Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$, and progesterone[1]

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ABSTRACT: We evaluated whether a fixed-time AI (TAI) protocol could yield pregnancy rates similar to a protocol requiring detection of estrus, or detection of estrus and AI plus a clean-up TAI for heifers not detected in estrus, and whether adding an injection of GnRH at controlled internal drug release (CIDR) insertion would enhance fertility in CIDR-based protocols. Estrus in 2,075 replacement beef heifers at 12 locations was synchronized, and AI was preceded by 1 of 4 treatments arranged as a 2 × 2 factorial design: 1) Estrus detection + TAI (ETAI) (n = 516): CIDR for 7 d plus 25 mg of prostaglandin $F_{2\alpha}$ (PG) at CIDR insert removal, followed by detection of estrus for 72 h and AI for 84 h after PG (heifers not detected in estrus by 84 h received 100 μg of GnRH and TAI); 2) G+ETAI (n = 503): ETAI plus 100 μg GnRH at CIDR insertion; 3) Fixed-time AI (FTAI) (n = 525): CIDR for 7 d plus 25 mg of PG at CIDR removal, followed in 60 h by a second injection of GnRH and TAI; 4) G+FTAI (n = 531): FTAI plus 100 μg of GnRH at CIDR insertion. Blood samples were collected (d −17 and −7, relative to PG) to determine ovarian status. For heifers in ETAI and G+ETAI treatments, a minimum of twice daily observations for estrus began on d 0 and continued for at least 72 h. Inseminations were performed according to the a.m.-p.m. rule. Pregnancy was diagnosed by transrectal ultrasonography. The percentage of heifers exhibiting ovarian cyclic activity at the initiation of treatments was 89%. Pregnancy rates among locations across treatments ranged from 38 to 74%. Pregnancy rates were 54.7, 57.5, 49.3, and 53.1% for ETAI, G+ETAI, FTAI, and G+FTAI treatments, respectively. Although pregnancy rates were similar among treatments, a tendency ($P = 0.065$) occurred for pregnancy rates in the G+ETAI treatment to be greater than in the FTAI treatment. We concluded that the G+FTAI protocol yielded pregnancy rates similar to protocols that combine estrus detection and TAI. Further, the G+FTAI protocol produced the most consistent pregnancy rates among locations and eliminated the necessity for detection of estrus when inseminating replacement beef heifers.

Key words: beef heifer, synchronization, timed artificial insemination

J. Anim. Sci. 2006. 84:3000–3009
doi:10.2527/jas.2006-220

INTRODUCTION

Synchronization of estrus can be an effective means of increasing the proportion of females that become pregnant early in the breeding season, resulting in shorter calving seasons and more uniform calf crops (Dziuk and Bellows, 1983). To stimulate greater use of estrus-synchronization protocols by beef producers,

[1]Partial funding for this project was provided by Select Sires Inc. We thank Pfizer Animal Health (New York, NY) for contributions of prostaglandin $F_{2\alpha}$ (Lutalyse) and CIDR inserts and Intervet, Inc. (Millsboro, DE) for donation of gonadotropin-releasing hormone (Fertagyl). Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA, the institutions listed herein, or the authors, and does not imply its approval to the exclusion of other products that may also be suitable. Appreciation also is expressed to W. Alexander, C. Bailey, G. Barquero, S. Bellows, C. Blevins, D. Brown, M. Constantaras, N. DiLorenzo, D. Eborn, I. Franciscon, D. Grum, T. Krizek, R. Lemenager, D. Llewellyn, M. Merrill, M. Meneghetti, M. Portaluppi, G. Perry, C. Riggins, and T. Towns, K. VanZant, and R. Wasson for their assistance with data collection and laboratory analysis.

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Received April 6, 2006.
Accepted June 29, 2006.

protocols must limit time and labor inputs. Reduced inputs can be achieved by using 1 of 2 strategies: 1) use protocols that minimize the number and frequency of handling of cattle through the handling facility and 2) use protocols that minimize or eliminate detection of estrus by employing fixed-time AI (**TAI**).

Recently developed TAI protocols (Dahlen et al., 2003) have not yielded pregnancy rates in heifers as consistently satisfactory as those in suckled cows (Lamb et al., 2001; Bader et al., 2005; Larson et al., 2006). The primary reason for their failure seems to be related to the inability to synchronize follicular waves with GnRH as successfully as in cows. After an injection of GnRH at random stages of the estrous cycle, 64 to 75% of postpartum beef and dairy cows ovulated a follicle (Geary et al., 1998; Thompson et al., 1999; El-Zarkouny et al., 2004), whereas only 48 to 60% of beef and dairy heifers ovulated follicles in response to the same treatment (Macmillan and Thatcher, 1991; Pursley et al., 1995; Moreira et al., 2000). Therefore, the objectives of the current study were to determine whether 1) a fixed-time AI protocol using PG and a CIDR could yield pregnancy rates similar to those requiring detection of estrus and 2) an injection of GnRH at CIDR insertion could enhance pregnancy rates compared with methods without GnRH at insertion.

