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Evaluation of the 14-d CIDR-PG and 9-d CIDR-PG protocols for synchronization of estrus in *Bos indicus*-influenced and *Bos taurus* beef heifers

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ABSTRACT

Two long-term, CIDR-based estrus synchronization protocols were evaluated among *Bos indicus*-influenced and *Bos taurus* beef heifers. Treatments were evaluated on the basis of estrous response and pregnancy rate resulting from fixed-time artificial insemination (FTAI), and these outcomes were analyzed retrospectively relative to reproductive tract score (RTS; Scale 1–5) at treatment initiation. Estrus was synchronized for 1139 heifers in three locations, and heifers were assigned to one of two treatments within each location based on RTS. Heifers assigned to the 14-d CIDR-PG protocol received a controlled internal drug release (CIDR) insert (1.38 g progesterone) on Day 0, CIDR removal on Day 14, administration of prostaglandin $F_{2\alpha}$ (PG; 25 mg im) on Day 30, and administration of gonadotropin-releasing hormone (GnRH; 100 μg im) concurrent with FTAI on Day 33, 66 h after PG. Heifers assigned to the 9-d CIDR-PG protocol received administration of PG concurrent with CIDR insertion on Day 5, administration of PG concurrent with CIDR removal on Day 14, administration of PG on Day 30, and administration of GnRH concurrent with FTAI on Day 33, 66 h after PG. Estrus detection aids were applied at CIDR removal on Day 14 and at PG on Day 30 to evaluate estrous response rate. Mean RTS differed ($P < 0.0001$) based on biological type due to higher rates of estrous cyclicity (RTS 4 and 5) among *Bos taurus* heifers (72%; 416/574) than among *Bos indicus*-influenced heifers (27%; 150/565). The proportion of heifers expressing estrus following CIDR removal was greater ($P = 0.01$) among heifers assigned to the 14-d CIDR-PG treatment (88%; 492/559) compared to the 9-d CIDR-PG treatment (83%; 480/580). Estrous response following CIDR removal was also higher ($P < 0.0001$) among *Bos taurus* (95%; 547/574) compared to *Bos indicus*-influenced (75%; 425/565) heifers. Rate of estrous response prior to FTAI did not differ significantly based on treatment but was higher ($P < 0.0001$) among *Bos taurus* heifers (60%; 344/574) than among *Bos indicus*-influenced heifers (45%; 253/565). However, the effect of biological type on estrous response was not significant when RTS was included in the model, as RTS significantly ($P < 0.0001$) affected the rate of estrous response both at CIDR removal and prior to FTAI. Across treatments and biological types, heifers that expressed estrus prior to AI achieved higher ($P < 0.0001$) AI pregnancy rates than heifers failing to express estrus. Pregnancy rates to FTAI did not differ significantly based on treatment in either biological type. Higher rates of estrous cyclicity among *Bos taurus* heifers resulted in higher FTAI pregnancy rates among *Bos taurus* (51%; 290/574) compared to *Bos indicus*-influenced heifers (39%; 218/565). However, pregnancy rates of respective RTS did not differ based on biological type. In summary, long-term CIDR-based protocols provide a simple, effective method of estrus synchronization in *Bos indicus*-influenced and *Bos taurus* beef heifers. Moreover, these results highlight the importance of management practices that result in high rates of estrous cyclicity prior to protocol initiation, particularly among later maturing breeds and biological types.

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1. Introduction

Development of protocols to synchronize estrus and ovulation prior to fixed-time artificial insemination in crossbred beef heifers of *Bos indicus* influence has been limited in comparison to *Bos taurus* beef heifers. *Bos indicus*-influenced cattle are generally regarded to exhibit increased susceptibility to stress associated events and are unique from *Bos taurus* females based on their distinct reproductive physiology [1,2]. *Bos indicus*-influenced heifers generally reach puberty at later ages than *Bos taurus* females and differ from *Bos taurus* females in preovulatory follicular diameter, timing of follicular wave emergence, and sensitivity to progesterone effects on LH pulsatility [3–5]. These differences between biological types complicate the development of systems to effectively control the estrous cycles of *Bos indicus*-influenced cattle and have therefore limited the expanded use of AI in regions of the United States where these cattle are better adapted to tropical and subtropical environmental conditions [1,2]. Although several protocols have been developed in South America for synchronization of estrus in *Bos indicus* cattle [6], these protocols generally use estradiol products not currently approved by the United States Food and Drug Administration for use in estrus synchronization.

The 14-d CIDR-PG protocol is a long-term CIDR-based estrus synchronization protocol that has been widely adopted for use among *Bos taurus* beef heifers in many parts of the United States. Estrous response rate following this protocol has been well characterized among *Bos taurus* beef heifers [7–9], and the synchrony and rate of estrous response provides a platform for effective use of fixed-time artificial insemination (FTAI). However, despite extensive use of the 14-d CIDR-PG protocol among *Bos taurus* replacement beef heifers, there are no published studies to date involving use of the protocol in *Bos indicus*-influenced heifers.

