J. Dairy Sci. 97:4932–4941
http://dx.doi.org/10.3168/jds.2013-7887

Effect of early or late resynchronization based on different methods of pregnancy diagnosis on reproductive performance of dairy cows

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ABSTRACT

The aim of this study was to compare the reproductive performance of dairy cows subjected to early (ER) or late (LR) resynchronization programs after nonpregnancy diagnoses based on either pregnancy-associated glycoproteins (PAG) ELISA or transrectal palpation, respectively. In addition, the accuracy of the PAG ELISA for early pregnancy diagnosis was assessed. Lactating Holstein cows were subjected to a Presynch-Ovsynch protocol with timed artificial insemination (AI) performed between 61 and 74 DIM. On the day of the first postpartum AI, 1,093 cows were blocked by parity and assigned randomly to treatments; however, because of attrition, 452 ER and 520 LR cows were considered for the statistical analyses. After the first postpartum AI, cows were observed daily for signs of estrus and inseminated on the same day of detected estrus. Cows from ER that were not reinseminated in estrus received the first GnRH injection of the Ovsynch protocol for resynchronization 2 d before pregnancy diagnosis. On d 28 after the previous AI (d 27 to 34), pregnancy status was determined by PAG ELISA, and nonpregnant cows continued on the Ovsynch protocol for reinsemination. Pregnant cows had pregnancy status reconfirmed on d 46 after AI (d 35 to 52) by transrectal palpation, and those that lost the pregnancies were resynchronized. Cows assigned to LR had pregnancy diagnosed by transrectal palpation on d 46 after AI (d 35 to 52) and nonpregnant cows were resynchronized with the Ovsynch protocol. Blood was sampled on d 28 after AI (d 27 to 34) from cows in both treatments that had not been reinseminated on estrus and again on d 46 after AI (d 35 to 52) for assessment of PAG ELISA to determine the accuracy of the test. Cows were subjected to treatments for 72 d after the first insemination. Pregnancy per AI (P/AI) at first postpartum timed AI did not differ between treatments and averaged 28.9%. The proportion of nonpregnant cows that were resynchronized and received timed AI was greater for ER than for LR (30.0 vs. 7.6%). Cows in ER had a shorter interval between inseminations when inseminated following spontaneous estrus (21.7 ± 1.1 vs. 27.8 ± 0.8 d) or after timed AI (35.3 ± 1.2 vs. 55.2 ± 1.4 d). Nevertheless, the ER did not affect the rate of pregnancy (adjusted hazard ratio = 1.23; 95% confidence interval = 0.94 to 1.61) or the median days postpartum to pregnancy (ER = 132 vs. LR = 140). A total of 2,129 PAG ELISA were evaluated. Overall, sensitivity, specificity, and positive and negative predictive values averaged 95.1, 89.0, 90.1, and 94.5%, respectively, and the accuracy was 92.1%. In conclusion, PAG ELISA for early diagnosis of pregnancy had acceptable accuracy, but early resynchronization after nonpregnancy diagnosis with PAG ELISA did not improve the rate of pregnancy or reduce days open in dairy cows continuously observed for estrus.

Key words: dairy cow, pregnancy-associated glycoprotein, reproduction, resynchronization

INTRODUCTION

Reproductive performance is a major component of the economic viability in dairy herds and shortening the reinsemination interval for nonpregnant cows is expected to reduce the time to pregnancy and improve the pregnancy rate (Meadows et al., 2005; De Vries, 2006; Ribeiro et al., 2012). From 55 to 70% of lactating dairy cows fail to become pregnant in response to AI and need to be reinseminated (Santos et al., 2004). Programs for synchronization of the estrous cycle and timed AI have been widely used to increase submission to AI and to reduce the interval between inseminations (Fricke et al., 2003). However, nonpregnant cows need to be identified before being subjected to resynchronization protocols and the development of methods for early pregnancy diagnosis is critical to further reduce the interbreeding interval.

The diagnosis of pregnancy in cattle is traditionally performed starting around d 35 to 40 after insemination by transrectal palpation of the reproductive tract for identification of the amniotic vesicle (Warnick et

