Prostaglandin F_{2α} 7 d prior to initiation of the 7-d CO-synch + CIDR protocol failed to enhance estrus response and pregnancy rates in beef heifers

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ABSTRACT: To determine the effects of administration of 25 mg of PGF_{2α} 7 d prior to the initiation of the 7-d CO-Synch + controlled internal drug release (CIDR) fixed-time AI (TAI) protocol, 985 *Bos taurus* beef heifers were enrolled in a completely randomized design at 9 locations from April to July of 2016. Within location, all heifers were randomly assigned to 1 of 2 treatments: 1) CONTROL ($n = 496$); 100 µg injection of GnRH and a CIDR insert for 7 d [day 7], administration of 25 mg of PGF_{2α} at CIDR removal [day 0], followed by a second injection of GnRH and TAI 54 ± 2 h later; or 2) PRESYNCH ($n = 489$); same as CONTROL but heifers received an additional injection of 25 mg of PGF_{2α} 7 d prior [day 14] to CIDR insertion. Estrous detection patches were applied to all heifers on day 14 and were evaluated for estrual activity on day 7. Similarly, estrus alert patches were placed on all heifers on day 0 and evaluated for estrual activity at the time of TAI.

Pregnancy was diagnosed via transrectal ultrasonography between 35 and 55 d after TAI. The percentage of heifers exhibiting estrus between days 14 and 7 was greater ($P < 0.001$) for the PRESYNCH ($70.1 \pm 2.4\%$) than the CONTROL ($41.1 \pm 2.3\%$) treatment, whereas the percentage of heifers exhibiting estrus between day 0 and TAI was greater ($P < 0.001$) for the CONTROL ($55.6 \pm 2.4\%$) than the PRESYNCH ($39.7 \pm 2.5\%$) treatment. Estrus response rates differed ($P < 0.001$) among locations. Pregnancy rates to TAI differed ($P = 0.023$) among locations; however, they did not differ ($P = 0.739$) between CONTROL and PRESYNCH treatments (45.4 ± 2.5 vs. $43.2 \pm 2.5\%$, respectively). Final breeding season pregnancy rates did not differ ($P = 0.811$) between treatments. Therefore, an injection of PGF_{2α} 7 d prior to initiation of the 7-d CO-Synch + CIDR protocol failed to improve pregnancy rates to TAI in replacement beef heifers.

Key words: beef heifer, presynchronization, prostaglandin F_{2α}

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INTRODUCTION

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By controlling the bovine estrous cycle with exogenous hormones, fixed-time artificial insemination (TAI) may be facilitated, and the time and labor associated with the detection of estrus may be reduced. Presynchronization involves the administration of preliminary hormones prior to the initiation of a synchronization of

estrus protocol. The utilization of GnRH or PGF_{2α} before the commencement of a TAI protocol may increase the proportion of dominant follicles that respond to the first GnRH injection and may therefore improve the synchronization of the subsequent follicular growth waves (Kojima et al., 2000; Busch et al., 2007; Atkins et al., 2008). Presynchronization may also synchronize estrus more effectively, with resulting greater fertility (Patterson et al., 2003). In beef heifers, administration of PGF_{2α} 3 d prior to a TAI protocol increased the percentage of heifers initiating a new follicular wave and increased the synchrony of estrus after controlled internal drug release (CIDR) removal (Grant et al., 2011). Presynchronization with PGF_{2α} has the potential to improve follicle turnover (Perry et al., 2012; Hill et al., 2014), estrus synchrony (Grant et al., 2011), and pregnancy rates (Perry et al., 2012) in beef cattle.

The study was performed to evaluate the effects of an injection of PGF_{2α} 7 d prior to the initiation of the 7-d CO-Synch + CIDR protocol on fertility in replacement beef heifers. We hypothesized that an injection of PGF_{2α} 7 d before the initiation of the 7-d CO-Synch + CIDR protocol would improve pregnancy rates to TAI (PR/AI) by increasing the estrus response between days 14 and 7, thereby increasing the proportion of heifers that respond to the PGF_{2α} injection on day 0.

MATERIALS AND METHODS

All heifers were handled in accordance with procedures approved by each collaborating University's Animal Care and Use Committee.

