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# Fertility of Holstein heifers after two doses of PGF2 $\alpha$ in 5-day CO-Synch progesterone-based synchronization protocol

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#### ABSTRACT

The objective of the study was to determine the effect of three different PGF2a (PGF) treatments in the 5-day CO-Synch progesterone-based synchronization protocol on artificial insemination (AI) pregnancy rate (PR) in Holstein heifers in Turkey and the United States. We hypothesized that two doses of PGF administered concurrently or 6 hours apart would result in greater AI pregnancy compared with a single dose of PGF on Day 5 at controlled internal drug release (CIDR) removal. In Turkey, Holstein heifers (n = 450) from one farm in the province of Adana and another farm in the province of Bursa were included. In the US, Holstein heifers (n = 483) from two locations in the state of Idaho were included. Heifers within locations were randomly allocated to one of three protocol groups: 1PGF—received 25 mg IM of dinoprost at CIDR removal; 2Co-PGF—received 50 mg IM of dinoprost at CIDR removal, and 2PGF-received 25 mg IM of dinoprost at CIDR removal and an additional 25 mg IM of dinoprost 6 hours later. All heifers received a CIDR (1.38 g of progesterone) and GnRH (10 µg IM of Buserelin [Turkey] or gonadorelin hydrochloride [US]) on Day 0. The CIDRs were removed on Day 5, and each heifer was given PGF according to the assigned treatments. On Day 7, each heifer was given another dose of GnRH and concurrently inseminated at 56 hours after CIDR removal. Heifers in both experiments were examined for pregnancy status between 35 and 45 days after AI. Overall, controlling for age, the heifers in the 2PGF group had greater AI-PR (61.7% [192/311]) than heifers in 2Co-PGF (48.2% [149/309]; P < 0.001) or 1PGF (53.7% [168/313]; P < 0.05) groups. No difference was observed between 2Co-PGF and 1PGF groups (P > 0.1). In Turkey, the heifers in the 2PGF group had a greater AI-PR (60% [90/150]) than 2Co-PGF (45.3% (68/150); P < 0.01] group. No difference was observed between 2PGF and 1PGF (55.3% [83/150]) groups (P > 0.1). There was a trend for AI pregnancy between 1PGF and 2Co-PGF groups (P = 0.08). In the United States, the heifers in the 2PGF group had a greater AI-PR (63.4% [102/161]) than the heifers in 2PGF (50.9 [81/159]; P < 0.05) or 1PGF (52.1% [85/163]; P < 0.05) groups. Heifers that were 15- and 16-month old achieved greater AI-PR than 17- and 18-month-old heifers (59.2 [342/578] vs. 47.0% [168/355];  $P\,<\,0.01).$  In conclusion, administration of 2PGF at 6 hours apart on Day 5 at CIDR removal in a 5-day CO-Synch + CIDR protocol resulted in greater AI pregnancy. A greater number of 15and 16-month-old heifers became pregnant compared with 17- and 18-month-old heifers. © 2016 Elsevier Inc. All rights reserved.







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#### 1. Introduction

Replacement heifers are the foundation of any successful dairy operation. Improvement in herd genetic potential can be achieved when culled cows are replaced with genetically superior heifers. Artificial insemination (AI) is a tool available for producers to implement superior genetics into their herds that otherwise may not be available. Yet, a major constraint for use of AI is the time and effort related to daily estrous detection [1].

Ovulation-synchronization protocols, such as Ovsynch, permit a fixed timed AI (TAI) without the need for estrous detection. This protocol consists of administration of GnRH, followed by an injection of PGF2a (PGF) 7 days later. A second dose of GnRH is administered 48 hours later, and then TAI is completed 0 to 24 hours later [2]. When the Ovsynch protocol is modified with TAI performed at the same time as the second GnRH injection, it is referred to as CO-Synch [3]. Such programs have been used successfully in lactating dairy cows but inferior results were obtained in dairy heifers [2,4,5]. Because of differences in follicular dynamics between cows and heifers [5,6], dairy heifers could express estrus close to the PGF injection, thereby causing asynchrony at TAI [7]. Inclusion of controlled internal drug release (CIDR) insert containing progesterone in the Ovsynch or CO-Synch protocols suppresses estrus and/or ovulation during the days of CIDR insertion, thereby allowing submission of all heifers for TAI without affecting fertility [7].

