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Comparison of progestin-based estrus synchronization protocols before fixed-time artificial insemination on pregnancy rate in beef heifers[1]

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ABSTRACT: The objective of the experiment was to compare pregnancy rates resulting from fixed-time AI after administration of either 1 of 2 controlled internal drug release (CIDR)-based protocols. Heifers at 3 locations (location 1, n = 78; location 2, n = 61; and location 3, n = 78) were assigned to 1 of 2 treatments within reproductive tract scores (1 = immature to 5 = cycling) by age and BW. Heifers assigned to CIDR Select received a CIDR insert (1.38 g of progesterone) from d 0 to 14 followed by GnRH (100 μg, i.m.) 9 d after CIDR removal (d 23) and $PGF_{2\alpha}$ (PG, 25 mg, i.m.) 7 d after GnRH treatment (d 30). Heifers assigned to CO-Synch + CIDR were administered GnRH and received a CIDR insert on d 23 and PG and CIDR removal on d 30. Heifers at location 1 were fitted with a HeatWatch estrus detection system transmitter from the time of PG until 24 d after fixed-time AI to allow for continuous estrus detection. Artificial insemination was performed at predetermined fixed times for heifers in both treatments at 72 or 54 h after PG for the CIDR Select and CO-Synch + CIDR groups, respectively. All heifers were administered GnRH at the time of AI. Blood samples were collected 10 d before and immediately before treatment initiation (d 0) to determine pretreatment estrous cyclicity (progesterone ≥ 0.5 ng/mL). At location 1, the estrous response during the synchronized period was greater (P = 0.06; 87 vs. 69%, respectively), and the variance for interval to estrus after PG was reduced among CIDR Select- (P < 0.01) compared with CO-Synch + CIDR-treated heifers. Fixed-time AI pregnancy rates were significantly greater (P = 0.02) after the CIDR Select protocol (62%) compared with the CO-Synch + CIDR protocol (47%). In summary, the CIDR Select protocol resulted in a greater and more synchronous estrous response and significantly greater fixed-time AI pregnancy rates compared with the CO-Synch + CIDR protocol.

Key words: artificial insemination, beef heifer, estrus synchronization, pregnancy rate, progestin

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INTRODUCTION

The benefits of presynchronization with a progestin (Patterson et al., 1995) or $PGF_{2\alpha}$ (PG; Thatcher et al., 2001) followed by GnRH (Sheffel et al., 1982; Smith et al., 1987) and PG on follicular development and subsequent fertility in beef (Wood et al., 2001; Perry et al., 2002; Stegner et al., 2004a,b) and dairy (Thatcher et al., 2001; Portaluppi and Stevenson, 2004) cows is well-documented.

Kojima et al. (2004) compared the estrous response, timing of AI, and pregnancy rate among beef heifers that were presynchronized with a progestin [melengestrol acetate (**MGA**) or controlled internal drug-releasing (**CIDR**) inserts] for 14 d before initiating a GnRH-PG protocol. Distribution of estrus and AI was more synchronous among CIDR- than MGA-treated heifers, and pregnancy rate to AI was greater in CIDR- (63%) than MGA-treated heifers (47%; Kojima et al., 2004). These differences may result from a reduced interval to estrus (Macmillan and Peterson, 1993) and improved synchronization of follicular waves after CIDR removal compared with the end of MGA treatment.

Other studies in heifers indicate that the response to GnRH at CIDR insertion may be of limited value

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(Howard et al., 2006; Lamb et al., 2006). Atkins et al. (2005) reported that the ovulatory response to GnRH is influenced by day of the estrous cycle at administration, and the greatest ovulation rate occurred on d 5, followed by d 15, 10, 18, and 2. Similar data in beef (Kojima et al., 2004; Schafer et al., 2006) and dairy heifers (Moreira et al., 2000) support the concept that presynchronization before initiation of the GnRH-PG protocol may be of value to improve synchronization of follicular waves in heifers.

The objective of this experiment was to compare fixed-time AI (**FTAI**) pregnancy rates among beef heifers synchronized with the CO-Synch + CIDR or CIDR Select protocols and to characterize the estrous response and synchrony of estrus after treatment administration.

MATERIALS AND METHODS

The experimental procedures were approved by the University of Missouri—Columbia Animal Care and Use Committee.

