Comparison of progestin-based protocols to synchronize estrus and ovulation before fixed-time artificial insemination in postpartum beef cows[1]

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ABSTRACT: This experiment was designed to compare pregnancy rates in postpartum beef cows resulting from fixed-time AI (FTAI) after treatment with 1 of 2 protocols to synchronize estrus and ovulation. Crossbred, suckled beef cows (n = 650) at 4 locations (n = 210; n = 158; n = 88; and n = 194) were assigned within a location to 1 of 2 protocols within age group by days postpartum and BCS. Cows assigned to the melengestrol acetate (MGA) Select treatment (MGA Select; n = 327) were fed MGA (0.5 mg·head^{-1}·d^{-1}) for 14 d, GnRH (100 μg of Cystorelin i.m.) was injected on d 26, and prostaglandin $F_{2\alpha}$ (PG; 25 mg of Lutalyse i.m.) was injected on d 33. Cows assigned to the CO-Synch + controlled internal drug release (CIDR) protocol (CO-Synch + CIDR; n = 323) were fed a carrier for 14 d, were injected with GnRH and equipped with an EAZI-BREED CIDR insert (1.38 g of progesterone, Pfizer Animal Health, New York, NY) 12 d after carrier removal, and PG (25 mg of Lutalyse i.m.) was injected and the CIDR were removed on d 33. Fixed-time AI was performed at 72 or 66 h after PG for the MGA Select or CO-Synch + CIDR groups, respectively. All cows were injected with GnRH (100 μg of Cystorelin i.m.) at the time of insemination. Blood samples were collected 8 and 1 d before the beginning of MGA or carrier to determine estrous cyclicity status of the cows (estrous cycling vs. anestrus) before treatment [progesterone ≥ 0.5 ng/mL (MGA Select, 185/327, 57%; CO-Synch + CIDR, 177/323, 55%); P = 0.65]. There was no difference (P = 0.20) in pregnancy rate to FTAI between treatments (MGA Select, 201/327, 61%; CO-Synch + CIDR, 214/323, 66%). There was also no difference (P = 0.25) between treatments in final pregnancy rate at the end of the breeding period (MGA Select, 305/327, 93%; CO-Synch + CIDR, 308/323, 95%). These data indicate that pregnancy rates to FTAI were comparable after administration of the MGA Select or CO-Synch + CIDR protocols. Both protocols provide opportunities for beef producers to utilize AI and potentially eliminate the need to detect estrus.

Key words: beef cow, controlled internal drug release insert, estrus synchronization, fixed-time artificial insemination, melengestrol acetate, progestin

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INTRODUCTION

Artificial insemination offers beef producers the means to introduce proven superior genetics into their herds. Females conceiving to a synchronized estrus weaned calves that were on average 13 d older and 9.5 kg heavier than calves from nonsynchronized females (Schafer et al., 1990). However, surveys indicate only 10% of the beef cows in the United States are bred by AI (NAHMS, 1997), and a limited number of operations use estrus synchronization to facilitate their AI programs (NAHMS, 1997). The main reason beef producers cited for not implementing these practices was "lack of time and labor" (NAHMS, 1998). Development of methods to control estrous cycles in cattle that result in the expression of a highly synchronized fertile estrus and ovulation will more readily facilitate application of fixed-time AI (**FTAI**) and reduce or eliminate the time and labor required to detect estrus (Patterson et al., 2003a). Development of protocols that facilitate FTAI

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with associated high fertility would likely result in a dramatic increase in the adoption of AI in beef herds (Patterson et al., 2003b).

Previous research in our laboratory demonstrated the efficacy of using the melengestrol acetate (**MGA**) Select protocol to synchronize estrus and ovulation when FTAI was performed 72 h after administration of prostaglandin $F_{2\alpha}$ (**PG**; Perry et al., 2002; Stegner et al., 2004a; Bader et al., 2005). Other research showed an improvement in pregnancy rates resulting from FTAI after treatment with the CO-Synch + controlled internal drug release (**CIDR**) protocol when AI was performed 66 h as opposed to 48, 54, or 60 h after PG (Bremer et al., 2004). Larson et al. (2006) reported results from FTAI performed 60 h after administration of the CO-Synch + CIDR protocol, but comparisons with other time intervals were not evaluated. To date, a direct comparison of these protocols that facilitate FTAI has not been made.