MATERIALS AND METHODS

Locations and Animals

The use of animals in this experiment was in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999) and was approved by the University of Minnesota Institutional Animal Use and Care Committee. During the 2003 spring breeding season (April 1 to May 30), beef heifers used in this study were managed at 12 locations in 6 states. The herd size ranged from 34 to 435 heifers. A total of 2,090 replacement beef heifers were submitted initially for treatment; however, 15 heifers were eliminated from the study because they died, were culled, or became ill before the first pregnancy diagnosis. Breed types were British (n = 1,908), Continental (n = 39), British × Continental (n = 128), or undetermined (n = 15). Body condition scores (scale of 1 to 9; Whitman, 1975) for 8 of 12 locations were determined on d –17 relative to PG, and were 5.6 ± 0.6 (mean ± SD) with a range of 4 to 8. Data for individual locations are summarized in Table 1.

Treatments

Within location, all eligible heifers were inseminated artificially after being assigned randomly to 1 of 4 treatments (Figure 1), which were 1) Estrus detection + TAI (**ETAI**) (n = 516): heifers received a controlled internal drug release (**CIDR**) insert containing 1.38 g of progesterone (Pfizer Animal Health, New York, NY) for 7 d plus 25 mg of prostaglandin $F_{2\alpha}$ (**PG**; Lutalyse, dinoprost tromethamine, Pfizer Animal Health) at CIDR insert removal, followed by detection of estrus for 72 h and AI for 84 h after PG; heifers not detected in estrus by 84 h received 100 μg of GnRH (Fertagyl, gonadorelin, Intervet Inc., Millsboro, DE) and TAI; 2) **G+ETAI** (n = 503): ETAI plus 100 μg of GnRH at CIDR insertion; 3) Fixed-time AI (**FTAI**) (n = 525): heifers received a CIDR for 7 d plus 25 mg of PG at CIDR insert removal, followed in 60 h by a second injection of GnRH and TAI; and 4) **G+FTAI** (n = 531): FTAI plus 100 μg of GnRH at CIDR insertion.

For heifers in ETAI and G+ETAI treatments, a minimum of twice daily visual observations for estrus began on d 0 and continued for at least 72 h. Inseminations were performed according to the a.m.-p.m. rule (where heifers detected in estrus in the morning were inseminated artificially that evening or heifers detected in estrus in the evening were inseminated artificially the following morning) and were performed 8 to 14 h after first detected estrus. To determine the presence of a viable embryo, thereby assessing AI pregnancy rates, pregnancy was diagnosed by transrectal ultrasonography (5- or 7.5-MHz intrarectal transducer, Aloka 500V, Corometrics, Wallingford, CT) between 30 and 35 d after AI. A second pregnancy diagnosis to determine overall pregnancy rates was performed at 10 locations (IL-1, IL-2, KS-1, KS-2, MN-1, MN-2, MT-1, MT-2, MT-3, and ND) between 80 and 120 d after AI and was initiated a minimum of 30 d after clean-up bulls were removed. Exposure to natural-service bulls (clean-up bulls) was not initiated until a minimum of 10 d after AI to ensure a detectable difference in age between pregnancies initiated by AI compared with those resulting from natural matings.

Subsequent calving data at 7 locations (IL-1, IL-2, KS-2, MN-1, ND, OH-1, and OH-2) were recorded and resulted in 455 total births. Of the total births, 269 were the result of AI after initial treatments, whereas 186 births were the result of pregnancies initiated by clean-up bulls. Duration of gestation and calf gender ratios were assessed.

Blood Collection and RIA

At 8 of the 12 locations, blood samples (10 mL) were collected via venipuncture of jugular or tail veins on d –17 and –7 relative to the injection of PG (blood was not collected at the KS-1, ND, OH-1, or OH-2 locations). Blood was centrifuged at 3,000 × *g* for 10 to 15 min, and serum or plasma was recovered and stored at –20°C until RIA. Blood serum or plasma was analyzed for concentrations of progesterone in the laboratory of individual investigators according to validated procedures in each laboratory. Either serum or plasma was acceptable because the goal of assessing concentrations of progesterone was to determine cycling status. When either of the 2 blood samples had concentrations of progesterone ≥1 ng/mL, the heifer was considered to be

Table 1. Characteristics of heifers at each location, including number of heifers treated, breed composition, BCS, percentage having estrous cycles, and number of AI technicians and AI sires

Location[1]	No.	Breed origin	Mean BCS,[2] range	Cyclicity,[3] No./total, %	No. of AI technicians	No. of AI sires
IL-1	34	British, British × Continental	5.6 (5.0 to 6.0)	32/34 (94)	5	4
IL-2	56	Continental	5.5 (5.0 to 6.0)	49/56 (88)	1	2
KS-1	218	British	—	193/218 (89)	6	2
KS-2	160	British, British × Continental	—	—	2	1
MN-1	66	British	—	66/66 (100)	3	3
MN-2	435	British, British × Continental	6.0 (5.5 to 7.0)	413/416 (99)	4	4
MT-1	258	British	5.2 (4.5 to 8.0)	213/258 (83)	9	1
MT-2	320	British, Continental	5.2 (4.0 to 6.0)	250/320 (78)	4	9
MT-3	159	British	5.4 (4.0 to 6.0)	141/159 (89)	5	4
ND	146	British, British × Continental	—	—	1	3
OH-1	128	British	5.8 (5.0 to 8.0)	—	3	5
OH-2	95	British	5.7 (5.0 to 7.0)	—	3	5

[1]Location abbreviations refer to each of 12 herds among 6 states.
[2]BCS on 1 to 9 scale (1 = emaciated and 9 = obese; Whitman, 1975).
[3]Number of heifers cycling at initiation of treatments as a percentage of the total number of animals at each site.

