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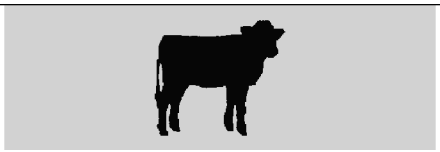

Evaluation of Gonadotropin-Releasing Hormone at Fixed-Time Artificial Insemination in Beef Heifers Synchronized Using a Modified CO-Synch Plus Controlled Internal Device Release Protocol

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Abstract

*The objective of this study was to determine whether a second injection of gonadotropin-releasing hormone (**GnRH**) at 54-h timed AI (**TAI**) of a CO-Synch/**CIDR** (controlled internal device release) protocol improves fertility in replacement beef heifers. Heifers (n = 375) at three locations (Colorado, Wyoming, and South Dakota) were stratified by BW within body condition score (**BCS**) and randomly allotted to one of two treatments. All heifers received 100 μg of GnRH and a CIDR insert (d –7), followed by CIDR removal and 25 mg of prostaglandin $F_{2\alpha}$ (**PG**; d 0). On d 2, control and treatment heifers underwent TAI at 54 h post-PG, and treatment heifers received a second 100-μg injection of GnRH. Blood samples were collected from all heifers at Colorado and Wyoming (d –17 and –7) to determine pubertal status. Ultrasonography was used to determine ovulation rate after TAI from a subsample of heifers (Colorado, n = 19; Wyoming, n = 49). No treatment × location interaction (P>0.10) occurred and pooled TAI pregnancy rates were similar (P>0.01) for control (46%) vs treatment (55%) heifers. Pubertal rates were greater (P<0.01) for heifers at Colorado (97.4%) than for heifers at Wyoming (46.4%); however, TAI pregnancy rates were similar (P> 0.10) for pubertal and prepubertal heifers. Ovulation rates tended to be different (P=0.10) for treatment (81.3%) than for control (62.5%) heifers. We conclude that the second injection of GnRH at TAI in the CO-Synch/CIDR protocol does not increase pregnancy rates to TAI in beef heifers, but that it may be economically viable and may guard against reduced fertility.*

(Key Words: Beef Heifers, Ovulation Synchronization, Fixed-Time Artificial Insemination, Controlled Internal Device Release.)

Introduction

Approaches to estrous synchronization in beef heifers have included the use of melengestrol acetate, gonadotropin-releasing hormone (GnRH), prostaglandin $F_{2\alpha}$ (PG), and combinations of these. Pregnancy rates from fixed-time AI (TAI) have varied widely because of day of cycle when synchronization begins, number of heifers ovulating (Ovsynch; Pursley et al., 1994; Moreira et al., 2000), and

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time of ovulation (GnRH/PG/GnRH; Pursley et al., 1995). Estrous synchronization protocols that involve GnRH/PG and administration of a second injection of GnRH at 48-h TAI (CO-Synch; Geary and Whittier, 1998) improved ovulation synchronization and fertility in beef cows (Thompson et al., 1999) but resulted in somewhat lesser fertility in beef heifers undergoing TAI at 48 h when compared with insemination following a standing estrus (Schmitt et al., 1994). Pregnancy rates from a GnRH/PG protocol increased when heifers received GnRH at 54-h TAI compared with no GnRH administered at TAI (Twagiramungu et al., 1995b). Twagiramungu et al. (1995a) reported that emergence of a new ovarian follicular wave occurs when either a large follicle continues its regression by atresia, GnRH administration causes ovulation, or a new wave emerges spontaneously before PG-induced luteolysis. A dominant follicle from the new ovarian follicular wave then ovulates following PG-induced luteolysis 24 to 32 h after the second injection of GnRH (Pursley et al., 1995). Addition of a controlled internal devise release (CIDR) insert to the GnRH/PG protocol improved fertility in beef heifers (Mapletoft et al., 2003); a majority of heifers expressed estrus between 48 and 72 h after PG (Schmitt et al., 1996; Martinez et al., 2002; Richardson et al., 2002). Also, increased progesterone concentrations decrease luteinizing hormone (**LH**) pulse frequency, allowing ovulation of a dominant follicle following its decline (Anderson et al., 1996). By administering a CIDR insert with a CO-Synch protocol, the value of a second GnRH injection at TAI for improving fertility in beef heifers is unknown. The objectives of this study were to determine whether the second injection of GnRH at 54-h TAI of the CO-Synch/CIDR protocol improves fertility in replacement beef heifers.

