 57.4                     |
| No. of standing events <sup>2</sup>      | 23.1                | 21.5                   | 5.6                      |
| Duration of stands <sup>1</sup> , sec    | 3.1                 | 2.9                    | 2.5                      |
| Duration of estrus, hr                   | 15.2                | 11.8                   | 6.1                      |
| Conception rate <sup>3</sup> , %         | 60.6                | 45.5                   | 49.7                     |
| Pregnancy rate <sup>3</sup> , %          | 50.0                | 49.4                   | 34.8                     |
| Calving rate, %                          | 92.2                | 98.6                   | 88.9                     |

<sup>1</sup>Replicate effect ( $P<.05$ ).

<sup>2</sup>Treatment effect ( $P<.05$ ).

<sup>3</sup>Estrus vs fixed-time AI ( $P<.01$ ; 82.5 vs 21.3%).

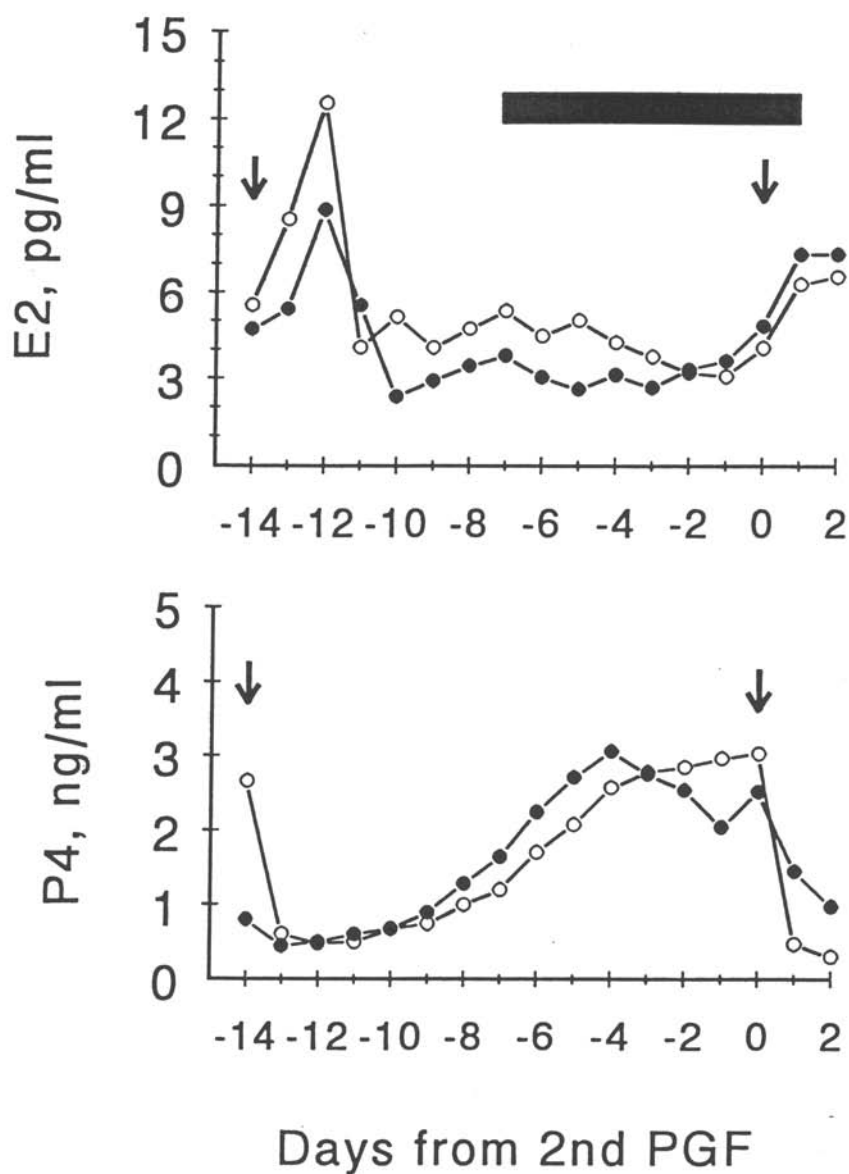
**Table 2. Reproductive Performance of Two Herds of New Mexico Holstein Heifers**

| Item                                     | Treatment           |                        |                          |
|------------------------------------------|---------------------|------------------------|--------------------------|
|                                          | 2×PGF <sub>2α</sub> | 2×PGF <sub>2α</sub> +N | 2×PGF <sub>2α</sub> +N+G |
| No. of heifers                           | 101                 | 92                     | 94                       |
| Estrus-detection rate, %                 | 94.4                | 93.1                   | 88.9                     |
| PGF <sub>2α</sub> to AI <sup>1</sup> , d | 3.5                 | 3.9                    | 3.4                      |
| Conception rate <sup>2</sup> , %         | 69.5                | 62.4                   | 70.0                     |
| Pregnancy rate <sup>3</sup> , %          | 69.5                | 61.6                   | 70.0                     |
| Calving rate, %                          | 84.5                | 86.3                   | 92.8                     |

<sup>1</sup>Herd effect ( $P<.05$ ;  $3.1 \pm .2$  vs.  $4.2 \pm .4$ )

<sup>2</sup>Herd ( $P<.05$ ) and sire ( $P = .07$ ) effects.

<sup>3</sup>Herd ( $P = .05$ ) and sire ( $P<.10$ ) effects.



**Figure 2.** Daily Concentrations of Estradiol-17 and Progesterone in Blood Serum of Dairy Heifers during 14 Days before and 2 Days after the Second of Two PGF<sub>2</sub> Injections (Arrows) in Controls (○; n = 10) or Norgestomet-Treated Heifers (●; n = 10). Concentrations were normalized to the peak of estradiol-17 that occurred near the onset of estrus after the second PGF<sub>2</sub> injection.  
 -- norgestomet treatment during 8 days.