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## The 9-day CIDR-PG protocol: Incorporation of PGF<sub>2α</sub> pretreatment into a long-term progestin-based estrus synchronization protocol for postpartum beef cows



Jordan M. Thomas<sup>a</sup>, Brianne E. Bishop<sup>a,b</sup>, Jillian M. Abel<sup>a,b</sup>,  
Mark R. Ellersieck<sup>c</sup>, Michael F. Smith<sup>a</sup>, David J. Patterson<sup>a,\*</sup>

<sup>a</sup> Division of Animal Sciences, University of Missouri, Columbia, Missouri, USA

<sup>b</sup> College of Veterinary Medicine, University of Missouri, Columbia, Missouri, USA

<sup>c</sup> Agriculture Experiment Station Statistician, University of Missouri, Columbia, Missouri, USA

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### ABSTRACT

A pilot experiment was designed to test the hypothesis that administration of PGF<sub>2α</sub> before progestin treatment would allow for a reduced duration of progestin treatment in a long-term progestin-based estrus synchronization protocol. A modified presynchronization treatment was compared with a standard long-term controlled internal drug release (CIDR) treatment, and treatments were compared on the basis of ovarian follicular dynamics, estrous response rate, synchrony of estrus expression, and pregnancy rates resulting from timed artificial insemination (TAI) in postpartum beef cows. Estrous was synchronized for 85 cows, with cows assigned to one of two treatments based on age, days postpartum, and body condition score. Cows assigned to the 14-day CIDR-PG protocol received a CIDR insert (1.38 g progesterone) on Day 0, CIDR removal on Day 14, and administration of PGF<sub>2α</sub> (25 mg im) on Day 30. Cows assigned to the 9-day CIDR-PG protocol received PGF<sub>2α</sub> concurrent with CIDR insertion on Day 5, PGF<sub>2α</sub> concurrent with CIDR removal on Day 14, and administration of PGF<sub>2α</sub> on Day 30. In both treatments, split-time AI was performed based on estrous response. At 72 hours after PGF<sub>2α</sub> (Day 33), cows having expressed estrus received TAI; cows that failed to express estrus by 72 hours received TAI 24 hours later (96 hours after PGF<sub>2α</sub> on Day 34), with GnRH (100 μg im) administered to nonestrous cows. Estrus-detection transmitters were used from CIDR removal until AI to determine onset time of estrus expression both after CIDR removal and after PGF<sub>2α</sub>. Ovarian ultrasonography was performed at CIDR removal on Day 14, PGF<sub>2α</sub> on Day 30, and AI on Days 33 or 34. At CIDR removal on Day 14, diameter of the largest follicle present on the ovary was similar between treatments. The proportion of cows expressing estrus after CIDR removal tended to be higher ( $P = 0.09$ ) among cows assigned to the 9-day CIDR-PG treatment (93%; 40 of 43) than among cows assigned to the 14-day CIDR-PG treatment (81%; 34 of 42). After PGF<sub>2α</sub>, a significantly higher proportion ( $P = 0.02$ ) of cows expressed estrus after synchronization with the 9-day CIDR-PG treatment (91%; 39 of 43) than the 14-day CIDR-PG treatment (69%; 29 of 42). Consequently, pregnancy rate to TAI tended to be increased ( $P = 0.09$ ) among the 9-day CIDR-PG treatment (76.7%; 33 of 43) compared with the 14-day CIDR-PG treatment (59.5%; 25 of 42). In summary, a long-term CIDR-based estrus synchronization protocol for postpartum beef cows was enhanced through administration of PGF<sub>2α</sub> at CIDR insertion and CIDR removal.

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\* Corresponding author. Tel.: +1 573 882 7519; fax: +1 573 884 4545.

E-mail address: [PattersonD@missouri.edu](mailto:PattersonD@missouri.edu) (D.J. Patterson).