Materials and Methods

Experimental Design. Nulliparous crossbred beef heifers from a cooperator herd in South Dakota [n = 211; BW = 392.3 ± 31.0 kg; body condition score (BCS) = 5.7 ± 0.30] and research herds in Colorado (n = 39; BW = 324.5 ± 29.5 kg; BCS = 5.7 ± 0.27) and Wyoming (n = 125; BW = 325 ± 27.8 kg; BCS = 5.4 ± 0.50) were synchronized with the CO-Synch plus EAZI BREED CIDR® (1.38 g of progesterone; Pfizer Animal Health, New York, NY) protocol. Heifers were stratified by BW within BCS and location to one of two treatment groups. All heifers received 100 μg (i.m.) of GnRH (Fertagyl®; Intervet., Inc., Millsboro, DE) concurrent with a CIDR insert on d –7, followed by CIDR removal and 25 mg (i.m.) of PG (Lutalyse®; Pfizer Animal Health) on d 0. On d 2, heifers in the control and treatment groups were inseminated 54 h post-PG administration, and heifers in the treatment group received a second injection (100 μg; i.m.) of GnRH at TAI. Heifers at Colorado and South Dakota were inseminated by one of two experienced AI technicians, and Wyoming heifers were inseminated by one of the same technicians or a third experienced technician. Service sires used for AI were different for each location and were assigned randomly to heifers within each location except where potential inbreeding was a concern (Colorado = 2, South Dakota = 13, and Wyoming = 4). Clean-up bulls were introduced 8 d (South Dakota) and 14 d (Colorado and Wyoming) after TAI for an additional 45 d. Heifers were diagnosed for pregnancy to TAI (Colorado, South Dakota, and Wyoming) at 45 d after TAI using transrectal ultrasonography (5-MHz intrarectal transducer, Aloka 500V; Corometrics, Wallingford, CT). Final breeding season pregnancy rates (only at Colorado and Wyoming) were determined using transrectal ultrasonography at approximately 120 d after TAI. First pregnancy diagnosis at 45 d was confirmed by the presence and size of embryo.

Determination of Ovulation Rate. Ovaries from a subset of heifers (randomly selected by BW within BCS from each treatment) at Colorado (n = 19) and Wyoming (n = 49) were examined by transrectal ultrasonography to characterize incidence of ovulation. Ovaries were scanned using an Aloka 500V with a 5-MHz intrarectal transducer at time of CIDR removal (d 0) to identify the presence of a large preovulatory dominant follicle and again at 48 h after CIDR removal (d 2; 6 h prior to TAI) to determine diameter of the largest dominant follicle. Follicles were classified as dominant if the diameter of that follicle was ≥10 mm (Ginther et al., 1989). At 40 h after TAI (d 4), ovaries were scanned for a third time to determine whether ovulation had occurred. Ovulation was defined as the disappearance of the largest dominant follicle present on the ovary at 48 h after CIDR removal. Follicular cysts were detected in one heifer from Colorado and in three heifers from Wyoming. Follicles were classified as cystic if the diameter of the largest follicle was >25 mm (Savio et al., 1990) at CIDR removal and at TAI. These heifers were removed from data estimating ovulation rate; thus, 18 (Colorado) and 46 (Wyoming) heifers remained for ovulation rate analysis.

Blood Sampling and Progesterone Analysis. Two jugular vein blood samples were collected with sterile vacuum tubes (Red Stopper®; Sherwood Medical, St. Louis, MO) from all heifers at Colorado and Wyoming on d –17 and –7 to determine serum concentrations of progesterone. Heifers were classified as pubertal before the onset of synchronization if at least one of the two serum samples contained concentrations of serum progesterone ≥1 ng/mL. All blood samples collected for progesterone analysis were allowed to clot on ice for 12 h. Samples were then centrifuged at 486 × g at 4°C for 15 min. Serum was collected and stored at –20°C until analyzed for progesterone concentration by solid-phase radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Serum samples were assayed in duplicate, and sensitivity of the assay was 0.08 ng/mL. Within and between

assay CV for serum samples were 12.9 and 9.6%, respectively, across two assays.

