Evaluation of the 5- vs. 7-day CIDR Program in Dairy Heifers Before Timed Artificial Insemination

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Summary

Our objectives were to determine: (1) the effectiveness of an injection of $PGF_{2\alpha}$ to regress the corpus luteum before initiating an timed artificial insemination (**TAI**) program, (2) ovulation response to gonadotropin-releasing hormone (**GnRH**), and (3) pregnancy outcomes in dairy heifers inseminated with conventional and gender-biased semen. Heifers (n = 545) from 3 locations (Florida, Kansas, and Mississippi) were assigned randomly to 1 of 2 treatments: (1) 25-mg prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) injection and controlled internal drug release (**CIDR**) insert on day −7 followed by 100 µg of GnRH administered on day −5, and a 25-mg $PGF_{2\alpha}$ injection at CIDR insert removal (**7D**) on day 0; or (2) 100 µg of GnRH and insertion of previously used autoclaved CIDR on day −5 and a 25-mg $PGF_{2\alpha}$ injection at CIDR removal (**5D**) on day 0. Artificial insemination occurred after detected estrus from days 0 to 3. Those heifers not detected in estrus were inseminated on day 3 (72 hours after $PGF_{2\alpha}$) and given a second 100-µg dose of GnRH (72 hours after CIDR removal). Blood collected on days −7 and −5 was assayed to determine concentrations of progesterone and presence of a CL (progesterone ≥1 ng/mL) on d −7. Blood progesterone concentrations on days 0 and 3 were used to determine if luteolysis occurred in all heifers. Pregnancy was determined on days 32 and 60 and intervening pregnancy loss was calculated. Of those heifers in the 7D treatment having progesterone ≥1 ng/mL on day −7, the proportion having progesterone <1 ng/mL 2 days later (luteolysis) was greater ($P < 0.05$) than that in the 5D treatment (43.0 vs. 22.9%), respectively. A treatment by location interaction was detected for pregnancies per AI. The Kansas location had no detectable treatment differences. In contrast, the 7D treatment produced more ($P < 0.05$) pregnancies in the first replicate of the Florida location and at the Mississippi location. We concluded that the 5D protocol was not more effective in producing acceptable luteolysis, pregnancy, and ovulation rates compared with the modified 7D protocol.

Key words: CIDR, progesterone, timed artificial insemination (TAI)

Introduction

Since 1997, it has been known that dairy heifers do not respond as well as lactating dairy cows to gonadotropin-releasing hormone + prostaglandin $F_{2\alpha}$ (**GnRH + $PGF_{2\alpha}$**) protocols to synchronize estrus, ovulation, or both. For example, a multi-site study demonstrated that heifers treated after Ovsynch (GnRH injection 7 days before and 48 hours after $PGF_{2\alpha}$ with timed AI administered 72 hours after $PGF_{2\alpha}$) averaged 35% conception compared with non-treated heifers inseminated after estrus (74%).

A recent study in suckled beef cows was the first to reduce the interval from GnRH to $PGF_{2\alpha}$ from 7 to 5 days. The authors hypothesized that reducing the interval between injections would allow the maturing ovulatory follicle to develop during a longer proestrus in an environment with decreased progesterone. Pregnancies per AI (**P/AI**) in the 7-day program were 59% and increased to 70% in the 5-day program. Furthermore, differences between the 7- and 5-day

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programs in beef heifers in another study showed a tendency for greater P/AI in the 5-day treatment (62.5%) compared with the 7-day program (52%).

When GnRH induced ovulation of follicles in a 5-day program, the resulting corpus luteum (**CL**) may not undergo luteolysis when an injection of $PGF_{2\alpha}$ is administered 5 days after GnRH. When a similar 5-day controlled internal drug release (**CIDR**) program was administered in dairy heifers, however, 1 injection of $PGF_{2\alpha}$ yielded similar P/AI (46.4%) when compared with 2 injections of $PGF_{2\alpha}$ given 12 hours apart (48.6%).