Experimental Design

Crossbred replacement beef heifers (n = 217) at 3 locations (location 1, n = 78; location 2, n = 61; and location 3, n = 78) were assigned within reproductive tract scores (**RTS**; 1 = immature to 5 = luteal phase; Anderson et al., 1991) by age and BW to 1 of 2 treatments (Table 1). Heifers assigned to the CIDR Select protocol (n = 108) received an Eazi-Breed CIDR (1.38 g of progesterone; Pfizer Animal Health, New York, NY) from d 0 to 14, GnRH (100 μg, i.m.; Cystorelin, Merial, Athens, GA) on d 23, and PG (25 mg, i.m.; Lutalyse, Pfizer Animal Health) on d 30 (Figure 1). The CO-Synch + CIDR-treated heifers (CO-Synch + CIDR; n = 109) received GnRH and a CIDR 23 d after d 0, which was the day of initiation of treatment, and PG and CIDR removal on d 30 (Figure 1).

Heifers at location 1 were fitted with a HeatWatch estrus detection system transmitter (DDx Inc., Denver, CO) at the time of PG to characterize the estrous response (estrus was defined as ≥ 3 mounts, each of which were ≥ 2 s in duration, within a 4-h period) during the synchronized period (0 to 144 h after PG). Transmitters were maintained on the heifers at location 1 until their subsequent return to estrus after the synchronized period or until 24 d after FTAI to characterize the return to estrus. Heifers at location 2 and 3 were not observed for estrus during the synchronized period; however, visual estrus detection was performed (twice daily, once each in the morning and evening, for ~1 h each time) until 24 d after FTAI to characterize the subsequent return to estrus. Although observations for behavioral estrus were conducted at location 1 during the synchronized period, all heifers were inseminated at the predetermined fixed time. Fixed-time AI was performed at 72 h after PG for heifers assigned to the CIDR Select treatment and 54 h after PG for heifers assigned to the CO-Synch + CIDR treatment (Figure 1). Time of PG administration and FTAI were recorded for each heifer. All heifers received GnRH at the time of insemination (Figure 1), and FTAI was performed by 1 of 2 experienced technicians. One AI sire was used at each location. The sire used at location 1 was the same sire used at location 2. The AI technicians were assigned to heifers within each treatment by heifer RTS, age, and BW. Twenty-four days after FTAI, the heifers were exposed to fertile bulls for the remainder of the 60-d breeding season.

Blood Collection and Progesterone Analysis

Blood samples were collected via jugular venipuncture 10 and 1 d before the initiation of treatment to determine pretreatment estrous cyclicity status. Blood samples were allowed to clot and were stored at 4°C for 24 h. Serum was collected by centrifugation and was stored at –20°C until hormone analyses were performed. Serum concentrations of progesterone were determined with a Coat-A-Count kit (Diagnostic Products Corp., Los Angeles, CA; Kirby et al., 1997), with intra- and interassay CV of 1.8 and 3.7%, respectively, and an assay sensitivity of 0.1 ng/mL. Heifers were considered estrous cycling if their progesterone concentrations were ≥ 0.5 ng/mL in one or both blood samples before treatment initiation.

Pregnancy Diagnosis

Pregnancy rate to AI was determined by transrectal ultrasonography (Aloka 500V equipped with a 5.0-MHz linear array transducer, Aloka, Wallingford, CT) 44 d (location 1) and 58 d (locations 2 and 3) after FTAI. Final pregnancy rates were determined by transrectal ultrasonography 80 to 95 d after the end of the breeding season at each location.

Statistical Analysis

Differences in age, BW, and RTS between treatments were analyzed by ANOVA using the linear statistical model of location, treatment, and the interaction of location × treatment (PROC GLM; SAS Inst. Inc., Cary, NC). Pretreatment estrous cyclicity status was analyzed by using χ^2 analysis (PROC GENMOD of SAS) using the model of location, treatment, and location × treatment interaction. Estrous response, pregnancy rate to FTAI, and final pregnancy rate at the end of the breeding season also were analyzed by using χ^2 analysis, with the model of location, treatment, AI technician, cyclicity status, and all relevant interactions. Differences in interval from PG to estrus (h; location 1) and FTAI to subsequent return to estrus (d) were determined by ANOVA using the linear statistical model of treatment, cyclicity, and the interaction of treatment × cyclicity (PROC GLM of SAS). Variances associated with intervals to estrus after PG administra-