Received December 30, 2013.
Accepted May 5, 2014.
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al., 1995), or at approximately 27 d after insemination with the aid of ultrasonography (Silva et al., 2007). Alternatively, chemical methods that detect the presence of conceptus-derived antigens can be used to identify pregnant cows early after AI (Green et al., 2009, 2011). Modern pregnancy-associated glycoproteins (**PAG**) are members of a large family of aspartic proteinases expressed by binucleate trophoblast cells in even-toed ungulates (Xie et al., 1991). These proteins are released into the uterine stroma after the fusion between binucleate and endometrial cells (Xie et al., 1991) and can be observed in the maternal circulation throughout gestation (Sasser et al., 1986; Green et al., 2005). The development of monoclonal antibodies specific for PAG produced during initial stages of gestation have allowed for the use of immunoassays such as ELISA for early pregnancy detection that can be performed in cows after 60 d postpartum (Silva et al., 2007; Green et al., 2009). Nevertheless, the concentrations of PAG in the blood of pregnant cows increase from d 24 to 30 after AI and then decrease toward d 60 of gestation (Thompson et al., 2010), suggesting that the accuracy of determining pregnancy status varies depending on the day after AI in which the blood is collected (Szenci et al., 1998; Green et al., 2009).

Previous studies have shown that diagnosing pregnancy starting on d 25 after AI based on PAG concentrations resulted in acceptable accuracy (Silva et al., 2007; Green et al., 2009) and reduced interbreeding interval and time to pregnancy in lactating cows resynchronized exclusively using timed AI (Silva et al., 2009). Nonetheless, the combination of estrous detection and timed AI for reinsemination of nonpregnant cows is used in the majority of dairy herds, as it often results in greater submission to AI and reduced time to pregnancy (Ribeiro et al., 2012; Giordano et al., 2013). A large portion of cows are expected to be observed in estrus and reinseminated before d 32 after insemination (Chebel et al., 2003; Bartolome et al., 2005; Galvão et al., 2007); therefore, the need for early pregnancy diagnosis will vary according to the ability to identify nonpregnant cows that return to estrus before any pregnancy diagnosis testing.

The main hypothesis of the present study was that an early resynchronization (**ER**) program starting at 28 d after AI using the PAG ELISA for pregnancy diagnosis and decision on resynchronization would reduce the interval between inseminations and, therefore, the time to pregnancy compared with the use of transrectal palpation in a late resynchronization protocol starting 46 d after AI. A second hypothesis was that the PAG ELISA would result in acceptable accuracy to determine pregnancy status in lactating dairy cows. Therefore, the objectives were to compare the time to pregnancy in cows subjected to ER versus late resynchronization (**LR**) programs based on nonpregnancy diagnosis using PAG ELISA or transrectal palpation, respectively, and to evaluate the accuracy of the PAG ELISA for pregnancy diagnosis.

MATERIALS AND METHODS

Animals, Housing, and Feeding

A total of 1,093 lactating Holstein cows from a commercial dairy herd located in central California were enrolled in this experiment from December 2004 to April 2005. Primiparous and multiparous cows were housed separately in freestall barns equipped with sprinklers and fans. Cows had ad libitum access to water and were fed a TMR to meet or exceed the requirements of lactating Holstein cows weighing 650 kg and producing 45 kg of milk per day with 3.5% fat and 3.2% true protein (NRC, 2001). Cows were fed multiple times daily and were milked 3 times daily.

Reproductive Management and Treatments

Weekly cohorts of cows were subjected to the Presynch-Ovsynch protocol (Moreira et al., 2001) for the first postpartum timed AI (Figure 1). Briefly, cows received an i.m. injection of $PGF_{2\alpha}$ (Lutalyse sterile solution, 25 mg of dinoprost as tromethamine salt, Zoetis, Madison, NJ) given on d 32 (25 to 38) and again on d 46 (39 to 52) postpartum. The Ovsynch protocol was initiated at 58 (51 to 64) DIM with an i.m. injection of GnRH (Cystorelin, 100 μg of gonadorelin diacetate tetrahydrate; Merial Ltd., Duluth, GA), which was followed 7 d later by an injection of $PGF_{2\alpha}$ and a final GnRH injection of $PGF_{2\alpha}$ 48 h later. Cows were inseminated 24 h after the last GnRH injection (61 to 74 DIM). The body condition of cows was scored using a 1 to 5 scale (0 = emaciated and 5 = obese; Ferguson et al., 1994) at 54 ± 3 DIM and those with BCS >2.00 were considered eligible for enrollment in the study. For purposes of statistical analyses, cows were categorized as having low (≤2.75) or moderate (≥3.00) BCS. The study followed a randomized complete block design. On the day of the first AI postpartum, cows were blocked by parity and, within each block, assigned randomly to either an ER (n = 548) or LR (n = 545) program (Figure 1). After the first AI postpartum, cows from both programs were observed for signs of estrus based on removal of tail chalk and those in estrus were inseminated on the same day.

Cows assigned to ER had pregnancy diagnosed weekly and they received the first GnRH injection of the Ovsynch protocol for resynchronization, on average, on