The hypothesis for the current study was that the 5-day CO-Synch + CIDR protocol would increase P/AI in treated dairy heifers and synchronization of estrus and ovulation would serve as a useful management tool to facilitate timed artificial insemination (**TAI**) in heifers. The objectives were to: (1) gauge the effectiveness of an injection of $PGF_{2\alpha}$ to regress the corpus luteum before initiating a TAI program, (2) gauge ovulation response to GnRH, (3) determine the ovulation response to the first injection of GnRH in both treatments, and (4) assess pregnancy outcomes.

Experimental Procedures

This experiment was conducted at 3 locations: (1) Kansas State University Dairy Teaching and Research Center in Manhattan, (2) a commercial dairy farm in Marianna, FL, and (3) Mississippi State University Bearden Dairy Research Center in Starkville. Heifers no less than 355 days of age were assigned randomly to receive either a 5- or 7-day synchronization program, both of which incorporated an intravaginal CIDR (Pfizer Animal Health, New York, NY) insert (Figure 1). The CIDR inserts were used once previously for 7 days and were then cleaned and autoclaved.

On day −7 of the experiment, heifers in the 7-day treatment (**7D**) received an injection of 25 mg $PGF_{2\alpha}$ i.m. (5 mL Lutalyse, Pfizer Animal Health). On day −5, heifers received a 100-μg injection of GnRH i.m. (2 mL Factrel, Pfizer Animal Health). In the 5-day treatment (**5D**), heifers received the first GnRH injection at the time of CIDR insertion on day −5. The CIDR insert was removed from heifers in both treatments on day 0 concurrent with a 25-mg injection of $PGF_{2\alpha}$. Blood samples were collected from all heifers via coccygeal venipuncture before administration of injections on days −7, −5, 0, and 3 to measure concentration of progesterone.

Heifers were inseminated artificially with frozen-thawed semen based on either standing estrus from days 0 to 3 or at 72 hours post-CIDR removal (TAI). Heifers receiving the TAI also received injection of GnRH. Heifers inseminated based on standing estrus did not receive a second GnRH injection. Pregnancy was diagnosed 32 days later by transrectal ultrasonography based on the presence of uterine fluid or presence of a viable embryo. Pregnancy was reconfirmed approximately 4 weeks later.

Holstein heifers in Kansas enrolled in the experiment averaged 402 ± 18 days of age and 942 ± 82 lb body weight at enrollment. Heifers were fed a total mixed ration consisting of prairie hay, corn, soybean meal, corn silage, minerals, and vitamins, with water provided ad libitum. Feed was delivered to feed bunks twice daily and heifers were housed in dirt lots with a concrete apron next to the feed bunk. The experiment was conducted in 19 replicates (n = approximately 10 heifers/replicate) from October 2009 through January 2011. Some heifers detected as not pregnant were re-enrolled in the same treatment up to 2 additional times. Heifers at this location were inseminated with gender-biased semen.

Jersey, Holstein, or Jersey × Holstein heifers in Florida were enrolled in the experiment. At enrollment, body condition scores (**BCS**; 1=thin, 5=fat) of heifers averaged 3.0 ± 0.3. The experiment was conducted in 3 replicates from January through April 2010. Heifers at this location were randomly assigned to receive either conventional or gender-biased semen. An additional group of Jersey, Holstein, or Jersey × Holstein heifers were treated in 3 replicates from December 2010 through March 2011. Heifers at this location were inseminated using conventional semen.

Holstein and Jersey heifers in Mississippi enrolled in the experiment averaged 476 ± 60 days of age and 746 ± 91 lb body weight. The experiment was conducted in 3 replicates from December 2010 through January 2011. Heifers at this location were inseminated using gender-biased semen.

Results and Discussion

Concentrations of serum progesterone on d −7, −5, 0, and 3 are represented in Figure 2. At the onset of treatments, progesterone concentrations did not differ on days −7 or −5. Of those heifers in the 7D treatment having progesterone ≥1 ng/mL on day −7, the proportion having progesterone <1 ng/mL 2 days later (luteolysis) was greater ($P < 0.05$) than that in the 5D treatment (43.0 vs. 22.9%, respectively).