The 9-d CIDR-PG protocol is a similar long-term CIDR-based estrus synchronization protocol that was recently developed for use in mature beef cows. Preliminary evaluation suggested that the incorporation of prostaglandin $F_{2\alpha}$ (PG) treatment at CIDR insertion and removal facilitated a decreased length of progestin treatment and enhanced estrous response [10], and subsequent work found that pregnancy rate to FTAI was increased among 9-d CIDR-PG treated compared to 14-d CIDR-PG treated cows [11]. However, the 9-d CIDR-PG protocol has not yet been evaluated among either *Bos indicus*-influenced or *Bos taurus* beef heifers.

Treatment of prepubertal heifers with exogenous progesterone hastens attainment of puberty and enhances conception rates among both *Bos taurus* [12–15] and *Bos indicus* [16] heifers. However, high levels of circulating progesterone decrease LH pulsatility and therefore have a suppressive effect relative to ovarian follicular development [17–20]. With the hypothesis that this effect is particularly consequential among *Bos indicus*-influenced females, previous studies evaluated strategies to reduce endogenous progesterone production or limit exogenous progesterone administration during treatment with a short-term progestin-based estrus synchronization protocol as a means of improving success among *Bos indicus*-influenced females [5,21–24].

This experiment was designed to characterize the relative effectiveness of long-term CIDR-based protocols among *Bos indicus*-influenced and *Bos taurus* heifers in their respective production systems in the midwestern and southeastern United States. We hypothesized that the 9-d CIDR-PG protocol would increase estrous response and FTAI pregnancy rates among *Bos taurus* and/or *Bos indicus*-influenced heifers in comparison to the 14-d CIDR-PG protocol.

2. Materials and methods

All experimental procedures were approved by the University of Missouri Animal Care and Use Committee.

2.1. Animals

On Day 0 prior to protocol initiation, individual animal weights and reproductive tract scores (RTS; Scale 1–5) [25–27] were obtained for beef replacement heifers (14–15 months of age) at three locations. In each location, heifers identified as having an abnormal or infantile (RTS 1) reproductive tract were culled and not exposed for estrus synchronization and AI (Location 1: n = 18; Location 2: n = 3; Location 3: n = 104). The remaining 1139 heifers were assigned within location to treatments (Fig. 1) based on source and RTS. Location 1 (n = 533) and Location 2 (n = 41) consisted of exclusively *Bos taurus* commercial Angus heifers, and Location 3 (n = 565) consisted of exclusively crossbred heifers of *Bos indicus* influence, ranging from 1/8th to 3/8th Brahman. Heifers assigned to the 14-d CIDR-PG protocol received an Eazi-Breed CIDR insert (1.38 g progesterone; Zoetis, Madison, NJ) on Day 0 and CIDR removal on Day 14. Heifers assigned to the 9-d CIDR-PG protocol were administered prostaglandin $F_{2\alpha}$ (PG; 25 mg im; Lutalyse, Zoetis, Madison, NJ) concurrent with CIDR insertion on Day 5 and concurrent with CIDR removal on Day 14. Heifers in both treatments were administered PG on Day 30, and fixed-time artificial insemination (FTAI) was performed on Day 33, 66 h after PG administration. Gonadotropin-releasing hormone (GnRH; 100 μg im; Cystorelin, Merial, Athens, GA) was administered to heifers in both treatments concurrent with FTAI. Time of PG administration on Day 30 and time of FTAI on Day 33 was recorded for each heifer. Within each location, insemination was performed using conventional frozen-thawed semen sourced from a single AI sire. In Location 1 and Location 3, two technicians were pre-assigned and balanced across treatments based on heifer source, RTS, and weight. In Location 2, a single technician performed all inseminations. Fourteen d after AI, heifers were exposed to fertile bulls for the remainder of the breeding season.

2.2. Estrus detection

Estrus detection aids (Estrotect, Rockway Inc, Spring Valley, WI) were applied at CIDR removal on Day 14 and evaluated at PG administration on Day 30 to determine rate of estrous response following CIDR removal. A new estrus detection aid was applied at PG administration on Day 30 and evaluated at FTAI on Day 33 to determine rate of estrous response following PG administration. Estrus was defined as removal of >50% of the scratch-off coating on the Estrotect.

2.3. Pregnancy diagnosis

Pregnancy rate to FTAI was determined by transrectal ultrasonography (SonoSite EDGE equipped with a L52 10.0–5.0 MHz linear-array transducer; SonoSite Inc., Bothell, WA) 62 d–90 d after FTAI. Pregnancies resulting from AI were distinguished from those resulting from natural service based on fetal size [28], as heifers were not exposed to natural service bulls until 14 d after AI.

2.4. Statistical analysis

Treatment differences for RTS, weight, and timing of insemination were analyzed using the TTEST procedure of SAS (SAS Inst. Inc., Cary, NC). Chi-square contingency tables (PROC FREQ; SAS Inst. Inc., Cary, NC) were used to initially assess potential differences in