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Effect of Synchronization Protocols on Follicular Development and Estradiol and Progesterone Concentrations of Dairy Heifers

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ABSTRACT

The objectives were to evaluate the effect of synchronization protocols on follicular development and estradiol 17-β (E_2) and progesterone (P_4) concentrations in dairy heifers. In experiment 1, 36 heifers were assigned to 1 of 6 synchronization protocols in a 3×2 factorial design: presynchronization with GnRH on study d –6 or –9 [study d 0 = initiation of the Cosynch + CIDR (controlled internal drug releasing insert containing P_4) protocol] or no presynchronization (control) and one injection of $PGF_{2\alpha}$ or not on study d 0. In experiment 2, 126 heifers were assigned to 1 of 4 synchronization protocols in a 2×2 factorial arrangement: presynchronization or not with GnRH on study d –6 and injection of $PGF_{2\alpha}$ or not on study d 0. In experiments 1 and 2, all heifers received a modified Cosynch protocol with CIDR for 7 d starting on study d 0. After the $PGF_{2\alpha}$ of the Cosynch and removal of the CIDR, heifers were detected in estrus and inseminated. Those not inseminated by study d 10 received an injection of GnRH and were timed-inseminated. Ovaries were scanned by ultrasound on d 0, 2, and 5, daily from d 7 to 14, and on d 16. Blood samples collected on d 0, 2, 7, 9, and 16 were analyzed for P_4, and the blood sample collected on d 9 was analyzed for E_2. Pregnancy was diagnosed at 28 and 40 ± 3 d after artificial insemination. In experiment 1, there was a tendency for the presynchronization protocol to affect the proportion of heifers ovulating in response to the first GnRH injection of the Cosynch + CIDR protocol. In experiment 2, a greater proportion of presynchronized heifers ovulated in response to the first GnRH injection. Although heifers receiving $PGF_{2\alpha}$ had larger ovulatory follicles on d 7 and before ovulation and shorter intervals to estrus and ovulation, these heifers tended to have decreased concentrations of E_2 during proestrus. Presynchronization of dairy heifers with GnRH increased ovulation in response to the first GnRH injection, and treatment of heifers with $PGF_{2\alpha}$ at initiation of the Cosynch + CIDR protocol increased the size of the ovulatory follicle and reduced the intervals to estrus and ovulation.

Key words: heifer, ovulation synchronization, follicle growth

INTRODUCTION

Although the use of ovulation synchronization protocols and fixed-time AI has resulted in acceptable conception rates in lactating dairy cows (Pursley et al., 1997; Moreira et al., 2001; Cerri et al., 2004), dairy heifers inseminated at a fixed time after the Ovsynch protocol have reduced conception rates as compared with heifers inseminated on detection of estrus (Schmitt et al., 1996; Pursley et al., 1997; Tenhagen et al., 2005). The pattern of follicular development of dairy heifers is different from that of lactating dairy cows (Sartori et al., 2004), and it could explain the differences in conception rates following fixed-time AI between these animal types.

Heifers inseminated at a fixed time after the Ovsynch protocol were more likely to experience a short luteal cycle than heifers inseminated on detection of estrus, probably because of inadequate gonadotropic stimulation of the dominant follicle and formation of a corpus luteum (CL) with a reduced life span (Schmitt et al., 1996). Growth of ovulatory follicles in the presence of reduced concentrations of progesterone ($\mathbf{P_4}$) results in greater exposure to LH, expedited maturation of follicles, increased concentrations of estradiol ($\mathbf{E_2}$) during proestrus, and shorter intervals to onset of estrus (Stegner et al., 2004).

Ovulation after the first GnRH injection of the Ovsynch protocol in dairy heifers and in lactating dairy cows is dependent on the stage of the estrous cycle when the synchronization protocol is initiated, and initiation of the Ovsynch protocol during early diestrus results

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in an improved ovulation response (Vasconcelos et al., 1999; Moreira et al., 2000). The lack of ovulation following the first injection of GnRH of the Ovsynch protocol results in compromised embryo quality (Cerri et al., 2005) and reduced conception rates (Chebel et al., 2006) following Ovsynch and fixed-time AI in lactating dairy cows.

