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Fertility of lactating Holstein cows submitted to a Double-Ovsynch protocol and timed artificial insemination versus artificial insemination after synchronization of estrus at a similar day in milk range

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ABSTRACT

Our objective was to compare the AI submission rate and pregnancies per artificial insemination (P/AI) at first service of lactating Holstein cows submitted to a Double-Ovsynch protocol and timed artificial insemination (TAI) versus artificial insemination (AI) to a detected estrus after synchronization of estrus at a similar day in milk range. Lactating Holstein cows were randomly assigned to receive their first TAI after a Double-Ovsynch protocol (DO; n = 294) or to receive their first AI after a synchronized estrus (EST; n = 284). Pregnancy status was determined 33 ± 3 d after insemination and was reconfirmed 63 ± 3 d after insemination. Data were analyzed by ANOVA and logistic regression using the MIXED and GLIMMIX procedures of SAS (SAS Institute Inc., Cary, NC). By design, days in milk at first insemination did not differ between treatments (76.9 ± 0.2 vs. 76.7 ± 0.3 for DO vs. EST cows, respectively), but more DO cows were inseminated within 7 d after the end of the voluntary waiting period than EST cows (100.0 vs. 77.5%). Overall, DO cows had more P/AI than EST cows at both 33 d (49.0 vs. 38.6%) and 63 d (44.6 vs. 36.4%) after insemination, but pregnancy loss from 33 to 63 d after insemination did not differ between treatments. Primiparous cows had more P/AI than multiparous cows 33 and 63 d after insemination, but the treatment by parity interaction was not significant. Synchronization rate to the hormonal protocols was 85.3%, which did not differ between treatments; however, synchronized DO cows had more P/AI 33 d after insemination than synchronized EST cows (54.7 vs. 44.5%). In summary, submission of lactating Holstein cows to a Double-Ovsynch protocol and TAI for first insemination increased the percentage of cows inseminated within 7 d after the end of the voluntary waiting period and increased P/AI at 33 and 63 d after first insemination resulting in 64 and 58% more pregnant cows, respectively, than submission of cows for first AI after detection of estrus at a similar day in milk range. We conclude that, because the proportion of synchronized cows did not differ between treatments, DO cows had more P/AI than EST cows because of an intrinsic increase in fertility after submission to a fertility program.

Key words: first artificial insemination, timed artificial insemination, estrus, fertility

INTRODUCTION

Hormonal synchronization protocols that allow for timed artificial insemination (**TAI**) have been incorporated widely into reproductive management programs by dairy farms (Caraviello et al., 2006; Norman et al., 2009); however, AI based on detection of estrus continues to be an important part of the overall reproductive management program on most dairy farms (Caraviello et al., 2006; Miller et al., 2007). Results from the first field trial evaluating the Ovsynch protocol on a commercial dairy farm reported that Ovsynch and TAI yielded similar pregnancies per artificial insemination (**P/AI**) to that of cows receiving AI after a detected estrus (39 vs. 37%; Pursley et al., 1997) but that median days to first AI (54 vs. 83) and days open (99 vs. 118) were decreased for cows submitted for first insemination using an Ovsynch protocol than for cows submitted for AI after a detected estrus, respectively. Thus, the initial effect of TAI protocols on 21-d pregnancy rates in US dairy herds was to increase the AI service rate, with little to no effect on P/AI (Norman et al., 2009).

Since the development of the Ovsynch protocol by Pursley et al. (1995), several modifications to the original Ovsynch protocol have been tested in an attempt to increase P/AI to TAI. These modifications include increasing ovulatory response to the first GnRH treat-

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ment (Carvalho et al., 2015b), presynchronization using 2 $PGF_{2\alpha}$ treatments (i.e., Presynch-Ovsynch; Moreira et al., 2001; Navanukraw et al., 2004; Ribeiro et al., 2011), presynchronization using a combination of GnRH and $PGF_{2\alpha}$ (i.e., G-6-G and Double-Ovsynch; Bello et al., 2006; Souza et al., 2008; Carvalho et al., 2014a), and addition of a second $PGF_{2\alpha}$ treatment 24 h after the first within the Ovsynch protocol to induce complete luteal regression (Carvalho et al., 2015a; Wiltbank et al., 2015; Santos et al., 2016). Taken together, these modifications have yielded P/AI at first service that exceed 50% in high-producing Holstein cows (Carvalho et al., 2014a, 2015b). Thus, the latest iterations of hormonal synchronization protocols for submitting lactating dairy cows for first service can be best described as fertility programs for high-producing dairy cows because all cows can be inseminated within 7 d of the end of the voluntary waiting period and yielding more P/AI at first service.