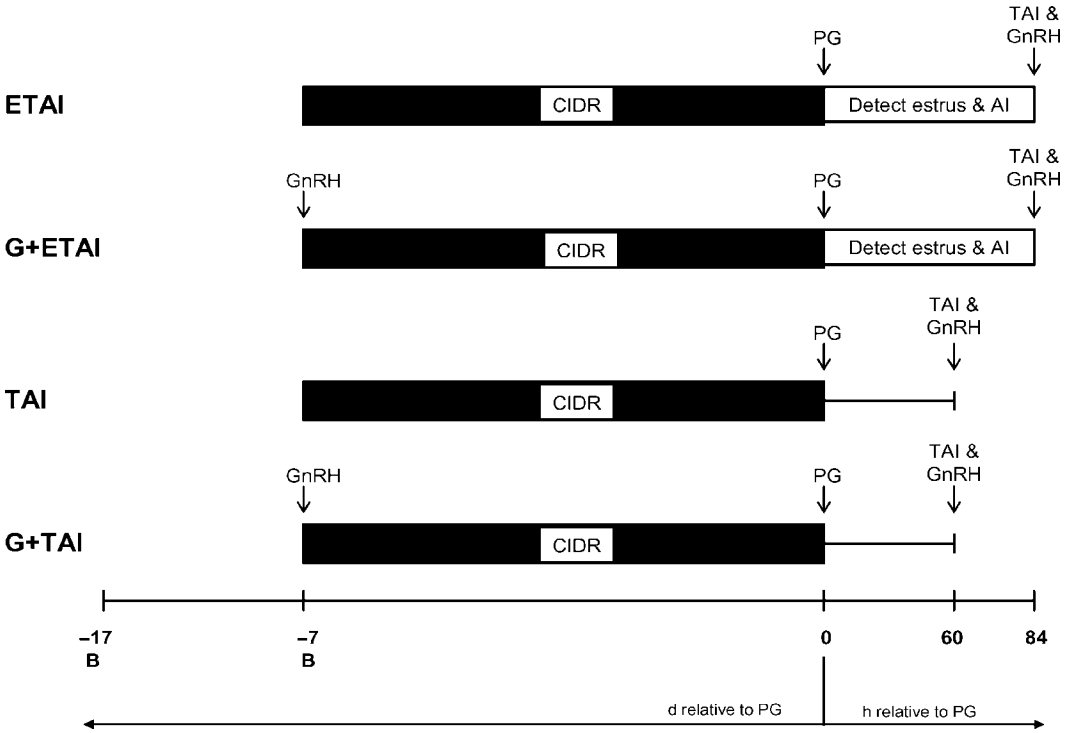

Figure 1. Experimental protocol for estrous synchronization treatments. Blood (B) samples were collected on d –17 and –7. PG = Prostaglandin $F_{2\alpha}$; CIDR = controlled internal drug release devise; and TAI = timed AI.

cycling at the initiation of treatments (Perry et al., 1991). The intraassay CV for the progesterone RIA ranged from 3.2 to 8.1% among locations, whereas the interassay CV ranged from 4.9 to 9.2%.

Definition of Terms

Estrous response: Percentage or number of heifers detected in estrus after PG.

Distribution of estrus: Percentage or number of heifers detected in estrus within specific time intervals following PG injection.

Clean-up TAI heifers: heifers that were not detected in estrus and were inseminated at a predetermined fixed time.

Conception rates: proportion of those heifers exhibiting estrus during the synchronization period that became pregnant to AI for the ETAI and G+ETAI treatments.

AI Pregnancy rates: proportion of heifers pregnant to AI of all heifers whose cycles were synchronized during the synchronized period.

Overall pregnancy rates: proportion of all heifers pregnant after AI and a period of exposure to natural-service bulls.

Statistical Analyses

The CATMOD procedure (SAS Inst. Inc., Cary, NC) was used to analyze all categorical data (conception rates, pregnancy rates, synchronization rates, cycling rates, and sex ratios). The GLM procedure of SAS was used to analyze noncategorical data (BCS, parity, time to estrus and AI, and gestation length) as a 2×2 factorial arrangement of treatments. The 2 factors being estrus AI plus a clean-up TAI vs. fixed-time AI (AI method) and GnRH vs. no GnRH at CIDR insertion (GnRH method). Proportions of heifers cycling at the onset of treatments were analyzed with location as a fixed effect. The model excluded the KS-1, ND, OH-1, and OH-2 locations because blood samples were not collected at those 4 locations. Within each model, means and SD were assessed to ensure that no biases existed among treatments based on BCS and cycling status.