The largest follicle detected in heifers on day −5 did not differ between the 7D and 5D treatments (12.7 ± 0.3 vs. 12.1 ± 0.3 mm), respectively, but more ($P < 0.001$) of the largest follicles ovulated in response to GnRH on day −5 in the 7D than the 5D treatment (47.2 vs. 27.6%). The second-largest follicle did not differ in diameter between the 7D and 5D treatments (10.0 ± 0.3 vs. 9.8 ± 0.3 mm), respectively, or in its ovulatory response to GnRH (16.1 vs. 10.9%). In contrast, total ovulatory response of the 2 largest follicles was greater ($P < 0.001$) in the 7D vs. 5D heifers (51.1 vs. 30.4%).

The 7D treatment had decreased ($P < 0.05$) progesterone concentrations on day 0 compared with the 5D treatment, but this difference did not persist through day 3 at TAI. Luteolysis in response to $PGF_{2\alpha}$ on day 0 indicated that luteolysis occurred in 90.1% of heifers in the 7D treatment and did not differ from that of 88.6% of heifers in the 5D treatment.

Pregnancies per AI determined 32 days post-AI are illustrated for the 3 locations in Figure 3. The Kansas location had no detectable treatment differences. In contrast, the 7D treatment produced greater ($P < 0.05$) pregnancy rates in the first replicate of the Florida location and at the Mississippi location (Figure 3).

With estrus detection being performed from d 0 until 72 hours after CIDR removal, 166 heifers (30.6%) were inseminated before TAI, and 39.2% of early-inseminated heifers became pregnant. The remainder of the 376 heifers submitted to TAI at 72 hours after CIDR removal and 26.2% became pregnant. The P/AI for heifers inseminated at estrus and for those receiving TAI differed ($P = 0.006$).

Pregnancies per AI in heifers having elevated (≥ 1 ng/mL) progesterone on day −7 were 36% (n = 345) and differed (P < 0.05) from those having decreased progesterone (< 1 ng/mL; 20%, n = 194). This difference also existed for heifers having elevated progesterone on day −5 (33%, n = 41 vs. 20%, n = 105). For heifers having decreased vs. increased progesterone at TAI, P/AI was increased (P = 0.006) from 12% (n = 51) to 32% (n = 460). Pregnancy loss calculated between 32 and 60 days post-AI was minimal (between 2.7 and 4.4%) and did not differ between treatments, although insufficient numbers of observations precluded detection of any differences.

We concluded that treatment of dairy heifers with the 5D Co-Synch + CIDR protocol failed to increase P/AI compared with the modified 7D protocol used in the current study. The P/AI were similar in one Florida and the Kansas locations, but favored the 7D treatment in the second Florida and Mississippi locations. With the majority of semen used in the study being gender-biased, P/AI were expected decrease as reported in other studies. The combination of synchronization and use of gender-biased semen are the probable cause of the reduced P/AI, because previous studies used conventional semen at TAI. The potential for increased pregnancy rates with the use of the 5D CIDR program has been shown in previous studies, but various protocols have produced mixed results. This variability indicates that further studies are required to identify a reliable TAI program for dairy heifers.