Therefore, the objective of this study was to compare pregnancy rates resulting from FTAI between cows assigned to the MGA Select or CO-Synch + CIDR protocols.

MATERIALS AND METHODS

Animals

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

Crossbred lactating beef cows (n = 650) at 4 locations (n = 210; n = 158; n = 88; and n = 194) were assigned within age group (2 to 14 yr) by calving date (days postpartum, **DPP**) and BCS (1 to 9 scale; 1 = emaciated and 9 = obese; Richards et al., 1986) to 1 of 2 treatments (Table 1). Cows assigned to the MGA Select treatment (MGA Select; n = 327) were fed MGA (Pfizer Animal Health, New York, NY; 0.5 $mg \cdot head^{-1} \cdot d^{-1}$) for 14 d, GnRH (100 μg of Cystorelin i.m., Merial, Athens, GA) was injected on d 26, and PG (25 mg of Lutalyse sterile suspension i.m., Pfizer Animal Health) was injected on d 33. The CO-Synch + CIDR treated cows (CO-Synch + CIDR; n = 323) were fed a carrier for 14 d, were injected with GnRH and equipped with an EAZI-BREED CIDR [1.38 g of progesterone (**P_4**), Pfizer Animal Health] 12 d after carrier removal, and PG (25 mg of Lutalyse i.m.) was injected and the CIDR was removed on d 33.

Artificial insemination was performed at 72 h after PG for cows assigned to the MGA Select treatment, and at 66 h after CIDR removal and PG administration for cows assigned to the CO-Synch + CIDR treatment (Figure 1). Time of PG administration and AI were recorded for each cow. All cows were injected with GnRH (100 μg of Cystorelin i.m.) at the time of insemination, and AI was performed by 1 of 3 experienced technicians. Three AI sires were used at location 1, and 1 sire was used at locations 2, 3, and 4. One of the sires used at location 1 was the same sire that was used at locations 3 and 4. The AI sire and technician were assigned to cows within each treatment by cow age, calving date, and BCS.

Blood Collection and RIA

Blood samples were collected via jugular venipuncture at 8 and 1 d before feeding of the MGA or carrier to determine pretreatment estrous cyclicity status. Blood samples were allowed to clot and stored at 4°C for 24 h. Serum was collected after centrifugation and stored at −20°C until hormone analyses were performed. Serum concentrations of P_4 were determined with a Coat-A-Count kit (Diagnostic Products Corporation, Los Angeles, CA; Kirby et al., 1997) with intra- and interassay CV of 2.7 and 13.8% and an assay sensitivity of 0.1 ng/mL. Cows were considered to be estrous-cycling if the concentrations of P_4 in serum were elevated (≥0.5 ng/mL) at one or both pretreatment sampling times.

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) at 40 to 45 d after FTAI. Final pregnancy rates were determined 50 to 60 d after the end of the breeding season at each location. Females were exposed to fertile bulls 14 d after FTAI at all locations. Cows were exposed to bulls for 31 d (i.e., a 45-d breeding season) at location 2, and 46 d (a 60-d breeding season) at locations 1, 3, and 4.

Statistical Analyses

Differences in age, days postpartum, and BCS 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, AI sire, AI technician, pregnancy rate to FTAI, and final pregnancy rate at the end of the breeding season were analyzed by using χ^2 analysis (PROC FREQ of SAS).

Pregnancy rates resulting from FTAI were analyzed using a generalized linear models method (PROC GENMOD of SAS). The model was arranged as a 4 × 2 × 2 factorial (location, treatment, estrous cyclicity status). The means were expressed as the logit [natural log (p/1 − p)]. The antilog of the average logit produced the odds. These models also included DPP, BCS, and age as separate covariates in the analyses.