Animals and Treatments

A total of 985 Angus-based *Bos taurus* beef heifers were enrolled across 9 locations (CO, ND,

SD-1, SD-2, SD-3, SD-4, SD-5, SD-6, and SD-7) in 3 states (South Dakota, North Dakota, and Colorado). Herd size ranged from 50 to 220 heifers. Within location, heifers were randomly assigned to 1 of 2 treatments (Figure 1): 1) estrous detection patches (Estroject; Rockway Inc., Spring Valley, WI) were applied on day 14, 100 µg of GnRH (Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parsippany, NJ) was administered at CIDR (Eazi-BREED CIDR; 1.38 g P4; Zoetis Animal Health) insertion on day 7, 25 mg of PGF_{2α} (Lutalyse, dinoprost tromethamine; Zoetis Animal Health) was administered at CIDR removal concurrently with a second estrous detection patch application on day 0, followed 54 ± 2 h later by the administration of 100 µg of GnRH and TAI (CONTROL; *n* = 496); or 2) heifers were treated the same as the CONTROL treatment with the addition of a 25 mg injection of PGF_{2α} 7 d prior to CIDR insertion on day 14 (PRESYNCH; *n* = 489). Heifer BW was recorded on day 7 of the protocol at 5 locations (ND, SD-1, SD-3, SD-4, and SD-7). Estrous detection patches were examined for activation on day 7 (EST1) and at the time of TAI (EST2). Estrous detection patches were considered activated when at least 50% of the rub-off coating was removed, or when the patch was absent. Conventional frozen semen was utilized for all inseminations. No less than 10 d after TAI, heifers were exposed to bulls for the remainder of the breeding season at 7 locations (CO, ND, SD-1, SD-2, SD-3, SD-4, and SD-7).

Pregnancy Diagnosis

Transrectal ultrasonography (5.0-MHz linear array transducer, Aloka 500V, Instrument of Science and Medicine, Vancouver, BC, Canada; or Ibex portable ultrasound 5.0-MHz linear multi-frequency transducer, Ibex, E.I. Medical Imaging, Loveland, CO) was performed by 2 experienced

Figure 1. Schematic of treatments, indicating that CONTROL (*n* = 496) heifers received an estrous detection patch (Estroject; Rockway Inc., Spring Valley, WI) on day 14, an injection of GnRH (Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parsippany, NJ) and a CIDR (Eazi-BREED CIDR; 1.38 g P4; Zoetis Animal Health) insert on day 7, an injection of PGF_{2α} (Lutalyse, dinoprost tromethamine; Zoetis Animal Health) and an estrous detection patch at CIDR removal on day 0, and an injection of GnRH followed by TAI 54 ± 2 h after CIDR removal. PRESYNCH heifers (*n* = 489) were treated the same as CONTROL with the addition of a PGF_{2α} injection administered on day 14. Estrous detection patches were examined for activation on day 7 (EST1) and at the time of TAI (EST2). Pregnancy diagnosis was performed by ultrasonography between days 35 and 55 after TAI. Final pregnancy diagnosis was performed at least 35 d after the end of the breeding season.

veterinarians between days 35 and 55 after TAI to determine the presence of a conceptus heartbeat, thereby assessing PR/AI. Final overall pregnancy rates were determined by transrectal ultrasonography at least 35 d after the end of the breeding season.

Statistical Analyses

The SAS (version 9.4; SAS/STAT, SAS Inst. Inc., Cary, NC, USA) statistical package was used for all statistical analyses. Pregnancy rates to TAI, EST1, EST2, and final pregnancy rates were analyzed using the GLIMMIX procedure of SAS. The first model accounted for the fixed effects of treatment (CONTROL and PRESYNCH), location ($n = 9$), and the treatment $\times$ location interaction. Artificial insemination sire and AI technician were distributed evenly among treatments; therefore, these variables were not included in the model. A second model to determine the impact of estrus status prior to insertion of a CIDR (EST1) and the impact of estrus between CIDR removal and TAI (EST2) on pregnancy rates and estrus expression rates included the fixed effects of EST1, EST2, and the EST1 $\times$ EST2 interaction. Heifer was considered the experimental unit. Statistical differences were considered significant

at $P \leq 0.05$, with $0.05 < P < 0.10$ considered a tendency.