The idea that fertility programs and TAI can yield greater fertility than AI to estrus at first insemination in high-producing dairy cows has not been definitively tested. Several experiments compared P/AI of cows inseminated after TAI with cows inseminated after a detected estrus at first AI, with some studies reporting no differences in P/AI (Pursley et al., 1997; Chebel and Santos, 2010; Dolecheck et al., 2016), whereas others reported more P/AI for cows receiving TAI (Gumen et al., 2012; Fricke et al., 2014; Stevenson et al., 2014). In all of these studies, however, DIM at first service differed between cows submitted to TAI and cows submitted to AI after a detected estrus. Further, DIM is a clear confounder because cows with more DIM at first service have more time for uterine involution and for resumption of cyclicity, both of which affect P/AI (Buch et al., 1955; Chebel and Santos, 2010).

Our objective was to compare the AI submission rate and P/AI at first service of lactating Holstein cows submitted to a Double-Ovsynch protocol and TAI versus AI to a detected estrus after synchronization of estrus at a similar DIM range. Our hypothesis was that the AI submission rate and P/AI at first service would be greater for cows submitted to first TAI after a Double-Ovsynch protocol than for cows receiving AI to a detected estrus after submission to a hormonal protocol for synchronization of estrus.

MATERIALS AND METHODS

All animal handling and experimental procedures were approved by the Animal Care and Use committee of the College of Agriculture and Life Sciences at the University of Wisconsin–Madison.

Cows, Housing, and Feeding

This study was conducted from October 2015 to June 2016 on 1 commercial dairy farm in Portugal. Lactating Holstein cows (n = 578) were milked twice daily at approximately 12-h intervals. Cows were fed twice daily a TMR consisting of corn silage and alfalfa hay as forage with corn and soybean meal-based concentrate formulated to meet or exceed the minimum nutritional requirements for high-producing dairy cows (NRC, 2001). Cows were housed in free-stall barns bedded with mattress and had ad libitum access to feed and water. Primiparous and multiparous cows were housed in separate pens, and barns were equipped with fans and sprinklers that were automatically activated when the temperature inside the barns exceeded 28 and 32°C, respectively. The rolling herd average and daily milk production were 10,719 kg and 35.6 kg/cow per day with 4.9% fat and 3.3% protein during the experiment.

Experimental Treatments

Each week, cows at 50 ± 3 DIM (d 0) were stratified by parity (primiparous vs. multiparous) and were randomly assigned to 2 treatments to receive first insemination (Figure 1). Cows (n = 294) in the first treatment were submitted for first TAI after a modified Double-Ovsynch (**DO**) protocol that included a second $PGF_{2\alpha}$ treatment 24 h after the first in the Breeding-Ovsynch portion of the protocol. Briefly, on d 0 cows received the first GnRH treatment of the Pre-Ovsynch portion of the Double-Ovsynch protocol, followed by treatment with $PGF_{2\alpha}$ 7 d later and GnRH 72 h after $PGF_{2\alpha}$. Seven days later, cows received a GnRH treatment followed by 2 $PGF_{2\alpha}$ treatments administered 7 and 8 d later, with the last GnRH treatment administered 56 h after the first $PGF_{2\alpha}$ treatment followed by TAI 16 to 20 h later. Cows (n = 284) in the second treatment were submitted to a hormonal synchronization protocol for synchronization of estrus and were inseminated if detected in estrus (**EST**). Briefly, 3 d after d 0 (Figure 1), cows were treated with GnRH followed by treatment with $PGF_{2\alpha}$ 7 d later. Fourteen days later, cows received 2 $PGF_{2\alpha}$ treatments administered 24 h apart. Cows detected in estrus from 2 d before until 7 d after the first of the 2 $PGF_{2\alpha}$ treatments at the end of the protocol were inseminated within 12 h of detection of estrus based on visual detection of estrus, which was aided using pedometers attached to the rear legs of the cows (Westfalia Separator, GEA, Lisbon, Portugal).

The GnRH (100 μg/dose of gonadoreline diacetate tetrahydrate, Ovarelin) and the $PGF_{2\alpha}$ (25 mg/dose of dinoprost tromethamine, Enzaprost-T) used in this