RESULTS

Pregnancy

The number of cows at each location, age, days postpartum, BCS, and estrous cycling status of cows before the initiation of treatments are shown in Table 1. There

Table 1. Number of cows at each location, days postpartum, BCS, and estrous-cycling status for cows before initiation of each treatment (means ± SE)

Location and treatment[1]	No.	Age, yr	Time postpartum,[2] d	BCS[3]	Cows with elevated progesterone[4]	
					Proportion	%
Location 1						
MGA Select	106	5.3 ± 0.3	46.4 ± 1.4	5.6 ± 0.06	62/106	58
CO-Synch + CIDR	104	5.4 ± 0.3	45.9 ± 1.4	5.7 ± 0.06	50/104	48
Combined	210	5.3 ± 0.2	46.1 ± 1.0[a]	5.7 ± 0.04[a]	112/210	53[a]
Location 2						
MGA Select	80	5.7 ± 0.3	32.7 ± 1.6	6.1 ± 0.07	29/80	36
CO-Synch + CIDR	78	5.7 ± 0.3	32.4 ± 1.6	6.0 ± 0.07	34/78	44
Combined	158	5.7 ± 0.2	32.5 ± 1.1[b]	6.0 ± 0.05[b]	63/158	40[b]
Location 3						
MGA Select	45	5.5 ± 0.4	44.6 ± 2.1	5.2 ± 0.10	16/45	36
CO-Synch + CIDR	43	5.4 ± 0.4	44.1 ± 2.1	5.3 ± 0.10	15/43	35
Combined	88	5.5 ± 0.3	44.4 ± 1.5[ac]	5.3 ± 0.07[c]	31/88	35[b]
Location 4						
MGA Select	96	5.2 ± 0.3	43.8 ± 1.4	5.3 ± 0.07	78/96	81
CO-Synch + CIDR	98	5.3 ± 0.3	41.7 ± 1.4	5.3 ± 0.07	78/98	80
Combined	194	5.2 ± 0.2	42.8 ± 1.0[c]	5.3 ± 0.05[c]	156/194	80[c]
Overall						
MGA Select	327	5.4 ± 0.2	41.9 ± 0.8	5.5 ± 0.03	185/327	57
Overall						
CO-Synch + CIDR	323	5.4 ± 0.2	41.0 ± 0.8	5.6 ± 0.03	177/323	55

[a–c]Within a column, means without a common superscript letter differ ($P < 0.05$).
[1]See Figure 1 for a description of the protocols.
[2]Number of days postpartum at the initiation of melengestrol acetate (MGA) feeding for MGA Select-treated cows and carrier feeding for CO-Synch + CIDR-treated cows.
[3]BCS of cows at the time of first blood sample before initiation of treatments (1 to 9 scale, where 1 = emaciated and 9 = obese).
[4]Estrous cyclicity = the percentage of cows with elevated (≥0.5 ng/mL) concentrations of progesterone in serum before treatment. Cows were considered to be cyclic if progesterone was elevated in either of 2 blood samples collected 8 or 1 d before treatment.

were no differences between treatments at the respective locations for age, DPP, BCS, or estrous cyclicity status at the initiation of treatment; however, there were differences among locations (Table 1).

There was no effect of treatment ($P = 0.20$), technician ($P = 0.63$), or sire ($P = 0.11$) on pregnancy rates resulting from FTAI (Table 2). In addition, pretreatment estrous cyclicity before the initiation of the MGA Select or CO-Synch + CIDR protocols did not affect (MGA Select, $P = 0.39$; CO-Synch + CIDR, $P = 0.31$; Table 3) pregnancy rates resulting from FTAI. Pregnancy rates at the end of the breeding season did not differ ($P = 0.25$) between treatments (Table 2).

Further analyses of the data using PROC GENMOD procedures of SAS indicated that pregnancy rates resulting from FTAI were not affected by location ($P = 0.37$), treatment ($P = 0.49$), the interaction of location × treatment ($P = 0.83$), estrous cyclicity status ($P = 0.52$), the interaction of location × estrous cyclicity status ($P = 0.60$), the interaction of treatment × estrous cyclicity status ($P = 0.49$), or the interaction of location × treatment × estrous cyclicity status ($P = 0.29$). These results did not change when DPP ($P = 0.85$) or BCS ($P = 0.94$) were included in the statistical model as separate covariates. Age was significant as a covariate ($P = 0.06$); however, cow age did not affect the factorial arrangement of treatments with respect to effect on pregnancy rate resulting from FTAI. Based on the odds ratio, cows synchronized with the MGA Select and CO-Synch + CIDR protocols were 1.70 and 1.94 times, respectively, more likely to become pregnant as a result of FTAI following treatment than not.

Pregnancy Loss

Twelve cows among the 4 locations that were determined to be pregnant at the first ultrasound failed to maintain pregnancies resulting from FTAI. These included 9 MGA Select treated cows and 3 CO-Synch + CIDR treated cows. Six of the 12 cows were, however, pregnant at the end of the breeding period including 5 of the MGA Select and 1 CO-Synch + CIDR treated cow.