Table 1. Number, age, BW, reproductive tract score (RTS), and estrous cyclicity of heifers at each location before initiation of treatment (means ± SE)[1]

Item	No. of heifers	Age,[3] d	BW,[4] kg	RTS[5]	Estrous cyclicity[2]	
					Proportion	%
Location 1						
CIDR Select	39	402 ± 2.1	404 ± 5	4.2 ± 0.14	31/39	79
CO-Synch + CIDR	39	400 ± 2.1	404 ± 5	4.2 ± 0.14	29/39	74
Combined	78	401 ± 1.5^{x}	404 ± 3^{x}	4.2 ± 0.10^{x}	60/78	77^{xy}
Location 2						
CIDR Select	30	390 ± 2.4	345 ± 5	3.6 ± 0.16	24/30	80
CO-Synch + CIDR	31	390 ± 2.4	346 ± 5	3.6 ± 0.16	21/31	68
Combined	61	390 ± 1.7^{y}	346 ± 4^{y}	3.6 ± 0.11^{y}	45/61	74^{x}
Location 3						
CIDR Select	39	405 ± 2.0	401 ± 5	4.1 ± 0.14	32/39	82
CO-Synch + CIDR	39	405 ± 2.0	400 ± 5	4.1 ± 0.14	36/39	92
Combined	78	405 ± 1.5^{z}	400 ± 3^{x}	4.1 ± 0.10^{x}	68/78	87^{y}
Combined						
CIDR Select	108	399 ± 1.3	383 ± 3	4.0 ± 0.09	87/108	81
Combined						
CO-Synch + CIDR	109	399 ± 1.3	383 ± 3	4.0 ± 0.08	86/109	79

$^{x–z}$Means within a column with different superscripts are different ($P < 0.05$).
[1]See Figure 1 for a description of the treatment protocols.
[2]Estrous cyclicity = the percentage of heifers with elevated (≥0.5 ng/mL) concentrations of progesterone in serum before initiation of the treatment. Heifers were considered to be cyclic if progesterone was elevated in either of 2 blood samples collected 10 d before and just before treatment initiation.
[3]Age (d) of the heifers at the initiation of the treatments.
[4]Body weight of the heifers at the time of the first blood sample before initiation of the treatments.
[5]Reproductive tract scores of the heifers at the time of the first blood sample before initiation of the treatments (1 to 5 scale, where 1 = immature and 5 = luteal phase).

