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Supplementation with Estradiol-17β Before the Last Gonadotropin-Releasing Hormone Injection of the Ovsynch Protocol in Lactating Dairy Cows

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ABSTRACT

The aim of this study was to determine whether an increase in circulating estrogen concentrations would increase percentage pregnant per artificial insemination (PP/AI) in a timed AI protocol in high-producing lactating dairy cows. We analyzed only cows having a synchronized ovulation to the last GnRH of the Ovsynch protocol (867/1,084). The control group (n = 420) received Ovsynch (GnRH – 7 d – $PGF_{2\alpha}$ – 56 h – GnRH – 16 h – timed AI). The treatment group (n = 447) had the same timed AI protocol with the addition of 1 mg of estradiol-17β (E2) at 8 h before the second GnRH injection. Ovarian ultrasound and blood samples were taken just before E2 treatment of both groups. In a subset of cows (n = 563), pressure-activated estrus detection devices were used to assess expression of estrus at 48 to 72 h after $PGF_{2\alpha}$ treatment. Ovulation was confirmed by ultrasound 7 d after timed AI. Treatment with E2 increased expression of estrus but overall PP/AI did not differ between E2 and control cows. There was an interaction between treatment and expression of estrus such that PP/AI was greater in E2-treated cows that showed estrus than in E2-treated or control cows that did not show estrus and tended to be greater than control cows that showed estrus. There was evidence for a treatment by ovulatory follicle size interaction on PP/AI. Supplementation with E2 improved PP/AI in cows ovulating medium (15 to 19 mm) but not smaller or larger follicles. The E2 treatment also tended to improve PP/AI in primiparous cows with low (≤2.5) body condition score, and in cows at first postpartum service compared with Ovsynch alone. In conclusion, any improvements in PP/AI because of E2 treatment during a timed AI protocol appear to depend on expression of estrus, parity, body condition score, and size of ovulatory follicle.

Key words: Ovsynch, estradiol, dairy cow, conception rate

INTRODUCTION

The increase in milk yield of lactating dairy cows has been associated with a decline in reproductive efficiency (Sartori et al., 2002a; Washburn et al., 2002; Lopez et al., 2004). One of the factors decreasing reproductive efficiency in high-producing herds is the decrease in expression and detection of estrus (Nebel et al., 1997; Dransfield et al., 1998; Lopez et al., 2004). Timed AI (**TAI**) protocols such as Ovsynch have been developed to decrease reliance on detection of estrus in reproductive management programs (Pursley et al., 1995). However, even with increased AI submission rates, the low fertility of lactating dairy cows continues to be a problem after TAI. One of the reasons for low fertility may be the lower circulating estradiol-17β (**E2**) concentrations near AI in high-producing cows (Sartori et al., 2002a, 2004; Lopez et al., 2004; Wolfenson et al., 2004) apparently because of increased metabolism of E2 (Sangsritavong et al., 2002). This problem is exacerbated during Ovsynch because the second GnRH treatment causes ovulation of the dominant follicle before the peak E2 concentrations in most cows. Absent or reduced expression of estrus during Ovsynch is probably caused by this reduction in circulating E2. In addition, other reproductive problems could be caused by reduced E2, including inefficient sperm transport, suboptimal oviductal or uterine environment, impaired oocyte fertilization, and poor embryonic quality (Hawk and Cooper, 1975; Ryan et al., 1993; Sartori et al., 2002b).

This study was designed to test the effects of increasing circulating E2 during Ovsynch. A previous study (Sellars et al., 2006) incorporated 0.25 mg of estradiol cypionate (**ECP**) into an Ovsynch protocol and there were no differences in fertility when ECP was administered at the same time as the second GnRH treatment

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of Ovsynch. In this study, we chose to use E2 rather than a longer-acting estrogen such as ECP. In a recent experiment (Souza et al., 2005), we compared several types of estrogens (estradiol benzoate, estradiol cypionate, and estradiol-17β) and doses (0, 0.5, and 1 mg) in the presence or absence of dominant follicles. We concluded that 1 mg of E2 produced a physiological increase in circulating E2 without disrupting the normal decline in E2 concentrations following the LH surge (basal concentrations by ~10 h after LH peak). We also chose to administer the E2 treatment 8 h before the final GnRH injection of Ovsynch to mimic more closely the final surge in E2 concentrations that normally precedes the onset of estrus and the GnRH/LH surge.