RESULTS AND DISCUSSION

Body weight differed ($P < 0.001$) between locations; however, it did not differ ($P = 0.753$) between treatments (405.5 ± 2.1 kg) or between treatments within location ($P = 0.337$).

Synchrony of Estrus

Estrus response for all heifers between days 14 and 7 is summarized in Table 1. Between estrus patch application on day 14 and patch examination on day 7, estrus expression differed ($P < 0.001$) between treatments. Estrus expression was greater in PRESYNCH compared with CONTROL treatments and differed among locations ($P < 0.001$). The percentage of heifers exhibiting estrus behavior between days 14 and 7 ranged from 47.3% to 79.7% among locations; however, no treatment $\times$ location interaction was detected ($P = 0.567$). These results are similar to those previously reported in which beef heifers were exposed to a single PGF_{2 α} injection at a random point in their estrous cycle, and estrus response (1 to 9 d after PGF_{2 α} administration) was significantly greater in heifers receiving

Table 1. Estrus response on day 7 in replacement beef heifers that either received or did not receive an injection of PGF_{2 α} 7 d before initiation of estrus synchronization

Item	Treatment ^a		Overall	SEM ^b	P-value ^c
	CONTROL	PRESYNCH			
	n/n (%)				
Location					
SD-1	9/25 (36.0)	17/25 (68.0)	26/50 (52.0) ^{yz}	13.2	0.016
SD-2	9/28 (32.1)	19/28 (67.9)	28/56 (50.0) ^{yz}	12.5	0.004
SD-3	29/70 (41.4)	44/70 (62.9)	73/140 (52.1) ^{yz}	7.9	0.007
SD-4	12/32 (37.5)	18/28 (64.3)	30/60 (50.0) ^{yz}	12.1	0.027
SD-5	20/64 (31.3)	47/63 (74.6)	67/127 (52.8) ^{yz}	8.3	<0.001
SD-6	14/41 (34.2)	28/42 (66.6)	42/83 (50.6) ^{yz}	10.3	0.002
SD-7	40/111 (35.9)	64/109 (58.6)	104/220 (47.3) ^z	6.4	<0.001
ND	46/67 (68.7)	60/66 (90.9)	106/133 (79.7) ^x	8.1	0.006
CO	25/58 (43.1)	46/58 (79.3)	71/116 (61.2) ^y	8.7	<0.001
Overall ^d	204/496 (41.1)	343/489 (70.1)		3.3	<0.001

^aCONTROL: heifers received a 100- μ g injection of GnRH (Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parsippany, NJ) at CIDR (Eazi-Breed CIDR; 1.38 g P4; Zoetis Animal Health) insertion [day 7], a 25-mg injection of PGF_{2 α} (Lutalyse, dinoprost tromethamine; Zoetis Animal Health) administered at CIDR removal [day 0], and an injection of GnRH and fixed-timed AI (TAI) 54 ± 2 h later. PRESYNCH: treated the same as CONTROL, but received an additional 25-mg injection of PGF_{2 α} 7 d prior to the first GnRH injection [day 14]. Estrous detection patches (Estroject; Rockway Inc., Spring Valley, WI) are applied to all heifers on day 14 and are examined for activation on day 7.

^bSEM: Standard error of the mean between CONTROL and PRESYNCH treatments within row.

^cP-value represents the difference between CONTROL and PRESYNCH treatments within row.

^dOverall estrus response on day 7 differed ($P < 0.001$) between treatments.

^{x,y,z}Percentages within column with different superscripts differ ($P \leq 0.05$).

a treatment compared with controls (Lauderdale, 2002). It has been well documented that administration of $\text{PGF}_{2\alpha}$ induces regression of the CL, and as a result the bovine female returns to estrus within approximately 3 d (Tervit et al., 1973; Lauderdale et al., 1974; Louis et al., 1974). Our results indicate that $\text{PGF}_{2\alpha}$ in the PRESYNCH heifers was successful at inducing CL regression, thereby inducing greater estrus expression during days 14 and 7 compared with CONTROL heifers.