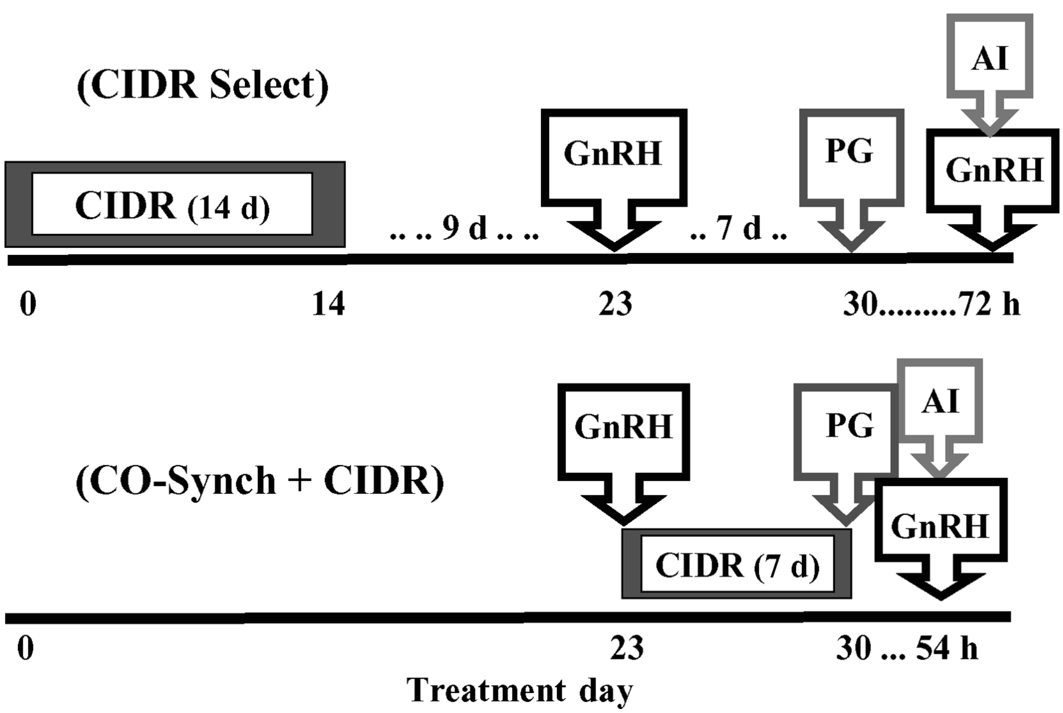

Figure 1. Treatment schedule for heifers assigned to the CIDR Select and CO-Synch + CIDR protocols. Heifers assigned to the CIDR Select protocol were equipped with an Eazi-Breed controlled internal drug release (CIDR; 1.38 g of progesterone) insert from d 0 to 14, GnRH (Cystorelin; 100 μg, i.m.) on d 23, and $PGF_{2\alpha}$ (PG; 25 mg, i.m.; Lutalyse) on d 30. At 72 h after PG, heifers received AI and GnRH. Heifers assigned to the CO-Synch + CIDR protocol were administered GnRH and received a CIDR insert on d 23. On d 30, CIDR were removed, and PG was administered; at 54 h after PG, heifers received AI and GnRH.

Table 2. Pregnancy rates of heifers at each location in response to fixed-time AI and at the end of the breeding season

	Pregnancy rate to fixed-time AI[1]		Pregnancy rate at the end of the breeding season[2]	
Item	Proportion	%	Proportion	%
Location 1				
CIDR Select	23/39	59	34/39	87
CO-Synch + CIDR	16/39	41	38/39	97
Location 2				
CIDR Select	19/30	63	29/30	97
CO-Synch + CIDR	15/31	48	28/31	90
Location 3				
CIDR Select	25/39	64	34/39	87
CO-Synch + CIDR	20/39	51	33/39	85
Combined				
CIDR Select	67/108	62[x]	97/108	90
Combined				
CO-Synch + CIDR	51/109	47[y]	99/109	91

[x,y]Means within a column with different superscripts are different ($P < 0.05$).

[1]Pregnancy rate to fixed-time AI determined by ultrasound 44 to 58 d after AI.

[2]Pregnancy rate at the end of the breeding season determined 80 to 95 d after the end of the 60-d breeding season.

tion (h) and after FTAI (d) were compared by dividing the greater variance by the lesser variance and performing an *F*-test (Snedecor and Cochran, 1989).

RESULTS

The number of heifers at each location, age, BW, RTS, and estrous-cycling status of heifers before the initiation of treatments are shown in Table 1. There was no difference between heifers in the treatment groups at the respective locations for age, BW, RTS, or estrous cyclicity status at the initiation of treatment; however, there were differences among locations (Table 1).

There was no effect of location ($P = 0.58$) or technician ($P = 0.13$) on pregnancy rates resulting from FTAI. However, there was a significant effect ($P = 0.02$) of treatment on pregnancy rates resulting from FTAI among all heifers (Table 2). Based on the odds ratio, heifers synchronized and inseminated with the CIDR Select protocol are 1.86 times more likely to conceive to FTAI than heifers synchronized and inseminated with the CO-Synch + CIDR protocol. Pretreatment estrous cyclicity did not affect (CIDR Select, $P = 0.98$; CO-Synch + CIDR, $P = 0.78$; Table 3) FTAI pregnancy rates. There was a treatment effect on FTAI pregnancy rate in cycling heifers ($P = 0.03$) but not in pre- and peripubertal heifers ($P = 0.39$; Table 3), possibly due to the low number of pre- and peripubertal heifers (CIDR Select, n = 21; CO-Synch + CIDR, n = 23). Final pregnancy rate at the end of the 60-d breeding season did not differ ($P = 0.61$) between treatments (Table 2).

One CIDR Select-treated heifer at location 1 failed to maintain the pregnancy that resulted from FTAI and was diagnosed as not pregnant at the final pregnancy diagnosis. One CO-Synch + CIDR-treated heifer at location 3 that was diagnosed as pregnant to the FTAI failed to maintain pregnancy and was diagnosed as not pregnant at the final pregnancy diagnosis.

