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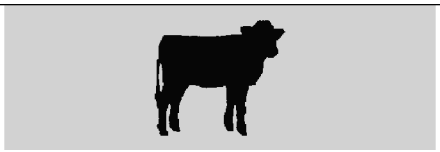

# Evaluation of Gonadotropin-Releasing Hormone at Fixed-Time Artificial Insemination in Beef Heifers Synchronized Using a Modified CO-Synch Plus Controlled Internal Device Release Protocol

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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 $\mu$g (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 $\mu$g; 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