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Comparison of 4- versus 5-day Co-Synch + controlled internal drug release (CIDR) + timed artificial insemination protocols in dairy heifers

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ABSTRACT

The objective of this study was to compare the pregnancy rate after timed artificial insemination (P/TAI) in dairy heifers treated with 4- versus 5-day Co-Synch + controlled internal drug release (CIDR) protocols. A total of 120 Holstein heifers were randomly assigned to one of two groups. The heifers received an intravaginal CIDR insert containing 1.38 g of progesterone for 4 days (Monday–Friday 4-day Co-Synch + CIDR; n = 60) or 5 days (5-day Co-Synch + CIDR; n = 60). At the time of CIDR removal, 25 mg of PGF_{2 α} was injected intramuscularly, and 72 hours after CIDR removal, the heifers received 100 µg of GnRH intramuscularly and were artificially inseminated. Artificial insemination was performed by an experienced technician, using commercial frozen-thawed semen from a single sire. Pregnancy diagnosis was performed by ultrasonography per rectum 32 days after TAI. Categorical data were analyzed using proc logistic and the chi-square test, whereas continuous variables were analyzed using the t-test of Statistical Analysis Systems. Heifers in the 4-day Co-Synch + CIDR group had an acceptable P/TAI₃₂ (55.0%, 33 of 60), which was not different (P = 0.35) from that observed in the 5-day Co-Synch + CIDR group (63.3%, 38 of 60). Progesterone concentration at CIDR insertion or estradiol concentration at TAI did not influence the pregnancy outcomes. Interestingly, estradiol concentration at TAI was greater in the 4-day Co-Synch + CIDR group compared to the 5-day Co-Synch + CIDR group (P < 0.01). In conclusion, the Monday to Friday 4-day Co-Synch + CIDR protocol resulted in adequate P/TAI in dairy heifers, which was similar to that of the 5-day Co-Synch + CIDR protocol. This novel protocol might represent a promising hormonal treatment for TAI in dairy heifers, facilitating their reproductive management routine, while maintaining an adequate fertility.

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1. Introduction

Poor heat detection is a major factor affecting reproductive efficiency in dairy herds [1]. Ovulation synchronization with timed artificial insemination (TAI) protocols using GnRH and $PGF_{2\alpha}$ (e.g., Ovsynch and Co-Synch) in combination with controlled internal drug release (CIDR) for 7 days have been successfully applied in lactating dairy cows, avoiding heat detection [2]. These protocols have resulted in acceptable pregnancy rates after TAI (P/TAI) ranging between 40% and 60% [2–5]. However, poorer ovulation synchronization and pregnancy rates (25.8%–45.5%) have been obtained in dairy heifers treated with 7-day protocols based on GnRH and PGF_{2α} [1,6–8]. The lower fertility in heifers treated with these protocols has been attributed to differences in follicular dynamics

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compared to cows [9]. Dairy heifers have shorter follicular waves and could express estrus close to the $PGF_{2\alpha}$ injection, thereby resulting in asynchrony at TAI [10].

Bridges et al. [11] modified the Co-Synch + CIDR protocol by shortening the interval for CIDR insertion and from the first GnRH injection to CIDR removal and PGF_{2a} injection from 7 to 5 days. In this protocol, a final injection of GnRH and TAI are administered 72 hours after CIDR removal and PGF_{2a} injection. This protocol also includes a second dose of $PGF_{2\alpha}$ 12 hours after the first injection of $PGF_{2\alpha}$. In a 5-day Co-Synch + CIDR protocol, the initial dose of GnRH induces ovulation and formation of accessory corpora lutea (CLs) which might not be responsive to a single $PGF_{2\alpha}$ treatment on Day 5. This makes it necessary to administer a second dose of $PGF_{2\alpha}$ 12 hours later when a dose of GnRH is administered at the time of CIDR insertion [11]. This 5-day Co-Synch + CIDR protocol has been investigated in dairy heifers resulting in pregnancy rates after TAI ranging from 52% to 61% [12], which were similar to the pregnancy rate obtained with AI after detection of estrus [13].