The model used to analyze conception, AI pregnancy rates, and overall pregnancy rates included AI method, GnRH method, location, cycling status assessed before the onset of treatment, and all 2- and 3-way interactions, with BCS as a regression covariate. Because breed composition, AI sires, and AI technicians were confounded with location, they were not included in the model, but they were distributed evenly among treatments at each location.

For the 8 locations at which BCS were determined (IL-1, IL-2, MN-2, MT-1, MT-2, MT-3, OH-1, and OH-2), an analysis was performed to determine whether differences in pregnancy rates occurred among treatments for well-conditioned heifers (BCS >5.5) vs. those heifers in poorer body condition (BCS ≤5.5). The model included AI method, GnRH method, location, and all 2-way interactions.

The models used to analyze the proportion of heifers that were observed in estrus and time to AI from PG included GnRH method, location, and cycling status at the onset of treatment, and all 2-way interactions with GnRH method. Because estrus was only detected as part of 2 treatment protocols, the FTAI and G+FTAI treatments were excluded from the model. Models used to analyze gestation length, interval from PG to calving, and sex ratios included AI method, GnRH method, and location, plus the 2- and 3-way interactions.

RESULTS

Cycling Status

Results of progesterone analyses of blood samples collected from heifers at d −17 and −7 indicated that an average of 88.9% (1,357 of 1,527) were cycling before treatment initiation. Proportions of cycling heifers among 8 locations ranged from 78 to 100% (Table 1). Pregnancy rates to AI did not differ between cycling (56.9%; 772 of 1,357) and noncycling heifers (59.4%; 101 of 170).

Characteristics of Estrus and AI

For heifers in the ETAI and G+ETAI treatments, estrus was detected for 72 h after the PG injection, at which time all heifers not detected in estrus were inseminated. Percentage of heifers detected in estrus was 74.0 ± 2.4% and was similar ($P = 0.558$) between the 2 treatments. However, differences ($P < 0.001$) were detected in the percentage of heifers detected in estrus among locations. The location with the least percentage of heifers detected in estrus was OH-2 (51.0%), whereas the IL-2 location had the greatest estrus-detection rates (93.1%).

Time from PG injection to estrus for those heifers exhibiting estrus did not differ between ETAI and G+ETAI (48.9 ± 0.8 h). Further, time from PG injection to AI for those heifers exhibiting estrus was similar among treatments (60.4 ± 0.8 h). Distribution of estrus between the injection of PG and before TAI was similar among treatments (Figure 2). Regardless of treatment, interval from 36 to 60 h after PG injection yielded the greatest proportion of sexual behavior. Of heifers detected in estrus, 62.3% exhibited estrus during this interval.

Our goal was to ensure that the clean-up TAI in ETAI and G+ETAI treatments occurred at 84 h after PG. Average interval to TAI was similar among the ETAI and G+ETAI treatments (78.9 ± 0.5 h). Although no location × treatment interaction was detected, a location effect ($P < 0.05$) was apparent in which the shortest interval occurred at the ND (69.8 ± 0.9 h) location and the longest interval was at the IL-1 (88.2 ± 2.0 h) location.

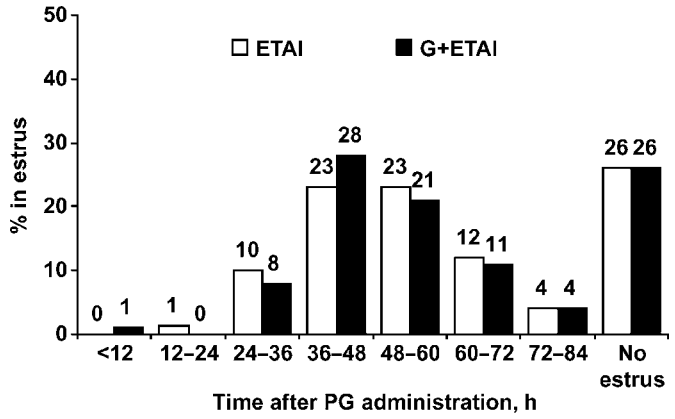

Figure 2. Distribution of estrus in ETAI heifers (open bars) and G+ETAI heifers (black bars). Numbers above bars represent the percentage of all females treated within each treatment. PG = Prostaglandin $F_{2\alpha}$; ETAI = heifers receiving a CIDR for 7 d plus PG at CIDR removal, followed by detection of estrus and AI (heifers not detected in estrus by 84 h received GnRH and timed AI); G+ETAI = ETAI plus GnRH at CIDR insertion.

Further, in the 2 TAI treatments (FTAI and G+FTAI), our aim was for the TAI to occur at 60 h after PG. Average interval was 56.3 ± 0.8 h for both treatments. No location × treatment interaction was detected, but a location effect ($P < 0.01$) was obvious in which the shortest interval to TAI occurred at the ND (47.9 ± 0.2 h) location and the longest interval at the IL-1 (64.0 ± 0.4 h) location.