Estrous response (location 1) during the synchronized period was greater ($P = 0.06$) among the CIDR Select-treated heifers (87%) than the CO-Synch + CIDR-treated heifers (69%; Figure 2). Mean interval from PG to estrus was shorter ($P = 0.03$) among CO-Synch + CIDR- (mean ± SE, 52.1 ± 4.5 h) compared with CIDR Select- (65.1 ± 4.0 h) treated heifers. Synchrony of estrus was greater in the CIDR Select-treated heifers (mean ± SD, 65.1 ± 15.6 h) compared with the CO-Synch + CIDR-treated heifers (52.1 ± 30.1 h), and variance associated with the interval from PG to estrus was less ($P < 0.01$) among CIDR Select heifers than CO-Synch + CIDR heifers. There was more variance ($P < 0.05$) associated with the interval from PG to estrus between pre- and peripubertal and estrous-cycling heifers synchronized with the CO-Synch + CIDR protocol (mean ± SD; 40.0 ± 10.4 h and 54.8 ± 5.0 h, respectively) compared with pre- and peripubertal and estrous-cycling heifers synchronized with the CIDR Select protocol (63.8 ± 8.8 h and 65.4 ± 4.5 h, respectively). Further analysis within treatment indicated more variance associated with the interval from PG to estrus between pre- and peripubertal and estrous-cycling heifers synchronized with the CO-Synch + CIDR protocol ($P < 0.05$) compared with the CIDR Select protocol.

The percentage of heifers exhibiting estrus after the synchronized period and before 24 d post-FTAI was similar ($P = 0.66$) among heifers synchronized with the CO-Synch + CIDR protocol (72%) and the CIDR Select protocol (68%; Figure 3). The mean interval from FTAI to the subsequent return to spontaneous estrus (according to HeatWatch and visual observation) was not different ($P = 0.26$) between the CIDR Select (mean ± SE; 20.2 ± 0.7 d) and CO-Synch + CIDR (19.2 ± 0.6 d) treatments (Figure 3). However, the variance associated with the interval from FTAI to subsequent spontaneous estrus was greater ($P < 0.05$) after the CO-Synch + CIDR protocol (mean ± SD; 19.2 ± 4.3 d) than the CIDR Select protocol (20.2 ± 3.0 d; Figure 3).

DISCUSSION

Hormonal treatment of heifers and cows to synchronize estrus has been commercially available to beef producers for over 30 yr; however, producers have been slow to adopt this management practice. Synchronization of estrous cycles has the potential to shorten the calving season, increase calf uniformity, and facilitate AI. Artificial insemination allows producers to use genetically superior proven sires and facilitates rapid improvement of US beef herds. Early estrous synchronization methods failed to manage follicular waves, re-

Table 3. Pregnancy rates after fixed-time AI based on estrous cyclicity before initiation of treatments

	CIDR Select				CO-Synch + CIDR			
	Estrous cycling		Pre- and peripubertal		Estrous cycling		Pre- and peripubertal	
Location	Proportion	%	Proportion	%	Proportion	%	Proportion	%
1	20/31	65	3/8	38	12/29	41	4/10	40
2	15/24	63	4/6	67	10/21	48	5/10	50
3	19/32	59	6/7	86	18/36	50	2/3	67
Combined	54/87	62^{x}	13/21	62	40/86	47^{y}	11/23	48

^{x,y}Combined means within a row with different superscripts are different ($P = 0.03$).

sulting in more days in the synchronized period and precluded fixed-time insemination with acceptable pregnancy rates (Wood et al., 2001; Patterson et al., 2003). Conversely, new methods of inducing and synchronizing estrus for postpartum beef cows and replacement heifers in which the GnRH-PG protocol is preceded by a progestin offers significant potential to more effectively synchronize estrus with resulting high fertility (Patterson et al., 2003).