DISCUSSION

Research of methods to effectively synchronize estrus in beef cattle has focused on the development of simple, cost-effective protocols that synchronize estrus and ovulation in order to facilitate prescheduled FTAI (Smith et al., 1984). One of the major challenges facing cow-calf producers in anticipation of a subsequent breeding season is the uncertainty in knowing the percentage of cows that have resumed normal estrous cyclicity following calving. Therefore, estrus synchronization protocols

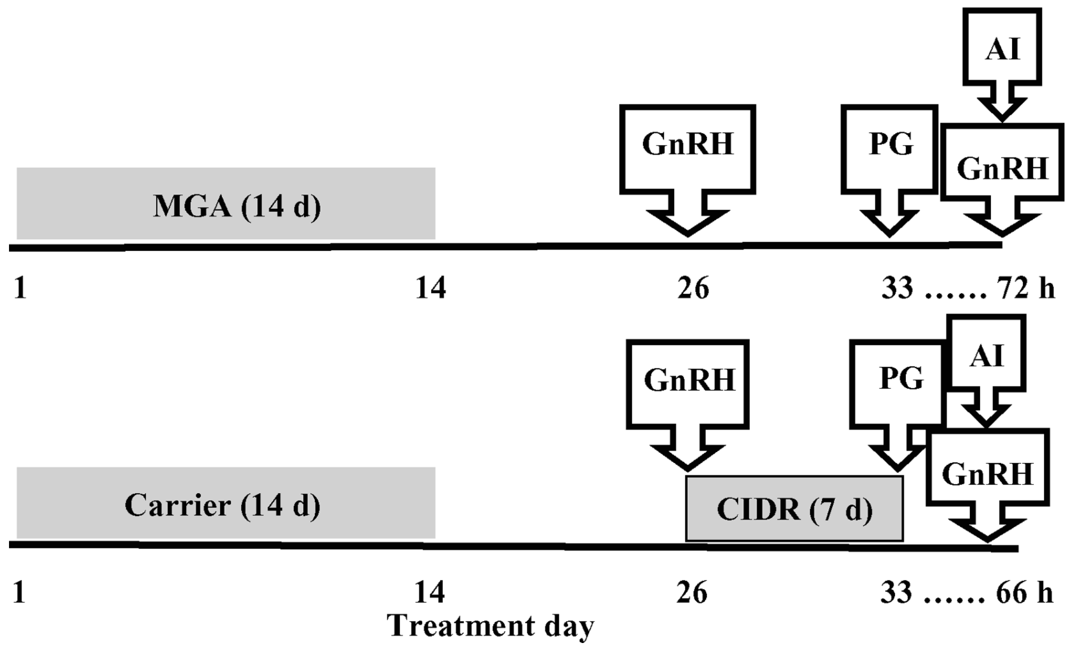

Figure 1. Treatment schedule for cows assigned to the MGA Select and CO-Synch + CIDR protocols. Cows assigned to the MGA Select protocol were fed melengestrol acetate (MGA; 0.5 mg·head^{-1}·d^{-1}) for 14 d, GnRH was administered (100 μg of Cystorelin i.m.) 12 d after MGA withdrawal, and $PGF_{2\alpha}$ (PG; 25 mg of Lutalyse i.m.) was administered 7 d after GnRH. Cows were inseminated 72 h after PG and received an injection of GnRH at AI. Cows assigned to the CO-Synch + CIDR protocol were fed a carrier for 14 d, were injected with GnRH and equipped with a controlled internal drug release insert (CIDR; 1.38 g of progesterone; Eazi Breed CIDR Insert) 12 d after carrier withdrawal (d 26), and the CIDR was removed 7 d later at the time that PG was administered (d 33). Cows were inseminated at 66 h after PG and received an injection of GnRH at AI.

that facilitate FTAI must be capable of synchronizing follicular development and luteal regression in estrous cycling cows, and effectively initiate estrous cyclicity in anestrous cows (Stevenson et al., 2003).