Conception and Pregnancy Rates

Pregnancy rates to AI (Table 2) were 57.5, 54.7, 49.3, and 53.1% for G+ETAI, ETAI, FTAI, and G+FTAI treatments, respectively. No AI method × GnRH method interaction was detected; however, heifers assigned to the estrus detection treatments [571/1,019 (56%)] had greater ($P < 0.05$) AI pregnancy rates than heifers receiving TAI treatments [541/1,056 (51%)]. However, AI pregnancy rates for heifers receiving GnRH at CIDR insertion [571/1,034 (55%)] were similar to those heifers that did not receive GnRH at CIDR insertion [541/1,041 (52%)]. An interesting observation occurred when determining the variation in pregnancy rates of each treatment among locations. The SD were 10.5, 11.7, 11.9, and 16.4% for the G+FTAI, ETAI, G+ETAI, and FTAI treatments, respectively, demonstrating that the range and distribution of pregnancy rates was less consistent in the FTAI treatment than the other treatments.

No interaction of location × treatment was detected; however, when AI pregnancy rates among the 4 treatments were combined within each location, a location effect ($P < 0.01$) on AI pregnancy rates was observed (Figure 3). Pregnancy rates to AI among locations ranged from 39% (ND location) to 74% (MN-1 location), whereas those at the remaining 10 locations were intermediate. No difference in AI pregnancy rates occurred among treatments when heifers were categorized into 2 BCS groups: ≤5.5 and >5.5. No interactions were detected between BCS group, AI method, and GnRH method.

Within protocols in which estrus was detected, conception rates were similar for ETAI (60.9%; 233 of 383) and G+ETAI (63.4%; 236 of 372) heifers. Further, TAI conception rates for those heifers that were not detected in estrus and inseminated at 84 h were similar between ETAI (36.8%; 49 of 133) and G+ETAI heifers (38.9%; 51 of 131).

Among the 10 locations in which a second pregnancy diagnosis was performed, overall pregnancy rates did not differ among treatments with the overall pregnancy rate of 85.5%.

Gestation and Sex Characteristics

Calving data during the subsequent calving season was assessed for 454 calves at 7 locations (Table 3). Of the 454 calvings, 269 calves (59.3%) resulted from AI after estrus synchronization. Average duration of gestation among all AI sired calves was 277.6 d, with a range of 255 to 299 d. Duration of gestation was similar among AI method or GnRH method, but a location effect ($P < 0.0001$) was detected, which may have included breed, sire, and management differences. Heifers at the IL-1 location had the shortest gestation period (277.0 ± 1.2 d), whereas those at OH-1 had the longest gestation period (280.6 ± 0.7 d). Period of gestation was greater ($P < 0.001$) for male (278.7 ± 0.4 d) than for female calves (276.6 ± 0.5 d).

For those heifers from which calving data were recorded, average interval from PG injection (d 0 of the study) to calving among heifers was 289.1 d with a range of 255 to 344 d (Figure 4). Although average calving interval was similar among treatments, a location effect ($P < 0.001$) was detected. Average calving interval was shortest ($P < 0.05$) at the MN-1 (277.2 ± 2.3 d) location, whereas the KS-2 (294.3 ± 1.4 d) location had the longest average calving interval from PG.

At calving, sex was recorded in 452 calves, with 234 (51.8%) males compared with 218 females. In addition, only 1 set of twins was recorded and was the result of AI. Sex ratio of calves that conceived to AI after synchronization of estrus favored ($P < 0.01$) bulls (i.e., 51.1% of 266 calves born were male). Further, of the 185 calves that conceived to clean-up bulls, 53.0% were male. No difference was detected in sex ratio for AI compared with natural-sired calves.

DISCUSSION

Synchronization of estrus and AI are reproductive management tools that have been available to beef producers for over 30 yr. Synchronization of the estrous cycle has the potential to shorten the calving season,

Table 2. First-service AI pregnancy rates in replacement beef heifers after a synchronized estrus using prostaglandin $F_{2\alpha}$ (PG), GnRH, and (or) a controlled internal drug release (CIDR) device

Item	Treatment[1]				
	ETAI	G+ETAI	FTAI	G+FTAI	Overall
	No. (%)				
Conception rates[2]					
At estrus	233/383 (61)	236/372 (63)	—	—	469/755 (62)
TAI	50/133 (37)	51/131 (39)	—	—	101/264 (38)
AI pregnancy rates[3]	282/516 (55)	289/503 (57)	259/525 (49)	282/531 (53)	1,112/2075 (54)
Cycling status[4]					
Cycling	195/341 (57)	201/317 (63)	189/353 (54)	185/346 (54)	770/1357 (57)
Noncycling	19/36 (53)	32/54 (59)	22/36 (61)	28/44 (64)	101/170 (59)
Overall pregnancy[5] rates	378/451 (84)	393/450 (87)	389/463 (84)	412/474 (87)	1,572/1,838 (86)
BCS[6]					
≤5.5	106/188 (56)	112/183 (61)	92/166 (55)	97/187 (52)	407/724 (56)
>5.5	93/180 (52)	103/175 (59)	113/216 (52)	98/190 (52)	407/761 (53)

[1]See experimental design of treatments in Figure 1; ETAI = heifers a CIDR for 7 d plus PG at CIDR removal, followed by detection of estrus and AI [heifers not detected in estrus by 84 h received GnRH and TAI; G+ETAI = ETAI plus GnRH at CIDR insertion; FTAI = heifers received a CIDR for 7 d plus PG at CIDR removal, followed in 60 h by a second injection of GnRH and TAI; G+FTAI = FTAI plus GnRH at CIDR insertion.