In the current study, synchronization of estrus and FTAI in replacement beef heifers with the CIDR Select protocol resulted in an improved estrous response, improved synchrony of estrus, and a greater FTAI pregnancy rate compared with the CO-Synch + CIDR protocol. Improved synchronization of estrus and ovulation with the CIDR Select protocol appears to be associated with an increased response to the first GnRH and more effective control of the emerging follicular wave. Schafer et al. (2006) reported an 86% response to GnRH after presynchronization with a 14-d CIDR in beef heifers; other reports range from 43 to 60% of beef and dairy heifers ovulating in response to GnRH with no presynchronization (Macmillan and Thatcher, 1991; Pursley et al., 1995; Moreira et al., 2000; Atkins et al., 2005). Presynchronization with a 14-d CIDR before the GnRH-PG protocol enhanced the estrous response and improved the pregnancy rate to FTAI. Pregnancy rates to FTAI after the CIDR Select protocol with FTAI at 72 h averaged 62%, whereas the CO-Synch + CIDR protocol with FTAI at 54 h averaged 47% across the 3 locations. To date, no FTAI results have been reported with the CIDR Select protocol; therefore, the timing of FTAI with the CIDR Select protocol was based on previously published reports by Schafer et al. (2006) and Kojima et al. (2004). Peak estrous response in these studies occurred 48 to 60 h after PG, and the peak AI date was 3 d after PG. Timing of insemination after the CO-Synch + CIDR protocol was based on recommendations from the pharmaceutical and AI industries of 54 ± 2 h (NCRBRTF, 2006) and other reports in which the timing of AI ranged from 48 to 60 h post-PG (Martinez et al., 2000, 2002a,b; Colazo et al., 2004; Walker et al., 2005; Lamb et al., 2006). Fixed-time AI pregnancy rates with the CO-Synch + CIDR protocol (47%) were lower than studies reported by Martinez et al.(65 and 68%; 2002 a,b) with FTAI at 48 h, however comparable

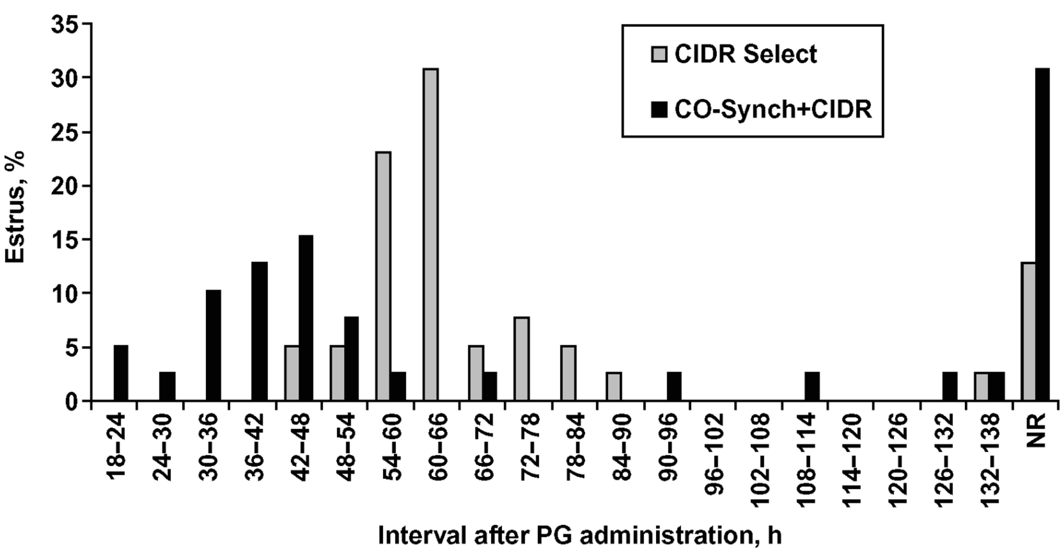

Figure 2. Percentage of heifers in estrus after $PGF_{2\alpha}$ (PG) at location 1; CIDR Select (gray bar) and CO-Synch + CIDR (black bar); NR = no response (no estrous response).