## 1. Introduction

The 14-day controlled internal drug release-prostaglandin (CIDR-PG) protocol is a long-term progestin-based estrus synchronization protocol currently seeing widespread industry adoption. Estrous response rate and synchrony of estrus expression following this protocol were characterized among heifers [1–3] and contribute to successful application of fixed-time artificial insemination (FTAI). Recent work has evaluated the 14-day CIDR-PG protocol in mature beef cows and noted several differences among cows compared with heifers. Nash et al. [4] reported that mature cows exhibit a less synchronous initial estrus after CIDR removal and an extended mean interval to estrus compared with the data previously published in heifers. Total estrous response after PG and before FTAI was also lower among cows (23%) compared with heifers (78%) after the 14-day CIDR-PG protocol [5,6]. Furthermore, in comparison to the 7-day CO-Synch + CIDR protocol, estrous response before FTAI was reduced in the 14-day CIDR-PG protocol (23% vs. 49%), although pregnancy rates to FTAI were comparable. Martin et al. [7] demonstrated an improvement among mature cows in estrous response rate before FTAI when using a 19-day interval to PGF<sub>2α</sub> after CIDR removal compared with a 16-day interval (47.4% vs. 29.7%;  $P < 0.01$ ). This extended interval to PGF<sub>2α</sub> also tended to improve TAI pregnancy rates among mature cows. However, this modification adds 3 days in length to the protocol. Currently, the 14-day CIDR-PG protocol has seen little industry use among mature suckled beef cows, largely because of the logistical challenge of using a protocol of longer duration in groups of cows with varying lengths of postpartum intervals. Therefore, modifications that decrease protocol length and enhance estrous response are of significant interest. In the absence of luteal progesterone, low concentrations of an exogenous progestin result in the formation of persistent follicles, characterized by an extended period of dominance and increased estradiol production [8–10]. This effect is attributed to an altered pulsatile pattern of gonadotropin release because of the decrease in endogenous progesterone production after PGF<sub>2α</sub>-induced luteolysis [11–17]. We hypothesized that a large dominant follicle would develop earlier during CIDR treatment after administration of PGF<sub>2α</sub> at CIDR insertion, and that by Day 9 of CIDR treatment, follicle size would be similar in comparison to Day 14 of CIDR treatment alone. Therefore, the present study was conducted as a pilot effort to evaluate the effect of administering PGF<sub>2α</sub> in conjunction with a long-term CIDR-based protocol. The study was designed to reduce length of the treatment schedule and compare and evaluate a modified long-term protocol on the basis of follicular dynamics and related reproductive end points.

## 2. Materials and methods

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

### 2.1. Animals

Estrus was synchronized for crossbred lactating beef cows ( $n = 85$ ). Cows were assigned to one of two

treatments (Fig. 1) based on age, days postpartum (DPP), and body condition score (BCS) [18] using a one to nine scale (1 = emaciated and 9 = obese). Cows assigned to the 14-day CIDR-PG protocol received an Eazi-Breed CIDR insert (1.38 g progesterone; Zoetis, Madison, NJ, USA) on Day 0, CIDR removal on Day 14, and PGF<sub>2α</sub> (25 mg im; Lutalyse, Zoetis, Madison, NJ, USA) on Day 30. Cows assigned to the 9-day CIDR-PG protocol received PGF<sub>2α</sub> concurrent with CIDR insertion on Day 5, PGF<sub>2α</sub> concurrent with CIDR removal on Day 14, and PGF<sub>2α</sub> on Day 30. In both treatments, split-time AI was performed based on estrous response [19–22]. At 72 hours after PGF<sub>2α</sub> (Day 33), cows that had expressed estrus received TAI. Cows that failed to express estrus by 72 hours received TAI 24 hours later (96 hours after PGF<sub>2α</sub> on Day 34), with GnRH (100 μg im; Cystorelin, Merial, Athens, GA, USA) administered to non-estrous cows concurrent with AI. Times of PGF<sub>2α</sub> administration, GnRH administration, and AI were recorded for each cow. Insemination was performed using conventional frozen semen sourced from one collection of a single AI sire. Two technicians were preassigned and balanced across treatments based on cow age, DPP, and BCS. Fourteen days after AI, cows were exposed to fertile bulls for the remainder of the 45-day breeding season.

### 2.2. Estrus detection

Cows were fitted with HeatWatch estrus-detection transmitters (DDx Inc., Denver, CO, USA) from the time of CIDR removal until FTAI for continuous estrus detection. Estrus was defined as cows receiving three or greater mounts of 2 or more seconds in duration within a 4-hour period, with the onset of estrus determined as the first mount within that period [23]. Transmitters were removed from cows expressing estrus by 72 hours after PGF<sub>2α</sub> but were not removed from the remaining cows until 96 hours to characterize estrus expression between 72 and 96 hours.

### 2.3. Ovarian ultrasonography

Transrectal ultrasonography (SonoSite EDGE equipped with a L52 10.0 to 5.0 MHz linear array transducer; SonoSite Inc., Bothell, WA, USA) was performed to assess ovarian follicular dynamics and the presence of CL. Dominant follicles were measured, and measurements reported are the largest follicle diameter (LFD). Ultrasonography was performed at CIDR removal on Day 14, at PGF<sub>2α</sub> administration on Day 30, and at AI on Days 33 and 34.

### 2.4. Pregnancy diagnosis

Pregnancy rate to AI was determined by transrectal ultrasonography (SonoSite EDGE equipped with a L52 10.0 to 5.0 MHz linear array transducer; SonoSite Inc.) 74 days after AI (Day 107).

### 2.5. Statistical analysis

Treatment differences for age, BCS, DPP, LFD measurements, and timing of estrus expression were analyzed using the TTEST procedure of SAS (SAS Institute Inc., Cary, NC,

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