[2]Conception rates for heifers inseminated after a detected estrus or at TAI at 84 h. Conception rates = percentage of cows pregnant compared with cows detected in estrus.

[3]AI Pregnancy rates = percentage of cows pregnant to AI compared with all cows estrous synchronized.

[4]Cycling status excludes KS-1, KS-2, MN-1, and ND locations.

[5]Overall pregnancy rates = percentage of cows pregnant during the breeding season compared with all cows estrous synchronized.

[6]BCS on 1 to 9 scale (1 = emaciated and 9 = obese; Whitman, 1975).

increase calf uniformity, and facilitate use of AI. Artificial insemination allows producers the opportunity to infuse superior genetics into their operations at costs far below the cost of purchasing a herd sire of similar standards. In addition, establishing pregnancy in replacement beef heifers with AI decreases the chance of

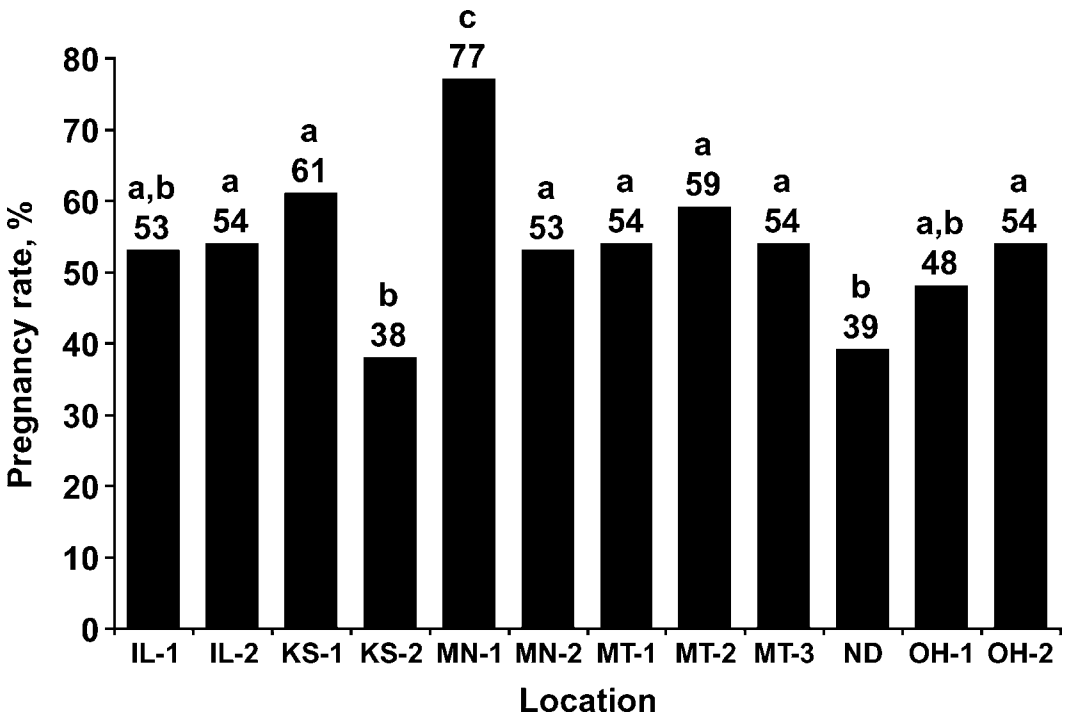

Figure 3. First-service AI pregnancy rates among locations. Numbers above each bar reflect actual first-service AI pregnancy rates among locations. Location abbreviations refer to each of 12 herds in 6 states. [a–c]Location effect ($P < 0.01$).

Table 3. Calving and sex characteristics after synchronization of estrus in replacement beef heifers using $PGF_{2\alpha}$ (PG), GnRH, a controlled internal drug release (CIDR) device, or a combination of these

Item	Treatment[1]				Overall	*P*-value
	ETAI	G+ETAI	FTAI	G+FTAI		
Calving length	d ± SE (No. of births)				d ± SD	
Average gestation length of AI sired calves[2]	277.1 ± 0.6 (74)	277.3 ± 0.3 (69)	277.7 ± 0.7 (60)	278.0 ± 0.6 (66)	277.6 ± 5.3 (269)	0.775
Average No. of days to calving after d 0[3]	303.7 ± 2.2 (49)	301.3 ± 2.1 (49)	304.3 ± 2.0 (51)	304.3 ± 2.4 (37)	305.9 ± 13.2 (186)	0.650
Sex ratios	Ratio (No. of calves)				ratio (No.)	
Sex ratio for AI sired calves[4]	0.39 (72)	0.51 (51)	0.62 (60)	0.55 (66)	0.51 (266)	0.067
Sex ratio for clean-up bull sired calves[5]	0.53 (49)	0.51 (49)	0.59 (51)	0.46 (37)	0.53 (186)	0.793