Statistical Analysis. Preliminary analysis of treatment, location, technician, and all possible interactions on TAI pregnancy rates revealed no significant differences in pregnancy rates to TAI for technician or technician × treatment interactions; therefore, AI technician was not included in the final analysis. Response variables included estrous cycling status, final pregnancy rate (Colorado and Wyoming), and TAI pregnancy rate. Fixed effects included in all models were treatment and location. Covariates included BW and BCS. For TAI pregnancy data, estrous cycling status was included as an additional main effect for Colorado and Wyoming data only. All possible two-way (estrous cycling status) and three-way (TAI pregnancy rate) interactions were modeled for each outcome. Statistical procedures were appropriate to binary observations using the Proc GENMOD procedure in SAS® (SAS Inst., Inc., Cary, NC). A sire effect was also included in all models as a random effect. Significance of main effects was determined using Chi-square at $P<0.05$.

Data collected on incidence of ovulation for heifers at the Colorado and Wyoming locations were analyzed using Proc GLM in SAS. Fixed effects included treatment, location, and treatment × location interaction on incidence of ovulation; significance was determined at $P<0.05$.

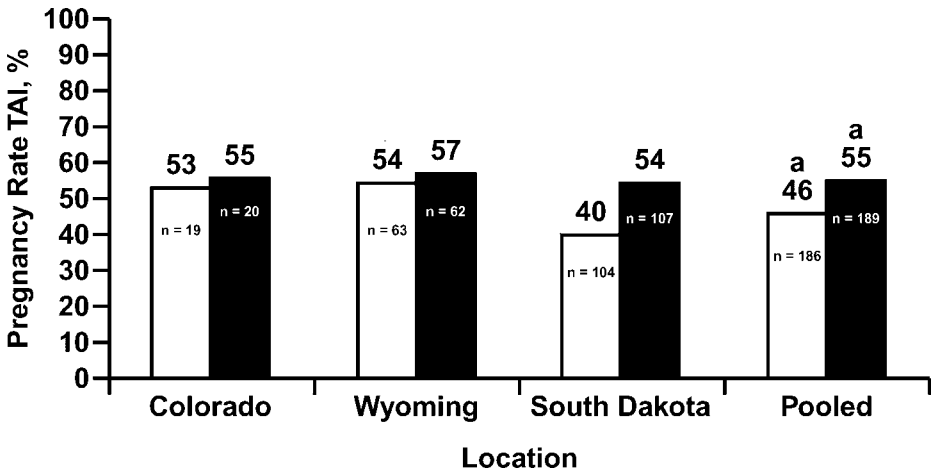

Figure 1. Effects of treatment (shaded bars) and control (open bars) protocols on timed AI (TAI) pregnancy rates synchronized with the controlled internal device release (CIDR)/CO-Synch protocol and 54-h TAI for each location and for all locations pooled. The CIDR/CO-Synch protocol consisted of 100 μg of gonadotropin-releasing hormone (GnRH) on d –7 given concurrently with a CIDR insert, CIDR removal, and 25 mg of prostaglandin $F_{2\alpha}$ on d 0. On d 2, control and treatment heifers are TAI at 54 h, and treatment heifers receive a second injection (100 μg) of GnRH at TAI. Raw mean percentages between pooled control and treatment heifers without a common letter differ (P<0.05).

Results and Discussion

Timed AI pregnancy rates for the two treatment groups for all three locations combined are summarized in Figure 1. Final breeding season pregnancy rates for heifers at Colorado (97.4%; 38 of 39) and Wyoming (89.1%; 106 of 119) were not different ($P>0.10$; data not shown), and final breeding season pregnancy rates were not determined at South Dakota because of management restrictions. The CIDR retention rate for all heifers at all locations was 100%. No treatment × location interaction occurred ($P>0.10$) and overall TAI pregnancy rates did not differ ($P>0.10$) among heifers at Colorado (53.9%), South Dakota (47.4%), and Wyoming (55.2%). Pregnancy rates to TAI were not different ($P>0.10$) for heifers in the control (46.2%) *vs* treatment (55.0%) group, respectively. Twagiramungu et al. (1995b) reported greater pregnancy rates when GnRH was administered at 54 h TAI compared with no GnRH given at TAI in beef heifers synchronized with a GnRH/PG protocol. However, a CIDR insert was not incorporated into the GnRH/PG protocol. With application of a CIDR insert in a GnRH/PG protocol, a majority of beef heifers expressed estrus between 48 and 60 h after PG and CIDR removal (Schmitt et al., 1996; Martinez et al., 2002), thus improving fertility of those heifers synchronized. If the majority of heifers in the present study would have expressed estrus 48 to 60 h after PG, an endogenous LH surge would have occurred 3 to 7 h later, followed by ovulation occurring 25 to 27 h later (Hendricks et al., 1970; Grieger et al., 1991). If such were the case, administering a second injection of GnRH at 54-h TAI would have occurred during the natural endogenous LH surge and may not have improved fertility. Perhaps induced ovulation at 54 h among treatment heifers that had not yet exhibited estrus resulted in a premature ovulation of smaller follicles with lesser pregnancy rates (Perry et al., 2005) than delayed ovulation and fertilization by aged spermatozoa, as may occur among some control heifers.