Estrus response rates for all heifers within location between day 0 and TAI are summarized in Table 2. Expression of estrus between estrus patch application on day 0 and patch examination at TAI differed ($P < 0.001$) between treatments, with greater estrus expression in CONTROL compared with PRESYNCH heifers. Although estrus response at TAI ranged from 35.4% to 65.1% among locations, no treatment $\times$ location interaction was detected ($P = 0.408$). Heifers that failed to express estrus at EST1 had greater ($P < 0.001$) estrus expression at EST2 compared with those that expressed estrus at EST1 (58.1 ± 2.5 vs. $38.7 \pm 2.1\%$).

Administration of $\text{PGF}_{2\alpha}$ in cows resulted in ovulation occurring approximately 95 ± 5 h after administration (Hafs et al., 1974; Louis et al., 1974). In the present study, heifers that exhibited estrus during the period of days 14 to 7 may have ovulated within the first few days after the

administration of $\text{PGF}_{2\alpha}$, subsequently initiating a new follicular wave, and inducing CL formation. Follicle size at the time of the first GnRH administration (day 7) was not determined in the current study; however, results from a previous study conducted in beef heifers indicate that smaller follicles (≤ 10.1 mm) are less likely to ovulate in response to exogenous GnRH than larger follicles (≥ 12.1 mm; Perry et al., 2007). Similarly, ovulation was only induced when follicles were approximately 9 mm or greater (Martinez et al., 1999). In the present study, if the follicle was not able to respond to the GnRH injection on day 7, it may have undergone atresia between days 7 and 0, and as a result, a new follicular wave may have been initiated (follicle turnover). We speculate that the dominant follicle resulting from this new follicular wave may not have developed sufficiently to induce behavioral estrus before TAI. Therefore, the dominant follicle may have required a longer interval between CIDR removal and TAI to induce expression of estrus.

Since a greater percentage of heifers in the PRESYNCH treatment expressed estrus prior to CIDR insertion, a greater proportion of heifers in that treatment also likely had a CL at CIDR removal when $\text{PGF}_{2\alpha}$ was administered. Thus, it is likely that concentrations of P4 were greater in PRESYNCH heifers at CIDR removal than in CONTROL heifers. Beef cows with elevated

Table 2. Estrus response at the time of TAI in replacement beef heifers that either received or did not receive an injection of $\text{PGF}_{2\alpha}$ 7 d before initiation of estrus synchronization

Item	Treatment ^a		Overall	SEM ^b	P-value ^c
	CONTROL	PRESYNCH			
	n/n (%)				
Location					
SD-1	15/25 (60.0)	12/25 (48.0)	27/50 (54.0) ^{wxy}	13.7	0.381
SD-2	19/28 (67.9)	13/28 (46.4)	32/56 (57.1) ^{wx}	12.9	0.098
SD-3	40/70 (57.1)	23/70 (32.9)	63/140 (45.0) ^{xyz}	8.2	0.003
SD-4	16/32 (50.0)	7/28 (25.0)	23/60 (38.3) ^{yz}	12.5	0.046
SD-5	31/64 (48.4)	14/63 (22.2)	45/127 (35.4) ^z	8.6	0.002
SD-6	26/41 (63.4)	28/42 (66.7)	54/83 (65.1) ^w	10.6	0.760
SD-7	45/111 (40.5)	36/109 (33.0)	81/220 (36.8) ^z	6.5	0.250
ND	44/66 (66.7)	32/66 (48.5)	76/132 (57.6) ^w	8.4	0.031
CO	39/58 (67.2)	29/58 (50.0)	68/116 (58.6) ^w	9.0	0.055
Overall ^d	275/495 (55.6)	194/489 (39.7)		3.4	<0.001

^aCONTROL: heifers received a 100- μg injection of GnRH (Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parsippany, NJ) at CIDR (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health) insertion [d -7], a 25-mg injection of $\text{PGF}_{2\alpha}$ (Lutalyse, dinoprost tromethamine; Zoetis Animal Health) administered at CIDR removal [day 0], and an injection of GnRH and fixed-timed AI (TAI) 54 ± 2 h later. PRESYNCH: treated the same as CONTROL, but received an additional 25-mg injection of $\text{PGF}_{2\alpha}$ 7 d prior to the first GnRH injection [day 14]. Estrous detection patches (Estrotect; Rockway Inc., Spring Valley, WI) are applied on day 0 and are examined for activation at the time of TAI.

^bSEM: Standard error of the mean between CONTROL and PRESYNCH treatments within row.

^cP-value represents the difference between CONTROL and PRESYNCH treatments within row.