The GnRH-PG protocol is ineffective in synchronizing estrus before FTAI due to the fact that 5 to 15% of the cyclic cows display estrus before administration of PG (Pursley et al., 1995; Twagiramungu et al., 1995; Kojima et al., 2000). As a result, more recent approaches to estrus synchronization in postpartum beef cows have included the use of a progestin to minimize or eliminate the proportion of animals that exhibit estrus prematurely. Progestin-based protocols that utilize MGA (MGA Select; Perry et al., 2002) or CIDR (Larson et al., 2006) have resulted in significantly greater pregnancy rates to FTAI when compared with CO-Synch (Geary et al., 1998).

Estrous response consistently peaks 72 h after PG following administration of the MGA Select protocol (Patterson et al., 2002; Stegner et al., 2004b). Furthermore, pregnancy rates to FTAI following administration of the MGA Select protocol are reported to be ≥60%, when FTAI was performed 72 h after PG (Perry et al., 2002; Stegner et al., 2004a; Bader et al., 2005). Published reports indicate that pregnancy rates to FTAI using the MGA Select protocol are greater (64 vs. 50%) when AI is performed at 72 vs. 80 h after PG, respectively (Stegner et al., 2004a). Other reports indicate that pregnancy rates resulting from FTAI after treatment with the MGA Select protocol were reduced (46%) when AI was performed 48 h after PG (Stevenson et al., 2003). Pregnancy rates resulting from FTAI reported in this study following treatment with the MGA Select protocol are consistent with other published reports when insemination was performed 72 h after PG (Perry et al., 2002; Stegner et al., 2004a; Bader et al., 2005).

Larson et al. (2006) demonstrated the potential for successful use of the CO-Synch + CIDR protocol followed by FTAI 60 h after CIDR removal and PG administration. This treatment schedule (Larson et al. 2006) resulted in pregnancy rates comparable with CIDR-based protocols that involve estrus detection and AI up to 84 h after PG followed by FTAI of nonresponders at 84 h (Larson et al., 2006). Other studies reported pregnancy rates following administration of the CO-Synch + CIDR protocol were optimized when insemination was performed at 66 h after PG compared with AI performed at 48 or 54 h (Bremer et al., 2004).

Inseminating cows 66 h following PG administration and CIDR removal offers more practical application of FTAI from a scheduling standpoint. These considerations led to the decision in this experiment to inseminate cows at 66 h following administration of the CO-Synch + CIDR protocol. The results from FTAI performed at 66 h reported here are comparable with those

Table 2. Pregnancy rates after fixed-time AI and at the end of the breeding season

Location and treatment[1]	Pregnancy rate to fixed-time AI[2]		Pregnancy rate at the end of the breeding season[3]	
	Proportion	%	Proportion	%
Location 1				
MGA Select	70/106	66	99/106	93
CO-Synch + CIDR	67/104	64	99/104	95
Location 2				
MGA Select	53/80	66	77/80	96[4]
CO-Synch + CIDR	56/78	72	76/78	97[4]
Location 3				
MGA Select	26/45	58	42/45	93
CO-Synch + CIDR	29/43	67	42/43	98
Location 4				
MGA Select	52/96	54	87/96	91
CO-Synch + CIDR	62/98	63	91/98	93
Overall				
MGA Select	201/327	61	305/327	93
Overall				
CO-Synch + CIDR	214/323	66	308/323	95

[1]Cows assigned to the MGA Select protocol were fed melengestrol acetate (MGA; 0.5 mg·head^{-1}·d^{-1}) for 14 d, GnRH was administered (100 μg of Cystorelin i.m.) 12 d after MGA withdrawal, and $PGF_{2\alpha}$ (PG; 25 mg of Lutalyse i.m.) was administered 7 d after GnRH. Cows were inseminated 72 h after PG and received an injection of GnRH at AI. Cows assigned to the CO-Synch + CIDR protocol were fed a carrier for 14 d, were injected with GnRH and equipped with a controlled internal drug release insert (CIDR; 1.38 g of progesterone; Eazi Breed CIDR Insert) 12 d after carrier withdrawal (d 26), and the CIDR was removed 7 d later at the time that PG was administered (d 33). Cows were inseminated at 66 h after PG and received an injection of GnRH at AI.

[2]Pregnancy rate to fixed-time AI determined by ultrasound 40 to 45 d after AI.

[3]Pregnancy rate at the end of the breeding season determined 50 to 60 d after the end of the breeding season.