The hypotheses of the present study were that presynchronization of dairy heifers with an injection of GnRH given before initiation of the synchronization protocol would result in an increased proportion of heifers ovulating in response to the first GnRH injection of the synchronization protocol and that the treatment of dairy heifers with a $PGF_{2\alpha}$ injection at the time of initiation of the synchronization protocol would result in reduced P_4 concentrations during the period of follicular development and expedited maturation of the ovulatory follicle. Therefore, the objectives of the present study were to evaluate the effect of presynchronization with GnRH on the ovulatory response of dairy heifers to the first injection of GnRH of the synchronization protocol and to evaluate the effect of reduced P_4 concentrations on follicular development and E_2 and P_4 concentrations in dairy heifers.

MATERIALS AND METHODS

Animals and Diet

One hundred and seventy-three (n = 173) Holstein heifers, between 11 and 12 mo of age and weighing approximately 360 kg, from a commercial feedlot located in the Treasure Valley of Idaho were used in these experiments. Heifers were housed in open lots and were fed a diet as a TMR twice a day. The diet was based on corn silage, alfalfa hay, soybean meal, steam-rolled corn, whole cottonseed, brewer's grain, and a mineral and vitamin supplement and was designed to meet or exceed the nutritional requirements of Holstein heifers weighing 360 kg and gaining 0.8 kg/d (NRC, 2001).

Of the 173 heifers initially enrolled in these experiments, 11 were removed because of lesions in the anus caused by frequent ultrasound examination (n = 9), loss of CIDR insert (n = 1), or for being moved to a pen with bulls before insemination (n = 1).

Treatments

Experiment 1. To determine the optimal interval between presynchronization with GnRH and initiation of the synchronization protocol, 36 heifers were blocked by weight and randomly assigned to 1 of 3 presynchronization protocols. Heifers in the control group (n = 14) received no presynchronization treatment, heifers in the presynchronization –6 (**PRES6**) group (n = 11) received one injection of GnRH (100 μg of gonadorelin diacetate tetrahydrate, Cystorelin, Merial Ltd., Iselin, NJ) on study d –6 (study d 0 = day of initiation of the synchronization protocol), and heifers in the presynchronization –9 (**PRES9**) group (n = 11) received one injection of GnRH on study d –9. Furthermore, heifers were blocked by presynchronization protocol and assigned to receive one injection of $PGF_{2\alpha}$ (25 mg of dinoprost tromethamine sterile solution, Lutalyse, Pfizer Animal Health, Kalamazoo, MI) on study d 0 (**PGF**) or not (**NPGF**). This resulted in 6 synchronization protocols (control-NPGF = 6, control-PGF = 8, PRES6-NPGF = 5, PRES6-PGF = 6, PRES9-NPGF = 6, PRES9-PGF = 5).

On study d 0, all heifers were initiated in a synchronization protocol (Cosynch + CIDR) and received one injection of GnRH (**G1**) and an intravaginal controlled internal drug releasing (**CIDR**) insert containing 1.38 g of P_4 (Eazi-Breed CIDR, Pfizer Animal Health); 7 d later the CIDR was removed and all heifers received one injection of $PGF_{2\alpha}$ (**PG**). From study d 7 to 10 heifers were inseminated on detection of estrus and those not inseminated by 72 h after CIDR removal received a second injection of GnRH (**G2**) concomitant with AI.

Experiment 2. Heifers (n = 126) were blocked by weight and randomly assigned to 1 of 4 synchronization protocols in a 2×2 factorial arrangement: presynchronization (**PRES**) or no presynchronization (**NPRES**) with GnRH on study d –6 (study d 0 = day of initiation of the synchronization protocol) and treatment (PGF) or no treatment (NPGF) with an injection of $PGF_{2\alpha}$ on study d 0. This resulted in 4 treatments (NPRES-NPGF = 32, NPRES-PGF = 29, PRES-NPGF = 33, and PRES-PGF = 32). Starting on study d 0, all heifers were submitted to the same Cosynch + CIDR protocol as described for experiment 1 (Figure 1).

Estrus Detection and Insemination

Heifers were observed daily in the morning for signs of behavioral estrus and for secondary signs of estrus based on tail chalk removal (Macmillan et al., 1988) by using paintsticks (All-Weather Paintstick, LA-CO Industries, Chicago, IL), and those observed in estrus were inseminated immediately. Heifers not inseminated by study d 10 received an injection of GnRH concomitant with AI. One technician inseminated heifers 6 d per week, which coincided with the majority of inseminations, and a relief technician inseminated heifers once a week.

Ovarian Ultrasonography and Ovulatory Responses

In experiment 1, heifers in the PRES9 group had their ovaries examined by ultrasonography (7.5 MHz