^dOverall estrus response at the time of TAI differed ($P < 0.001$) between treatments.

^{w,x,y,z}Percentages within column with different superscripts differ ($P \leq 0.05$).

concentrations of P4 were shown simultaneously to have smaller follicles at CIDR removal (Mercadante et al., 2015). Smaller follicles are associated with reduced estrogen release and thus reduced estrus expression. In a study conducted in dairy heifers, the concurrent use of 2 CIDRs resulted in the ovulatory follicle appearing later in the estrous cycle compared with heifers receiving 1 or no CIDR (Sirois and Fortune, 1990). The greater concentrations of serum P4 may have resulted in an ovulatory follicle requiring a longer interval to ovulation from CIDR removal.

The CONTROL heifers were at random stages of the estrous cycle at the time of GnRH administration on day 7, and previous reports indicate that the response to GnRH, and subsequent initiation of a new follicular wave, can range from 38% to 60% in heifers (Macmillan and Thatcher, 1991; Pursley et al., 1995; Dahlen et al., 2011a; Dahlen et al., 2011b). CONTROL heifers that did not have follicles responding to the GnRH injection likely had larger follicles at CIDR removal, resulting in dominant follicles large enough to induce an estrus response between day 0 and TAI. We speculate that a larger proportion of the PRESYNCH heifers may have had smaller follicles on day 0 compared with CONTROL heifers, and as a result may have required a longer interval between CIDR removal and TAI to exhibit estrus.

Fertility

Pregnancy rates to TAI for all heifers are summarized in Table 3. Although heifers in the PRESYNCH treatment had a lower estrus response between day 0 and TAI compared with those in the CONTROL treatment, PR/AI did not differ ($P = 0.740$). In contrast, there was an effect of location ($P = 0.023$) on PR/AI, which ranged from 37.8% to 61.7%. No treatment $\times$ location interaction was detected ($P = 0.686$). At the conclusion of the breeding season, final pregnancy rates did not differ ($P = 0.811$) between CONTROL and PRESYNCH treatments, respectively. Final pregnancy rates differed ($P < 0.001$) among location and ranged from 78.3% to 97.9% (Table 4).

Treatment of cycling cows and heifers with low doses of a progestin (between 1 and 2 ng/mL) may result in the formation of persistent follicles (Zimbelman and Smith, 1966; Sirois and Fortune, 1990). When persistent follicles ovulate, they have been associated with reduced fertility (Mihm et al., 1994). Similarly, follicular development has been altered in heifers treated with a single CIDR. The low concentrations of P4 maintained by the presence of a single CIDR in dairy heifers have been reported to promote the prolonged development of a follicle and resulted in the complete absence of follicular recruitment (Sirois and Fortune, 1990). Greater concentrations of serum P4 resulting from

Table 3. Pregnancy rates to TAI in replacement beef heifers that either received or did not receive an injection of PGF_{2 α} 7 d before initiation of estrus synchronization

Item	Treatment ^a		Overall	SEM ^b	P-value ^c
	CONTROL	PRESYNCH			
	n/n (%)				
Location					
SD-1	13/25 (52.0)	8/25 (32.0)	21/50 (42.0) ^{xz}	14.0	0.154
SD-2	12/28 (42.9)	13/28 (46.4)	25/56 (44.6) ^{xy}	13.2	0.787
SD-3	34/70 (48.6)	29/70 (41.4)	63/140 (45.0) ^{xyz}	8.4	0.394
SD-4	17/32 (53.1)	20/28 (71.4)	37/60 (61.7) ^x	12.8	0.154
SD-5	24/64 (37.5)	24/63 (38.1)	48/127 (37.8) ^z	8.8	0.946
SD-6	21/41 (51.2)	23/42 (54.8)	44/83 (53.0) ^{xy}	10.9	0.745
SD-7	46/111 (41.4)	38/109 (34.9)	84/220 (38.2) ^z	6.7	0.325
ND	28/67 (41.8)	28/66 (42.4)	56/133 (42.1) ^{yz}	8.6	0.941
CO	30/58 (51.7)	28/58 (48.3)	58/116 (50.0) ^{xyz}	9.2	0.708
Overall	225/496 (45.4)	211/489 (43.2)		3.5	0.740

^aCONTROL: heifers received a 100- μ g injection of GnRH (Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parsippany, NJ) at CIDR (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health) insertion [d -7], a 25-mg injection of PGF_{2 α} (Lutalyse, dinoprost tromethamine; Zoetis Animal Health) administered at CIDR removal [day 0], and an injection of GnRH and fixed-timed AI (TAI) 54 $\pm$ 2 h later. PRESYNCH: treated the same as CONTROL, but received an additional 25-mg injection of PGF_{2 α} 7 d prior to the first GnRH injection [day 14]. Pregnancy diagnosis is performed by ultrasonography between days 35 and 55 after TAI.