[4]Pregnancy rate after the 45-d breeding season.

published by Bremer et al. (2004). Collectively, these various studies suggest that a critical window of time exists over which insemination should be performed to optimize pregnancy rate resulting from FTAI.

It is important to note that there were no differences within or between treatments for pregnancy rates resulting from FTAI between cows that were classified as estrous cycling or anestrus before treatment initiation. From a practical standpoint it is important to accurately compare the efficacy of these protocols with regard to induction of cyclicity in anestrous cows, measured by estrus, ovulation, and pregnancy outcome. The authors acknowledge the potential for misclassification of cows on the basis of 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 cows as anestrus using a progesterone concentration of 0.5 ng/mL as the cutoff.

Successful application and compliance in administering these protocols requires careful consideration of the advantages and disadvantages that accompany each of them. Based on these data, both protocols appear to work effectively in mixed populations of estrous cycling and anestrous beef cows despite differences reported recently by Perry et al. (2004). The CO-Synch + CIDR protocol may have broader application in comparison with the MGA Select protocol due to shorter treatment duration and the practical allowance in being able to assign more cows to treatment based on later calving date. Successful outcomes following administration of either protocol require careful compliance with each step of the respective treatment. Arguably the CO-Synch + CIDR protocol offers more precise control of progestin treatment, which should allow for more con-

Table 3. Pregnancy rates after fixed-time AI based on estrous cyclicity before initiation of the treatments[1]

Location	MGA Select				CO-Synch + CIDR			
	Estrous cycling[2]		Anestrus[2]		Estrous cycling		Anestrus	
	Proportion	%	Proportion	%	Proportion	%	Proportion	%
1	38/62	61	32/44	73	30/50	60	37/54	69
2	20/29	69	33/51	65	25/34	74	31/44	70
3	11/16	69	15/29	52	8/15	53	21/28	75
4	41/78	53	11/18	61	50/78	64	12/20	60
Combined	110/185	59	91/142	64	113/177	64	101/146	69

[1]Cows assigned to the MGA Select protocol were fed melengestrol acetate (MGA; 0.5 mg·head^{-1}·d^{-1}) for 14 d, GnRH was administered (100 μg of Cystorelin i.m.) 12 d after MGA withdrawal, and $PGF_{2\alpha}$ (PG; 25 mg of Lutalyse i.m.) was administered 7 d after GnRH. Cows were inseminated 72 h after PG and received an injection of GnRH at AI. Cows assigned to the CO-Synch + CIDR protocol were fed a carrier for 14 d, were injected with GnRH and equipped with a controlled internal drug release insert (CIDR; 1.38 g of progesterone; Eazi Breed CIDR Insert) 12 d after carrier withdrawal (d 26), and the CIDR was removed 7 d later at the time that PG was administered (d 33). Cows were inseminated at 66 h after PG and received and injection of GnRH at AI. Estrous cyclicity status before the MGA Select or CO-Synch + CIDR protocols did not affect pregnancy rate resulting from fixed-time AI (MGA Select, = 0.39; CO-Synch + CIDR, P = 0.31).

[2]Estrous cyclicity = the percentage of cows with elevated (≥0.5 ng/mL) concentrations of progesterone in serum before treatment. Cows were considered to be cyclic if progesterone was elevated in either of 2 blood samples collected 8 or 1 d before treatment.

sistent results in comparison with MGA. Furthermore, the labor associated with feeding MGA in most circumstances exceeds the labor required to insert and remove the CIDR device. However, the cost of the CIDR insert may be viewed as a disadvantage associated with this protocol in addition to the need to restrain animals one additional time.

Both progestin-based protocols performed comparably in this experiment in successfully facilitating FTAI following treatment irrespective of location, estrous cylicity status, BCS, or DPP. The results reported here regarding use of the CIDR device in postpartum beef cows demonstrates that a viable alternative to MGA is available and approved for use. Producers that have used MGA in the past to synchronize cows should transition to CIDR to comply with US Food and Drug Administration regulations concerning extralabel use of medicated feeds.

These results indicate that estrus synchronization with the MGA Select and CO-Synch + CIDR protocols produce comparable pregnancy rates to FTAI when inseminations were performed at 72 and 66 h after PG, respectively. The results reported here present beef producers a choice and means for expediting genetic improvement and reproductive management. Improvements in methods to synchronize estrus create the opportunity to significantly expand the use of AI in US beef herds.

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