[1]ETAI = heifers a CIDR for 7 d plus PG at CIDR removal, followed by detection of estrus and AI, heifers not detected in estrus by 84 h received GnRH and TAI; G+ETAI = ETAI plus GnRH at CIDR insertion; FTAI = heifers received a CIDR for 7 d plus PG at CIDR removal, followed in 60 h by a second injection of GnRH and TAI; G+FTAI = FTAI plus GnRH at CIDR insertion.
[2]Determined on heifers pregnant after estrous synchronization, estrous detection and AI, or TAI.
[3]Includes all heifers calving during subsequent calving season.
[4]Percentage of males sired by AI after estrous synchronization.
[5]Percentage of males sired by clean-up bulls.

Figure 4. Distribution of calvings for AI-sired (black bars) and clean-up bull-sired (open bars) calves as a percentage of treated heifers calving during the subsequent calving season. PG = Prostaglandin $F_{2\alpha}$.

dystocia because proven sires may be selected that have high reliability for producing calving ease and small birth weights (Bennett and Gregory, 2001). Early estrus-synchronization methods failed to manage follicular waves, resulting in more days during the synchronized period in which detection of estrus was necessary (Brown et al., 1988). This ultimately precluded fixed-time AI with acceptable pregnancy rates (Pursley et al., 1995).

Cyclicity of heifers before the onset of treatments in this study ranged from 78 to 100% at 8 locations. These results are consistent with the percentage of pubertal heifers reported in other studies (Lynch et al., 1997; Lammoglia et al., 2000; Lamb et al., 2004) and indicate the variability in the percentage of cycling females among cattle operations. Although variation in percentage of heifers cycling occurred among locations in our study, a greater percentage of heifers were cycling compared with suckled beef cows at a similar interval before the breeding season (Stevenson et al., 2000; Lamb et al., 2001; Larson et al., 2006). Conception rates were unaffected by pubertal status, perhaps because the CIDR, GnRH, or both induced cyclicity. For example, Thompson et al. (1999), using ultrasonography, scanned the ovaries of 40 early postpartum, suckled beef cows before, during, and after treatments with GnRH, norgestomet, or both and reported that luteal structures were induced from dominant follicles in 75% of the noncycling cows, resulting in elevated progesterone after 7 d. Similarly, noncycling suckled beef cows treated with a CIDR in a TAI protocol had increased pregnancy rates compared with those cows not receiving a CIDR (Lamb et al., 2001).

Estrus-detection rate was 74% and was similar between ETAI and G+ETAI heifers. Distribution of estrus between the 2 treatments and interval from PG to estrus resembled that observed in dairy heifers that were treated with GnRH and PG 6 d apart (Richardson et al., 2002). Heifers receiving a 7-d CIDR with or without

GnRH at CIDR insertion and PG 1 d before CIDR removal had longer intervals to estrus after PG than those treated with GnRH and PG 6 d later (Richardson et al., 2002). In our study, however, the CIDR was removed concurrent with the PG injection, resulting in heifers exhibiting estrus earlier than would have occurred had the CIDR been removed on d 8. Average interval from PG to estrus in heifers in which estrus was synchronized with the MGA + PG protocol ranged from 44 to 71 h among 3 studies, demonstrating variation in response when synchronizing estrus with MGA + PG (Lamb et al., 2001, 2004; Wood et al., 2001). Further, among the 12 locations in our study, average interval from PG to estrus ranged from 42 to 60 h.

Pregnancy rates to the clean-up AI in the 2 estrus-detection treatments were 36.8 and 38.9% for ETAI and G+ETAI, respectively. Because of the clean-up TAI, pregnancy rates were increased by 9.7 and 10.1% points for ETAI and G+ETAI, respectively, that would not have occurred had nondetected heifers not been time-inseminated. Although clean-up TAI pregnancy rates were less than those achieved after detected estrus, clean-up TAI was essential to achieve pregnancy rates similar to those achieved by the G+FTAI protocol in which no detection of estrus was necessary.

Timed AI protocols using combinations of GnRH, PG, and progesterone for beef heifers have not yielded results as consistently acceptable as those reported for mature cows (Bader et al., 2005; Larson et al., 2006), especially considering that at the time of treatment initiation, a majority (88.9% in our study) of the heifers were pubertal and AI pregnancy rates did not differ between cycling and noncycling heifers (57 vs. 59%, respectively). The primary reason for TAI system failure seems to be the inability to synchronize follicular waves with the same rate of success as that achieved in cows. After an injection of GnRH, 64 to 75% of postpartum beef and dairy cows ovulated a follicle after an injection of GnRH (Geary et al., 1998; Thompson et al., 1999; El-Zarkouny et al., 2004), whereas only 48 to 60% of beef and dairy heifers ovulated follicles in response to the same treatment (Macmillan and Thatcher, 1991; Pursley et al., 1995; Moreira et al., 2000). In our study, no difference was detected in the synchrony of estrus or pregnancy rates between the ETAI and G+ETAI treatments, indicating that response to GnRH in heifers at CIDR insertion may be of limited value. Similarly, no difference in pregnancy rates existed between the 2 TAI treatments (FTAI and G+FTAI), but the variation in pregnancy rates among locations for the FTAI treatment demonstrates that perhaps a GnRH injection at CIDR insertion is necessary in a TAI program.

