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Incorporation of a rapid pregnancy-associated glycoprotein ELISA into a CIDR-Ovsynch resynchronization program for a 28 day re-insemination interval

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Abstract

The objective was to compare two resynchronization programs; one that used a blood-based ELISA for pregnancy-associated glycoproteins (PAG) for pregnancy diagnosis so that non-pregnant cows were re-inseminated at 28 d after first TAI, and another that used transrectal ultrasonography for pregnancy diagnosis so that non-pregnant cows were re-inseminated at 35 d after first TAI. The PAG_resynch cows (n = 103) began CIDR-Ovsynch resynchronization on Day 18 after first TAI (Day 0). On Day 25, the CIDR was removed and pregnancy diagnosis with a PAG ELISA was performed. If a cow was not pregnant on Day 25, she was treated with $PGF_{2\alpha}$, treated with GnRH 2 d later (Day 27), and TAI on Day 28. Control cows (n = 99) were observed for estrus until Day 25, when they began an identical CIDR-Ovsynch program with pregnancy diagnosis by transrectal ultrasonography on Day 32. If a cow was not pregnant on Day 32, then she was treated with $PGF_{2\alpha}$, treated with GnRH 2 d later (Day 34), and TAI on Day 35. There was no difference in pregnancy per AI (P/AI) for either group at first or second insemination. For cows without pregnancy loss, the interval between first and second ($P < 0.001$) or second and third ($P < 0.016$) TAI was shorter for PAG_resynch cows compared with Control cows. The interval between first and second or second and third TAI was not different if pregnancy loss cows were included in the analysis. Plasma progesterone concentrations were similar at $PGF_{2\alpha}$ treatment, and plasma estradiol concentrations increased similarly after $PGF_{2\alpha}$ treatment for PAG_resynch and Control cows. In conclusion, the 28 d CIDR-Ovsynch resynchronization protocol was comparable to a 35 d CIDR-Ovsynch resynchronization protocol that also included estrus detection. Shortened resynchronization protocols that do not require estrus detection may improve reproductive efficiency in dairy cattle.
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1. Introduction

Artificial insemination in dairy cattle is typically managed in one of three ways: AI cattle approximately 12 h after an observed estrus, enroll cattle in a timed AI (TAI) protocol, or combine the first two approaches by allowing AI of cows that are detected in estrus while enacting a TAI protocol for cows not detected in estrus. Regardless of the approach, most cows (> 50%) will require more than one insemination (re-insemination) before they become pregnant. Timely re-insemination of non-pregnant cattle requires either excellent estrus detection (so that cattle are observed at their "natural" return to estrus) or a method of pregnancy detection that can be used soon (< 35 d) after the previous AI

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(Day 0), so that non-pregnant cows can be identified within a reasonable timeframe. The most commonly used technique that satisfies the latter approach is transrectal ultrasonography [1,2]. A second approach that meets these criteria is assaying for pregnancy-associated glycoproteins (PAG). There are commercially-available assays for PAG such as the BioPRYN test (Biotracking LLC, Moscow, ID, USA) that assays specifically for pregnancy-specific protein B (boPAG1). Another possibility is a rapid PAG ELISA that has comparable diagnostic capability to transrectal ultrasonography and can detect pregnancies a few days earlier after breeding [3].

Studies in which cows are resynchronized at ≤ 35 d generally employ Ovsynch [4] or Ovsynch-like resynchronization following transrectal ultrasonography [5–7]. One study used a PAG ELISA to diagnose pregnancy on Day 27 after AI to resynchronize cows with Ovsynch at Day 35 [8]. These studies did not incorporate a CIDR (Controlled Internal Drug Release, 1.38 g progesterone, Pfizer, New York, NY, USA) in the resynchronization program. A CIDR can provide beneficial effects when included in the Ovsynch program [9] and may possess some capacity to improve pregnancy per AI (P/AI) of the preceding insemination when used in a resynchronization program [10,11]. The objective of this research was to apply the PAG ELISA described by [3] for pregnancy diagnosis on Day 25 after TAI within a CIDR-Ovsynch resynchronization program that used no estrus detection and re-inseminated cows at a 28 d interval. This "PAG only no estrus detection" approach was compared with a Control resynchronization that involved estrus detection, transrectal ultrasound examination, and a 35 d CIDR-Ovsynch resynchronization program.