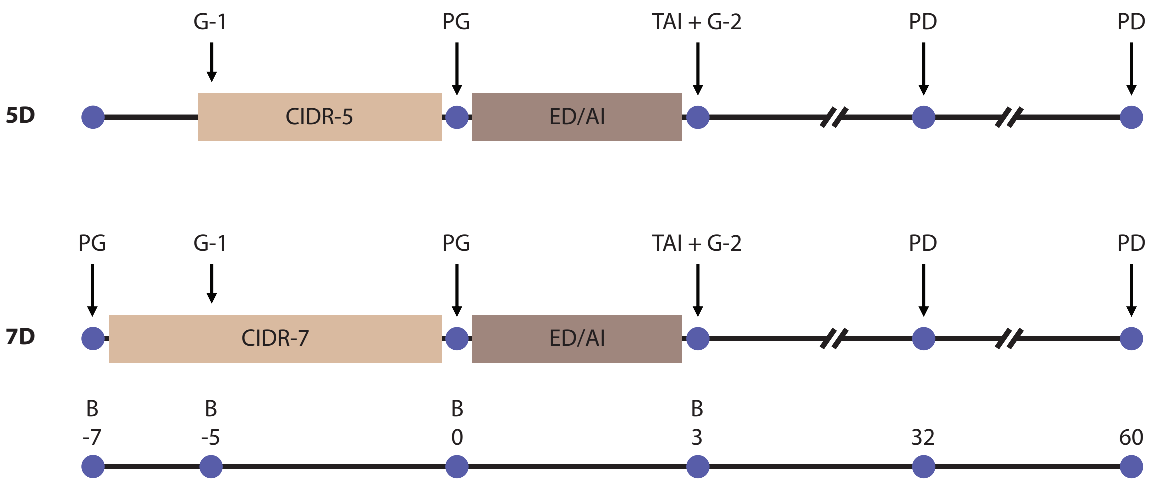

Figure 1. Experimental design of treatments.
Both treatments consisted of a gonadotroin-releasing hormone (GnRH; G1) injection at day -5 with $PGF_{2\alpha}$ injection on day 0. The 7D treatment also included a controlled internal drug release (CIDR) insert on day -7 concurrent with a prostaglandin $F_{2\alpha}$ (PG) injection, whereas the 5D treatment included a CIDR on day -5 at GnRH-1. Estrus detection (ED) and AI at detected estrus occurred from days 0 to 3. Those heifers not detected in estrus were time-inseminated (TAI) on day 3 (72 hours after PG) and concurrent with the second GnRH (GnRH-2) injection. Pregnancy was diagnosed on day 32 by ultrasound and heifers detected as not pregnant were re-treated up to 2 times on the same treatment in some cases. A second pregnancy diagnosis was conducted 4 weeks later to calculate pregnancy loss since the first positive pregnancy diagnosis. GnRH = 100 µg of GnRH, PG = 25 mg of $PGF_{2\alpha}$, US = transrectal ultrasonography, and B = blood collection.