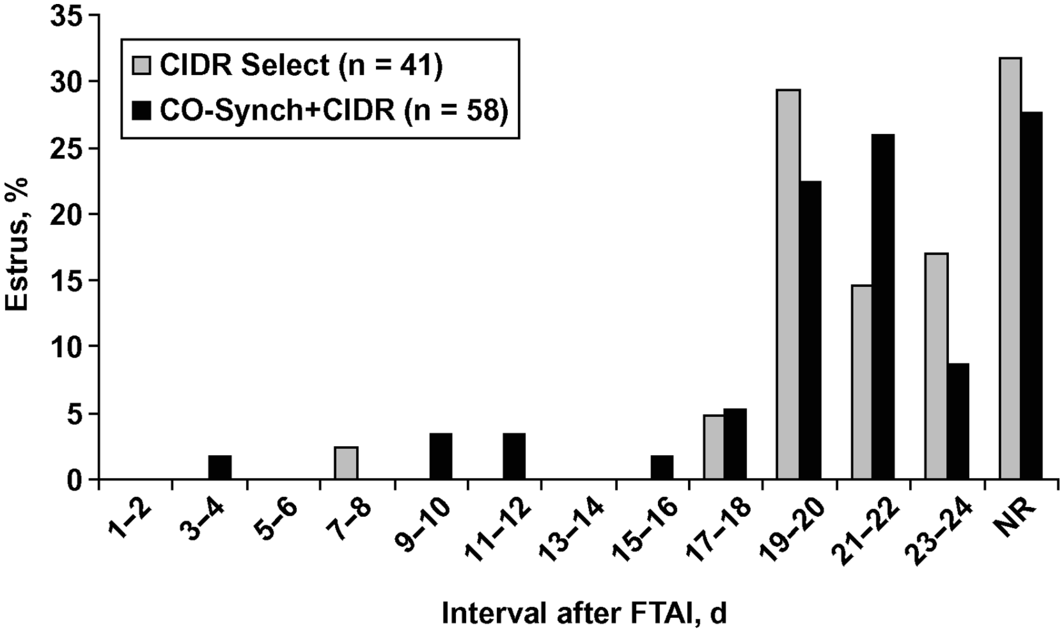

Figure 3. Percentage of heifers in estrus after fixed-time AI (FTAI); CIDR Select (gray bar), and CO-Synch + CIDR (black bar); NR = no response (no estrous response).

to others that performed FTAI at 52 (54%, Colazo et al., 2004), 54 (48%, Martinez et al., 2000; 55%, Walker et al., 2005), or 60 h (53%, Lamb et al., 2006) after PG and CIDR removal.

It is important to note that there were no differences within treatment for pregnancy rates resulting from FTAI between estrous-cycling and prepubertal heifers. From a practical standpoint, it is important to accurately compare the efficacy of these protocols to induction of cyclicity in prepubertal heifers, measured by estrus, ovulation, and pregnancy outcome. We acknowledge the potential for misclassification of heifers by cyclicity determined from 2 blood samples before treatment initiation and the use of progesterone values ≥ 0.5 ng/mL to confirm cyclicity. However, the potential for committing a type II error is greatly minimized if not negated in describing heifers as prepubertal using a 0.5 ng/mL cutoff.

Estrous response after the CO-Synch + CIDR protocol (69%) was similar to previous reports by Martinez et al. (65%; 2002b), in which FTAI was performed at 48 h after PG, and in the study by Lamb et al. (74%, 2006), in which estrus was detected for 72 h, and heifers not detected in estrus were FTAI at 84 h post-PG. However, mean interval to estrus after the CO-Synch + CIDR protocol was slightly longer in the current study (52.1 h) compared with previous reports (47.1 h, Martinez et al., 2002b; 48.9 h, Lamb et al., 2006). Schafer et al. (2006) reported the peak estrous response in heifers after the CIDR Select protocol occurred 48 to 60 h after PG, whereas the peak estrous response in the current study occurred 54 to 66 h after PG.

In GnRH-based protocols, the second GnRH treatment at the time of AI is intended to synchronize ovulation and induce the start of a normal estrous cycle in most treated cattle. However, in the current study, 5 heifers in the CO-Synch + CIDR protocol and 1 heifer in the CIDR Select protocol that received the second GnRH to synchronize ovulation at FTAI were observed in estrus 4 to 14 d later. This suggests that GnRH did not induce ovulation in these heifers. In addition, the increased variance associated with the interval from FTAI to subsequent return to estrus and reduced FTAI pregnancy rates after the CO-Synch + CIDR protocol indicate a reduced synchrony of estrus compared with CIDR Select. Reduced variance associated with the interval from FTAI to subsequent return to estrus after CIDR Select allows producers to concentrate their time detecting estrus within a 4- or 5-d window and achieve acceptable AI pregnancy rates during a second AI period.

In summary, synchronizing estrus and AI of replacement beef heifers with the CIDR Select protocol resulted in a greater and more synchronized estrous response, significantly greater FTAI pregnancy rates, and a greater degree of synchrony of estrus during the subsequent return to estrus after FTAI compared with CO-Synch + CIDR-treated heifers. This improvement in pregnancy rate may offset the 2 additional trips through the chute associated with the CIDR Select protocol compared with the CO-Synch + CIDR protocol.

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