In beef cows, Bridges et al. [8] modified the 7-day CO-Synch + CIDR protocol to a 5-day interval from the first GnRH to PGF injection, with a final injection of GnRH and TAI 72 hours from CIDR removal. This approach resulted in higher AI-pregnancy rate (PR) than a 7-day Co-Synch + CIDR. Kasimanickam et al. [9] found that administration of two 25 mg doses of PGF given 7 hours apart on Day 5 at CIDR removal in a 5-day CO-Synch + CIDR resulted in higher PRs in beef cows than single dose, suggesting two doses of PGF are required to ensure complete leutolysis. Furthermore, Peterson et al. [10] observed similar results in beef heifers. These results support the use of the 5-day protocol for use of FTAI in beef heifers, but unfortunately, this protocol has a practical limitation. Because of the shortened interval from the initial GnRH to PGF, this protocol requires heifers to be handled twice for PGF administration on the day of CIDR removal, thus increasing time, labor costs, and stress of handling to the animals. A study in beef cows proved that while two 25 mg of PGF given at same time (2Co-PGF) was required to maximize AI-PR, the timing of administration (concurrently versus 8 hours apart after CIDR removal) did not affect PRs [11], whereas White et al. [12] observed that the 2PGF treatment (at 6 hours interval) resulted in a greater AI pregnancy (63.6%) compared to the 2Co-PGF (51.9%) and 1PGF (54.9%) treatments in beef heifers. In dairy heifers, a recent study observed that the AI pregnancy between 1PGF and 2PGF treatments were 53.9% and 54.5% [13]. If greater AI-PR after administration of 2Co-PGF treatment is proven then it will reduce time, labor costs, and stress of handling to the animals.

The objective of the study was to determine the effect of three different PGF treatments (2PGF, 2Co-PGF, and 1PGF)

in 5-day CO-Synch progesterone-based synchronization protocol on AI-PR in Holstein heifers in Turkey and the United States.

#### 2. Materials and methods

#### 2.1. Animals

In Turkey, the farms were located in the provinces of Adana and Bursa. Adana is situated in southern Turkey with typical Mediterranean climate, whereas Bursa is positioned in northwest Turkey with transitional climate between the Mediterranean and Black Sea. The heifers (age:  $16.4 \pm 0.11$ ) were housed in open barns and were fed total mixed ration (consisting of alfalfa, corn silage, and concentrates with 14% crude protein, straw, or wheat hay) twice daily with ad libitum access to water. In the United States, the farms were located in Idaho. Idaho is situated in northwestern region of the US with maritime, semi-arid continental climate. The heifers (age:  $15.9 \pm 0.08$ ) were housed in dry lots with headlocks and fed a total mixed ration, corn-based, diet twice daily.

#### 2.2. Treatment

In the Turkey experiment, Holstein heifers (n = 450)from one farm in the province of Adana (n = 60) and another farm in the province of Bursa (n = 390) were included. Heifers were blocked by number of services and randomly allocated to one of three protocol groups, including: 1PGF (n = 150) received 25 mg IM of dinoprost (PGF2a, Enzaprost-T, 5 mL, Ceva Animal Health, Istanbul, Turkey); 2Co-PGF (n = 150) received 50 mg IM of dinoprost at CIDR removal; and 2PGF (n = 150) received 25 mg IM of dinoprost at CIDR removal and an additional 25 mg IM of dinoprost 6 hours later. All heifers received a CIDR (1.38 g of progesterone; Eazi-Breed CIDR Cattle Insert, Zoetis Animal Health, Istanbul, Turkey) and 10 µg IM of Buserelin on Day 0 (Receptal, 2.5 mL, MSD Animal Health, Istanbul, Turkey). The CIDRs were removed on Day 5, and each heifer was given PGF based on the assigned protocol group (1PGF, 2Co-PGF, or 2PGF). Each heifer was given another dose of Buserelin (10  $\mu$ g, IM) and concurrently inseminated, at 56 hours after CIDR removal.

In the US experiment, Holstein heifers (n = 483) from two farms in ID (location 1: n = 258 and location 2: n =225) state that used synchronization protocols as a reproductive management tool were included. Heifers within locations were blocked by BCS, age and number of services, and randomly allocated to one of three protocol groups, including: 1PGF (n = 163) received 25 mg IM of dinoprost (PGF2a, Lutalyse, 5 mL, Zoetis Animal Health, New York, NY, USA); 2Co-PGF (n = 159) received 50 mg IM of dinoprost at CIDR removal, and 2PGF (n = 161) received 25 mg IM of dinoprost at CIDR removal and an additional 25 mg IM of dinoprost 6 hours later. All heifers received a CIDR (1.38 g of progesterone; Eazi-Breed CIDR Cattle Insert, Zoetis Animal Health, USA) and 100  $\mu$ g IM of GnRH on Day 0 (Factrel, 2 mL, Zoetis Animal Health, USA). CIDRs were removed on Day 5, and each heifer was given PGF based on the assigned protocol group (1PGF, 2Co-PGF, or

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