Rabaglino et al. [12] also reported that the 5-day Co-Synch + CIDR protocol may be applied in dairy heifers for a synchronized TAI using only one injection of $PGF_{2\alpha}$ at the time of CIDR removal (instead of two injections of $PGF_{2\alpha}$ 12 hours apart), which resulted in an effective reproductive management program for first and second TAI in dairy heifers. Further studies revealed that fertility of dairy heifers was not improved by administration of the first GnRH on Day 0 within the 5-day Co-Synch + CIDR protocol [14,15]. Therefore, when heifers are subjected to the 5-day Co-Synch + CIDR TAI program with a single treatment of PGF_{2α}, the initial GnRH may not be necessary, obtaining a similar pregnancy rate compared to heifers receiving GnRH at the beginning of the protocol (52.5% vs. 49.8%, respectively) [15].

A major impediment affecting the success of these programs in dairy farms is to overlook the significance of administering the hormonal injections at the prescribed day and time according to the specific protocol. This limiting aspect becomes more critical when farmers and veterinarians try to avoid cattle management during weekends. The establishment of a 4-day Co-Synch + CIDR protocol performed on a Monday to Friday schedule would simplify the routine reproductive management of heifers in dairy farms because it would facilitate treatment administration (Monday: CIDR insertion; Friday: CIDR withdrawal + $PGF_{2\alpha}$; next Monday: GnRH + TAI). In a recent pilot study, heifers treated with a 4-day Co-Synch + CIDR protocol showed an adequate P/TAI (66.7%, 10 of 15), which was not different from that observed in the 5-day CIDR + TAI protocol (46.7%, 7 of 15) or AI after $PGF_{2\alpha}$ injection and heat detection (46.7%, 7 of 15). However, despite the numerical differences, in that study, the sample size was not sufficient to show statistical differences among groups [16].

In the present study, we hypothesize that shortening the length of the Co-Synch + CIDR protocol by 1 day (4-day Co-Synch + CIDR) results in similar P/TAI to that obtained with the 5-day Co-Synch + CIDR protocol in dairy heifers. The objective of this study was to compare the P/TAI in dairy heifers treated with 4-day Co-Synch + CIDR versus 5-day Co-Synch + CIDR and TAI 72 hours after CIDR removal.

2. Materials and methods

2.1. Heifers, diets, and housing

The experimental protocols applied in this study were previously revised and approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine at the University of Georgia. A total of 120 nulliparous Holstein heifers, aged 12 to 14 months, from a commercial dairy farm located in Quitman, GA, USA, were used. Heifers were managed on pasture, with access to portable shades and trees, and fed a total mixed ration once daily that met or exceeded the nutritional requirements of Holstein heifers weighing 360 kg and gaining 0.8 kg/day [17]. The diet was based on a mixture of lactating cow ration balanced for energy, protein, fiber, mineral, and vitamin supplement. For implementation of synchronization protocols, insemination, blood collection, and pregnancy diagnosis, heifers were handled in head-locking stanchions.

2.2. Experimental design and treatments

This study represents a randomized controlled study. A total of 120 Holstein heifers were randomly assigned to one of two groups:

- 1 4-day Co-Synch + CIDR (n = 60): The heifers received an intravaginal CIDR insert (Eazi-Breed CIDR; Zoetis Animal Health, Florham Park, NJ, USA) containing 1.38 g of progesterone (P₄) for 4 days. On the day of CIDR removal, 25 mg of PGF_{2α} (Lutalyse; Zoetis Animal Health) was injected intramuscularly (IM); 72 hours after CIDR removal, the heifers received 100 μ g of GnRH (Factrel; Zoetis Animal Health) IM and TAI (Fig. 1):
- 2 5-day Co-Synch + CIDR (n = 60): The heifers received an intravaginal CIDR insert containing 1.38 g of P₄ for 5 days. The heifers were administered 25 mg of PGF_{2α} IM at the time of CIDR removal and 100 µg of GnRH IM + TAI 72 hours after CIDR removal (Fig. 1).

Heifers with different ages (12, 13, or 14 months) were evenly distributed in both groups. Signs of standing estrus behavior were monitored daily for 3 days after CIDR removal. Heifers with a red heatmount detector device were considered to have displayed signs of standing estrus. Blood samples from all heifers were collected at the time of CIDR insertion and TAI for determination of serum concentration of P_4 and estradiol (E_2). The study was conducted during two consecutive weeks including 60 heifers on each week (30 heifers/group/wk).

2.3. Ultrasonography of the reproductive tract

Ultrasonography per rectum was performed on all heifers immediately before CIDR insertion. The cervix,

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