The influence of the size of the ovulatory follicle in TAI programs has been investigated in a number of studies (Vasconcelos et al., 1999, 2001; Lamb et al., 2001; Perry et al., 2005) with somewhat inconsistent results. No previous study has provided adequate information on the effects of ovulatory follicle size on conception rate in high-producing dairy cows. Moreover, previous studies have not provided information on the mechanisms producing any observed changes in fertility because of differences in ovulatory follicle size. For example, lower fertility in cows ovulating smaller follicles may be caused by reduced circulating E2 concentrations. In this study, we evaluated the ovulatory follicle size in a large number of high-producing dairy cows and tested whether supplementing E2 concentrations would increase fertility in cows that ovulate different-sized follicles.

This experiment, therefore, was not designed to test a practical protocol for synchronizing ovulation in dairy cattle but was specifically designed to test whether low circulating E2 concentrations were an important component of the reduced fertility in lactating dairy cows, particularly during the Ovsynch protocol. Another objective was to compare percentage pregnant per AI (**PP/AI**) by ovulatory follicle size in cows that were submitted to Ovsynch or Ovsynch with E2 supplementation. Our first hypothesis was that cows receiving E2 treatment 24 h before TAI would express more estrus near AI and would have increased PP/AI compared with untreated cows receiving Ovsych. A second hypothesis was that cows ovulating medium-sized follicles would have greater PP/AI than cows ovulating smaller or larger follicles and that an E2-induced increase in PP/AI would be greatest in cows ovulating smaller and medium-sized rather than larger follicles.

MATERIALS AND METHODS

Materials

Prostaglandin $F_{2\alpha}$ (dinoprost tromethamine, 25 mg/dose; Lutalyse) was from Pfizer Animal Health (Kalamazoo, MI). The GnRH (gonadorelin diacetate tetrahydrate; 100 μg/dose; Ovacyst) was from Phoenix Scientific Inc. (St. Joseph, MO). Kamar heat mount detectors were from Kamar Inc. (Steamboat Springs, CO). Sesame oil and E2 were from Sigma Chemical Co. (St. Louis, MO). Benzyl alcohol was from EM Science (Cherry Hill, NJ). The E2 solution was prepared as follows: E2 was weighed and benzyl alcohol added to bring the solution to 5 mg/mL. Sesame oil was then added to the preparation to obtain a final solution of 0.5 mg/mL.

Animals, Management, and Experimental Design

Seven hundred seventeen lactating Holstein cows (466 multiparous and 251 primiparous), during 1,084 Ovsynch treatments, were housed in free-stall barns on a commercial dairy farm in Juneau, Wisconsin. Only synchronized breedings (criteria described subsequently) were analyzed in this study (867/1,084). The experimental period began in April 2004 and ended in October 2004. Cows were 105.2 ± 1.4 DIM and produced 39.4 ± 0.3 kg of milk/d. Cows in the study started to receive bST (500 mg/dose; Posilac, Monsanto Co., St. Louis, MO) at about 60 d postpartum. Cows were milked thrice daily and fed a TMR twice daily that consisted of corn silage and alfalfa silage as forage with a corn-soybean meal-based concentrate. The TMR was balanced to meet or exceed minimum nutritional requirements for lactating dairy cows (NRC, 2001).

Before first postpartum insemination, cows were presynchronized with 2 $PGF_{2\alpha}$ treatments given 14 d apart, with the first given on d 37 to 43 postpartum and the second on d 51 to 57 postpartum. This was followed by the first GnRH of the Ovsynch protocol 11 d later (d 62 to 68 postpartum). Cows detected in estrus by tail chalking (twice-daily examination of mounting activity followed by rechalking) between the second $PGF_{2\alpha}$ of the presynch and the first GnRH of the Ovsynch were inseminated. Animals were assigned to 2 groups in a completely randomized experimental design. The control group (n = 420) received Ovsynch (GnRH – 7 d – $PGF_{2\alpha}$ – 56 h – GnRH – 16 h – TAI). The treatment group (n = 447) had the same TAI protocol with the addition of 1 mg of E2 (i.m.; Ovsynch + E2) at 8 h before the second GnRH injection. Randomization of cows to treatment occurred at 48 h after the $PGF_{2\alpha}$ treatment (time of E2 treatment). Pregnancy diagnosis was performed by palpation of uterine contents per rectum at 35 to 41 d after AI and again at 58 to 64 d after AI. Pregnancy losses were calculated based on these 2 pregnancy exams. Cows diagnosed not pregnant were rerandomized to receive Ovsynch or Ovsynch + E2 without presynchronization treatments. To evaluate expres-