Overall, 97.4 and 46.4% of Colorado and Wyoming heifers, respectively, were pubertal ($P<0.01$; Table 1). No differences were observed ($P>0.10$) in the proportion of heifers cycling between control (54.9%) and treatment (57.3%) groups. The proportion of pubertal and prepubertal heifers pregnant to TAI did not differ ($P>0.10$) between the control (53.1 and 54.5%) and treatment (57.5 and 54.3%) groups, respectively (Table 1). These results coincide with reported advantages of progestogens administered to cattle not cycling at the beginning of the breeding season (Odde, 1990). Increased progesterone

TABLE 1. Estrous cycling and timed AI pregnancy rates (PR) of heifers bred at the Colorado and Wyoming locations for each treatment group and treatment groups combined[a].

Item	Control	Treatment	Overall
	[no./no. (%)]		
Percentage estrous cycling[b]			
CO	19/19 (100)	19/20 (95)	38/39 (97.4)[y]
WY	30/63 (47.6)	28/62 (45.2)	58/125 (46.4)[z]
Overall	45/82 (59.8)	47/82 (57.3)	96/164 (58.5)
PR, estrous cycling[c]			
CO	10/19 (52.6)	11/19 (57.9)	21/38 (55.3)
WY	16/30 (53.3)	16/28 (57.1)	32/58 (55.2)
Overall	26/49 (53.1)	27/47 (57.5)	53/96 (55.2)
PR, prepubertal			
CO	0/0 (0.0)	0/1 (0.0)	0/1 (0.0)
WY	18/33 (54.5)	19/34 (55.9)	37/67 (55.2)
Overall	18/33 (54.5)	19/35 (54.3)	37/68 (54.4)

[a]Control heifers were synchronized with 100 μg of gonadotropin-releasing hormone (GnRH) on d –7 plus a controlled internal device release (CIDR) insert for 7 d and 25 mg of prostaglandin $F_{2\alpha}$ (PG) on d 0 with CIDR removal, followed by timed AI (TAI) 54 h after PG. Treatment heifers were synchronized with same protocol as control heifers, plus incorporation of a second injection of GnRH (100 μg) at 54 h TAI.

[b]Percentage of heifers cycling at both Colorado (CO) and Wyoming (WY) locations based on progesterone concentrations of blood serum ≥1 ng/mL for either d –17 or –7. Estrous cycling data were not available for heifers at the South Dakota location.

[c]Pregnancy rates for heifers determined to be pubertal and prepubertal.

[y,z]Raw mean percentages within a column for percentage estrous cycling for both treatments combined (overall) with a common letter differ ($P<0.05$).

concentrations, such as with a CIDR insert, are known to decrease LH pulse frequency (Twagiramungu et al., 1995a), which should allow continued follicular growth and result in increase LH pulse frequency and ovulation following its removal (Anderson et al., 1996) for both pubertal and prepubertal heifers.

The incidence of ovulation by 40 h after TAI was not different ($P>0.10$) between Colorado and Wyoming heifers (66.7 and 73.9%). Ovulation rates tended to be different ($P=0.10$) between treatment (81.3%) and control (62.5%) heifers (Figure 2). Higher ovulation rates among treatment heifers did not result in greater overall pregnancy rates, suggesting again that perhaps addition of a second GnRH injection might have resulted in premature ovulation of small follicles in some heifers with lower fertility (Perry et al., 2005). Although ovulation rates for control heifers were high, perhaps increasing the number of heifers scanned for incidence of ovulation per treatment group would have increased the power to detect real differences. The doubling of ovulation rate among Colorado heifers, most of which were cyclic, suggests the second GnRH injection at TAI may be more important among groups of cyclic heifers. Heifers ovulating by 40 h after TAI at Colorado and Wyoming, regardless of treatment, had greater ($P<0.10$) TAI pregnancy rates (58.7%; 27 of 46) than heifers that had not ovulated (16.7%; 3 of 18). Of the heifers not ovulating by 40 h after TAI, one heifer in the control group at Colorado and one heifer in each of the two treatment groups at Wyoming did not have a large preovulatory dominant follicle on either the right or the left ovary at the time of second scanning on d 9 (48 h after PG). It is evident from these data that incidence of ovulation was marginal and variable between herds, when a second injection of GnRH was not administered at TAI.