^bSEM: Standard error of the mean between CONTROL and PRESYNCH treatments within row.

^cP-value represents the difference between CONTROL and PRESYNCH treatments within row.

^{x,y,z}Percentages within column with different superscripts differ ($P \leq 0.05$).

Table 4. Final breeding season pregnancy rates in replacement beef heifers that either received or did not receive an injection of PGF_{2α} 7 d before initiation of estrus synchronization

Item	Treatment ^a		Overall	SEM ^b	P-value ^c
	CONTROL	PRESYNCH			
	n/n (%)				
Location					
SD-1	14/16 (88.2)	13/17 (75.0)	27/33 (81.8) ^{yz}	8.6	0.125
SD-2	28/29 (96.4)	26/27 (96.4)	54/56 (96.4) ^x	6.6	1.000
SD-3	69/70 (98.6)	68/70 (97.1)	137/140 (97.9) ^x	4.2	0.732
SD-4	22/32 (68.8)	25/28 (89.3)	47/60 (78.3) ^z	6.4	0.001
SD-5 ^d	—	—	—	—	—
SD-6 ^d	—	—	—	—	—
SD-7	105/111 (94.6)	103/109 (94.5)	208/220 (94.6) ^x	3.3	0.976
ND	26/29 (89.7)	26/28 (92.9)	52/57 (91.2) ^{xy}	6.5	0.625
CO	55/58 (94.8)	55/58 (94.8)	110/116 (94.8) ^x	4.6	1.000
Overall	319/345 (92.5)	316/337 (93.8)		2.3	0.571

^aCONTROL: heifers received a 100-μg injection of GnRH (Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parsippany, NJ) at CIDR (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health) insertion [d -7], a 25-mg injection of PGF_{2α} (Lutalyse, dinoprost tromethamine; Zoetis Animal Health) administered at CIDR removal [day 0], and an injection of GnRH and fixed-timed AI (TAI) 54 ± 2 h later. PRESYNCH: treated the same as CONTROL, but received an additional 25-mg injection of PGF_{2α} 7 d prior to the first GnRH injection [day 14]. Final pregnancy diagnosis was performed at least day 35 after the end of the breeding season.

^bSEM: Standard error of the mean between CONTROL and PRESYNCH treatments within row.

^cP-value represents the difference between CONTROL and PRESYNCH treatments within row.

^dHeifers at SD-5 and SD-6 are not exposed to clean-up bulls after TAI; therefore, they are not included in overall pregnancy diagnosis analyses.

^{x,y,z}Percentages within column with different superscripts differ ($P \leq 0.05$).

the use of 2 CIDRs were shown to reverse persistent follicle growth (Sirois and Fortune, 1990). In this study, it is plausible that CONTROL heifers had lower concentrations of P4 between days 7 and d 0, and thus a greater incidence of persistent follicles, which may have led to a reduction in PR/AI. In contrast, the presence of both a CL and a CIDR between days 7 and 0 as a result of the PGF_{2α} injection on day 14 in PRESYNCH heifers may have resulted in increased concentrations of serum P4. The increased concentration of P4 likely curtailed the incidence of persistent follicles and may be one reason contributing to similar PR/AI achieved in the two treatments.

Expression of estrus prior to TAI has been reported to increase PR/AI in beef heifers (Perry et al., 2007). In this study, expression of estrus was greater in CONTROL heifers, and thus, PR/AI should have been reduced in the PRESYNCH heifers that had poorer estrus expression between day 0 and TAI. Because similar PR/AI were achieved, it is possible that by increasing the length of proestrus before TAI in the PRESYNCH group, an increase in estrus expression could have occurred and potentially resulted in increased PR/AI.