2. Material and methods

2.1. Animals and management

Holstein dairy cows were housed in freestall barns at the University of Missouri Foremost dairy farm from December 2008 to April 2010. Cows were fed a totally mixed ration that was formulated according to their stage of lactation and level of milk production. All cows (n = 202) were enrolled in a Presynch-Ovsynch program [$PGF_{2\alpha}$ (dinoprost, Lutalyse, Pfizer); wait 14 d, $PGF_{2\alpha}$; wait 14 d, GnRH (gonadorelin, Cystorelin, Merial, Duluth, GA, USA); wait 7 d, $PGF_{2\alpha}$; wait 2 d, GnRH; wait 16 h, TAI). Some cows [PAG (n = 16) and Control (n = 16)] had been previously inseminated, but were diagnosed as non-pregnant using transrectal ultrasonography at ≥ 30 d after AI before being enrolled in this experiment. All other cows in this experiment were not previously inseminated. Cows were randomly assigned to a resynchronization protocol that was used after first TAI (Fig. 1). Cows were either resynchronized with a CIDR-Ovsynch program, so that second TAI was 28 d after first TAI [PAG_resynch; cows began CIDR-Ovsynch on Day 18 after first TAI (Day 0); n = 103] or they were assigned to an estrus detection plus CIDR-Ovsynch program, so that second TAI was 35 d after first TAI (Control; cows began CIDR-Ovsynch on Day 25 after first TAI; n = 99). The CIDR-Ovsynch program was GnRH treatment and CIDR insertion followed by CIDR removal and pregnancy diagnosis 7 d later. Cows that were not pregnant were treated with $PGF_{2\alpha}$, GnRH (2 d later) and TAI (16 h after GnRH).

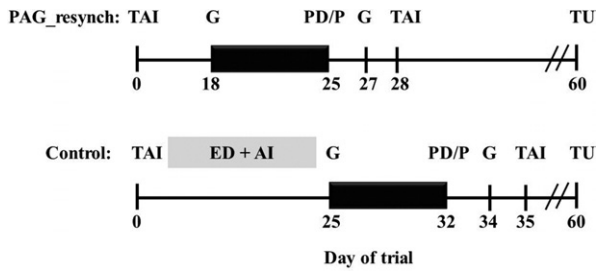

Fig. 1. Resynchronization timeline in days for PAG_resynch and Control groups. The numbers depicted are for days relative to the first TAI. Estrus detection followed by AI (ED + AI) was done in the Control group. Cows were treated with GnRH (Cystorelin; G), treated with a CIDR (Controlled internal drug release, 1.38 g progesterone, Pfizer) on Days 18 or 25 (solid rectangle indicates time of CIDR insertion). A pregnancy-associated glycoprotein (PAG) enzyme-linked immunosorbent assay (Day 25) or transrectal ultrasonography examination (Day 32) was done at CIDR removal for first pregnancy diagnosis (PD). Non-pregnant cows were treated with $PGF_{2\alpha}$ (P) and GnRH (G) for timed artificial insemination (TAI). A final pregnancy recheck examination (transrectal ultrasonography; TU) was done on Day 60.

Estrus detection for Control cows consisted of routine daily observation. Control cows observed in estrus were inseminated approximately 12 h after the initial observation. Most cows (n = 120) underwent one round of resynchronization. A small number of cows (n = 18 for PAG_resynch and n = 17 for Control) that were not pregnant after second AI stayed on their respective programs for a third resynchronized TAI.

2.2. Pregnancy diagnosis

Control cows were diagnosed for pregnancy by using transrectal ultrasonography on Day 32 after TAI. An Aloka 500-SSD equipped with a 5 MHz transducer

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