The number of service sires used was different and varied between locations. There was no sire by location effect ($P>0.10$) and differences in pregnancy rate to TAI were not observed for Colorado heifers inseminated to two different AI sires. However, one of four sires used at Wyoming and three of 13 sires used at South Dakota collectively resulted in heifer pregnancy rates of 37%. The remaining 13 sires from both South Dakota and Wyoming collectively resulted in heifer pregnancy rates of 56%. Dalton et al. (2001) reported significant differences among AI bulls on fertility rates when used for insemination at 0, 12, and 24 h after first standing estrus in dairy cows, and Saacke (2002) emphasized that sire differences can significantly impact pregnancy rates in TAI programs. Identifying sires that produce superior fertility rates in cattle that are synchronized for TAI would be expected to enhance pregnancy rates with this method.

In summary, addition of the second GnRH injection at 54-h TAI in beef heifers receiving a CO-Synch/CIDR protocol resulted in insignificant improvements in fertility. Although we can state that there were not differences between control and treatment heifers from a scientific standpoint, the value of GnRH given at TAI is unknown and, thus, may be economically viable to guard against reduced fertility. Given the increase in ovulation rate for treatment heifers and the variation in bull fertility in this study, use of semen from bulls proven to have high TAI pregnancy rates may increase the significance and the value of the second GnRH at TAI with this protocol.

Implications

Administration of a second injection of GnRH at 54-h TAI in a Co-

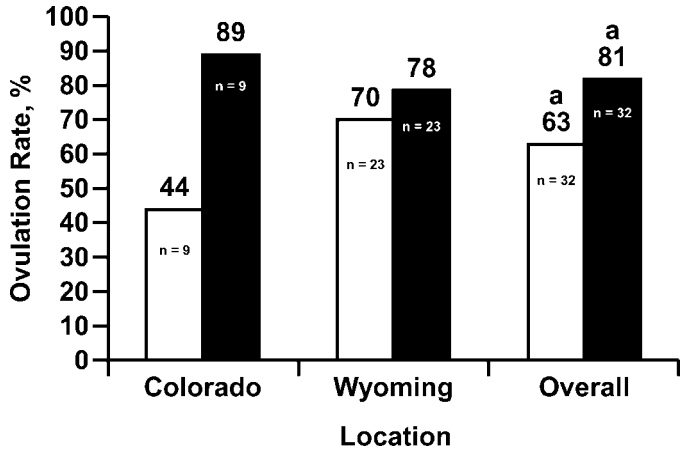

Figure 2. Effects of treatment (shaded bars) and control (open bars) protocols on ovulation rates in heifers scanned by transrectal ultrasonography at controlled internal device release (CIDR) removal (d 0) and at 48 h (d 2) and 88 h after CIDR removal (d 4) from Colorado and Wyoming locations only. The CIDR/CO-Synch protocol consisted of 100 μg of gonadotropin-releasing hormone (GnRH) on d –7 given concurrently with a CIDR insert, CIDR removal, and 25 mg of prostaglandin $F_{2\alpha}$ on d 0. On d 2, control and treatment heifers are TAI at 54 h, and treatment heifers receive a second injection (100 μg) of GnRH at TAI. Raw mean percentages for ovulation rates between overall control and treatment heifers without a common letter (a) differ (P<0.05).

Synch/CIDR protocol did not statistically improve pregnancy rates in replacement beef heifers, and heifer pubertal status does not appear to affect pregnancy rates to this protocol. The economic value of incorporating a second injection of GnRH at 54-h TAI remains questionable. Addition of the second GnRH injection at TAI may be an option to decrease the risk of low fertility. Depending on the cost of inputs, fertility of semen, and ultimately the cost of a low pregnancy rate outcome, it may be considered as a means of reducing risk. These results warrant further investigation to determine factors leading to the cause of pregnancy rate variations observed in response to GnRH administration at TAI.

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