Another contributing factor for the similar PR/AI between the treatments is that PR/AI in the PRESYNCH group may have been reduced as a result of smaller follicles being present at the time

of GnRH administration at TAI. As previously mentioned, follicles in beef heifers were only able to undergo induced ovulation by exogenous GnRH when they were ≈9 mm or greater (Martinez et al., 1999). In addition, follicle size has been shown to be associated with oocyte maturity (Driancourt and Thuel, 1998) and fertility in heifers (Perry et al., 2005). Gonadotropin-releasing hormone-induced ovulation of smaller follicles has resulted in decreased pregnancy rates and decreased embryonic survival in beef cows (Lamb et al., 2001; Perry et al., 2005; Atkins et al., 2013). Therefore, it is plausible that PR/AI may have increased in PRESYNCH heifers if the interval between day 0 and TAI had been increased to allow follicles to reach a suitable maturity to more effectively respond to the second GnRH at TAI.

When evaluating the effects of estrus response on PR/AI, an EST1 × EST2 interaction was detected ($P = 0.038$; Figure 2). Pregnancy rates to AI were greater in heifers that did not express estrus during EST1 but expressed estrus at EST2 compared with heifers that only exhibited estrus at EST1 but not at EST2, heifers that exhibited estrus at both EST1 and EST2, and heifers that did not exhibit estrus at either EST1 or EST2.

Pregnancy rates to AI were greater in heifers that only expressed estrus at EST2 compared with those that expressed estrus at both EST1 and

Figure 2. Pregnancy rates to AI (PR/AI) according to expression of estrus. Heifers were synchronized using the 7-d CO-Synch + CIDR protocol. Estrous detection patches (Estroject; Rockway Inc., Spring Valley, WI) were applied to all heifers on day 14 and were examined for activation on day 7 at CIDR (Eazi-Breed CIDR; 1.38 g P4; Zoetis Animal Health) insertion (EST1). Heifers received a second estrous detection patch on day 0 at CIDR removal which was examined for activation at the time of TAI; EST2. Pregnancy diagnosis was performed by ultrasonography between days 35 and 55 after TAI. An EST1 $\times$ EST2 interaction ($P = 0.038$) was detected on PR/AI. ^{a,b}Percentages above bars with different superscripts differ ($P < 0.05$).

EST2. Heifers exhibiting estrus between days 14 and 7 would be in diestrus at CIDR removal on day 0. Therefore, it is plausible to believe that the duration of proestrus in those heifers would be extended, resulting in later ovulation than heifers not exhibiting estrus between days 14 and 7. A large proportion of heifers that failed to exhibit estrus between days 14 and 17 would likely have undergone CL regression during the period of time that the heifers had a CIDR, between days 7 and 0. Therefore, at CIDR removal, these heifers would have had a shorter proestrus and ovulated earlier than heifers that were in diestrus on day 0, resulting in greater PR/AI.

IMPLICATIONS AND CONCLUSIONS

Management practices at each of the locations differed and thus location effects may contribute to synchrony of estrus and fertility in this study. The operations at these locations were producer-owned and differed in animal handling, facilities, nutrition, and production goals. The management practices and environmental conditions within each location may have contributed to the reported ranges in estrus response, PR/AI, and final pregnancy rates observed among locations.

This study was designed to determine the effects of PGF_{2 α} administration 7 d before the induction of the 7-d CO-Synch + CIDR protocol in beef replacement heifers. We hypothesized that an injection of PGF_{2 α} 7 d prior to initiation of the 7-d CO-Synch + CIDR protocol would improve PR/AI. Our objectives were to determine whether the presynchronization treatment would increase the proportion of heifers exhibiting estrus before the initiation of

estrus synchronization, and prior to TAI, resulting in improved PR/AI. The PRESYNCH treatment succeeded in increasing estrus response between days 14 and 7, however failed to increase estrus response between day 0 and TAI, and consequently failed to increase PR/AI. However, investigation into the optimum timing of TAI of heifers submitted to the presynchronization protocol should be conducted, as there is potential to increase follicle size at the time of GnRH administration and TAI. By increasing follicle size, and consequently improving both responsiveness to the GnRH injection as well as estrus expression between day 0 and TAI, greater PR/AI may be realized.

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