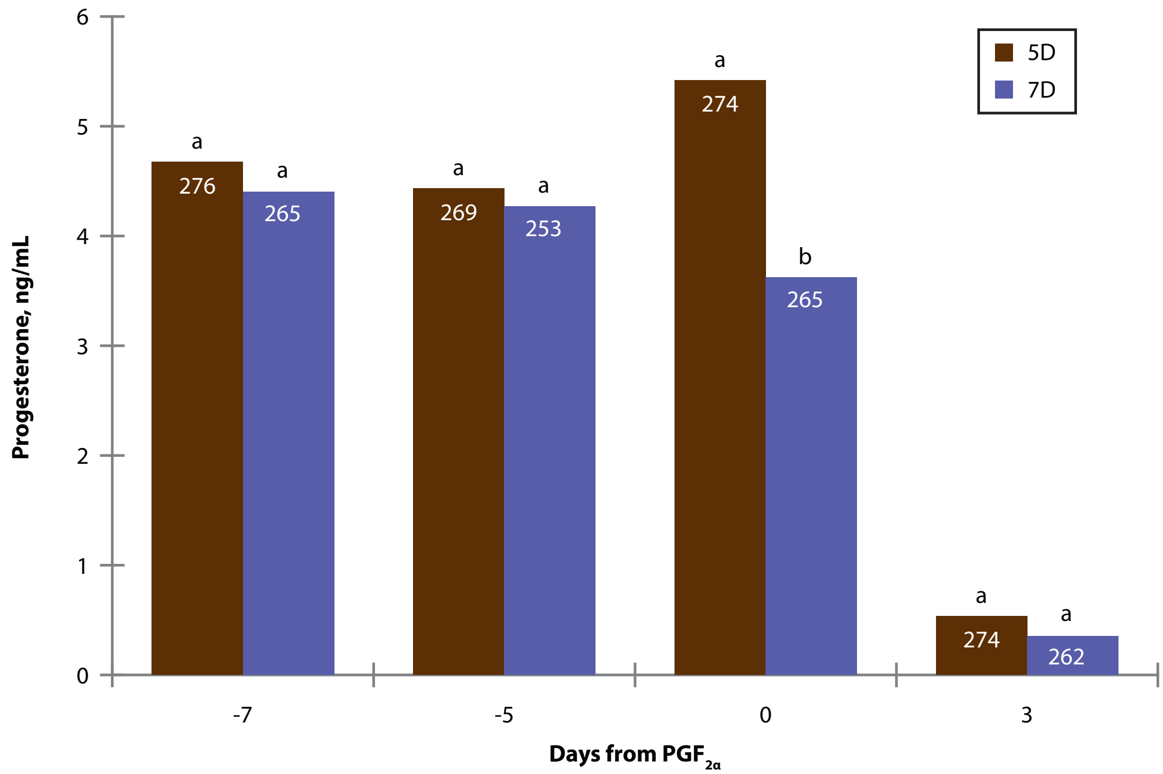

Figure 2. Concentrations of serum progesterone in heifers from day of common $PGF_{2\alpha}$ treatment (day 0) in the 5D and 7D treatments.
[a-b] Means within experimental day having different letters differ ($P < 0.05$).

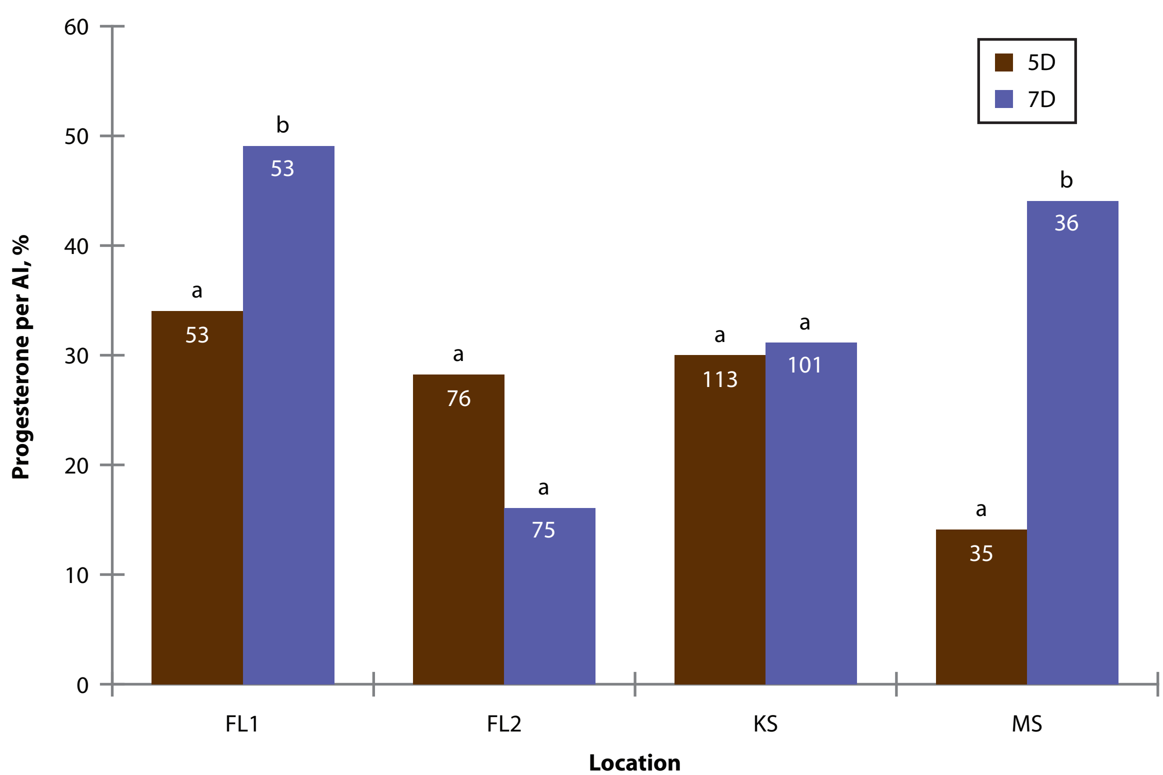

Figure 3. Pregnancy rates of heifers at 32 days post-AI.
[a-b] Means within location having different letters differ ($P < 0.05$).