Two locations (KS-1 and ND) had AI pregnancy rates that were significantly less than the remaining 10 locations. The poor results at the KS-1 location did not seem to be related to an excessive proportion of immature heifers. A large percentage (83%) of heifers was detected in estrus in the ETAI and G+ETAI treatments, which was greater than the overall estrus-detection rates (74%) of all the locations combined. However, the 4-d period (July 8 to July 11, 2003) in which heifers were exposed to AI also was excessively warm and temperatures exceeded 34°C, with a heat index of greater than 36°C during all 3 d. Although more common in dairy herds (de la Sota et al., 1998; Rensis and Scaramuzzi, 2003), heat stress may have been the primary reason for poor fertility in this group of heifers. Percentage of heifers detected in estrus at the ND location was 69%, which was poorer than the overall percentage of heifers detected in estrus, indicating that a greater percentage of heifers may not have attained puberty at the time of PG injection and were unable to conceive. However, an additional reason for poor pregnancy rates at the ND location may be related to the interval between PG and TAI or clean-up TAI, which was the shortest of all 12 locations.

Duration of gestation is influenced by numerous factors including; breed, parity, sex of calf, year of birth, and single vs. multiple progeny (Gregory et al., 1979; Bourdon and Brinks, 1982; Cundiff et al., 1998). In the current study, gestation length was similar among treatments, averaged 278 d among all AI-sired calves, and was similar to previous reports (Bourdon and Brinks, 1982; Azzam and Nielsen, 1987; Sacco et al., 1990). However, average gestation length was shorter than the 286 d (Cundiff et al., 1998) and 287 d (Reynolds, et al., 1990) reported in earlier studies of multiparous cows. Shorter gestation periods in the current study likely resulted from all females giving birth to their first calf or because the genetic origin of the cows was primarily of British descent. A majority of the females in our study were of British or British × Continental origins, which tend to have shorter gestation periods than Continental cows and those of *Bos indicus* origin (Gregory et al., 1979; Cundiff et al., 1998).

Sex ratio at calving revealed no differences among treatments. Overall, 51% of calves sired by AI were male and 53% of clean-up-bull sired calves were male. Sex ratio favored males regardless of whether their mothers conceived to AI after estrus synchronization or after natural mating. These results confirm previous studies (Gardner, 1950; Ballinger, 1970; Larson et al., 2006) indicating that sex ratio was not altered by time of insemination and a greater proportion of male calves was born.

Comparison of FTAI and G+FTAI treatments indicated that addition of a GnRH injection at CIDR insertion did not substantially improve pregnancy rates after a fixed TAI at 60 h, but there was a greater SD in pregnancy rates among locations for the FTAI treatment. Further, comparison among ETAI and G+ETAI revealed that injecting GnRH at CIDR insertion did not alter the percentage of heifers detected in estrus or distribution of estrus after PG. The main effects of AI method demonstrated that a combination of detecting estrus and AI before a clean-up TAI at approximately 84 h after PG proved effective in reducing the time and labor associated with this estrus-synchronization

protocol and enhanced pregnancy rates over TAI at 60 h by approximately 5%. In addition, the G+FTAI treatment yielded pregnancy rates similar to those achieved after detection of estrus before a clean-up TAI at 84 h, indicating that a TAI protocol without detecting estrus seems to be a suitable option for synchronization of estrus in replacement beef heifers. However, an injection of GnRH at CIDR insertion may be necessary to achieve consistent pregnancy rates similar to those in the estrus detection treatments. In addition, our results also indicated that factors associated with individual location, which could include differences in pasture and diet, climate, and geography, may affect the success of estrous synchronization with PG, GnRH, a CIDR, or a combination of these.

IMPLICATIONS

Optimal pregnancy rates in beef cattle require more than compliance to a sound estrus-synchronization protocol. We have demonstrated that beef producers can effectively utilize 2 strategies to enhance the ease and efficiency of implementing estrus-synchronization protocols while optimizing pregnancy rates in beef production systems: 1) a strategy that reduces detection of estrus by combining detection of estrus with a clean-up timed artificial insemination; and 2) a fixed timed artificial insemination protocol that includes a controlled internal drug release eliminates detection of estrus with all heifers inseminated artificially at a single predetermined time. Both strategies are of short duration (<10 d) and limit the frequency that heifers are handled, allowing artificial insemination to be utilized as a suitable reproductive management tool for producers. It is important to note that 88.9% of heifers used in this study were cycling before treatment initiation. It is well established that proper management of heifers preceding their first breeding season is critical to their reproductive success.

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