Effects of Method of Presynchronization and Source of Selenium on Uterine Health and Reproduction in Dairy Cows

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ABSTRACT

The objectives of this study were to evaluate the effects of method of presynchronization and source of supplemental Se on uterine health and reproductive performance of lactating dairy cows. Holstein cows ($n = 512$) were assigned randomly to 2 methods of presynchronization, Presynch (2 PGF_{2α} given 14 d apart) or CIDR-PS (controlled internal drug releasing inserted for 7 d with an injection of PGF_{2α} at removal) and 2 sources of Se, sodium selenite (SS) or selenized yeast (SY) supplemented at 0.3 mg/kg from 25 d before calving to 80 d in milk (DIM) arranged in a 2×2 factorial. Cows were inseminated following the Ovsynch protocol (d 0 GnRH, d 7 PGF_{2α}, d 9 GnRH, timed artificial insemination (AI) 12 h after the final GnRH) starting at 12 and 3 d after Presynch and CIDR-PS, respectively. Cows were diagnosed for pregnancy at 28, 42, and 56 d after AI. Source of Se did not influence uterine health and resumption of cyclicity, but fewer CIDR-PS than Presynch cows were cyclic at the beginning of the Ovsynch, although differences in the proportion cyclic may have been caused by the timing when corpus luteum evaluations were performed in the different presynchronization treatments. Ovulatory responses were not influenced by source of Se. However, the CIDR-PS increased ovulation to the first GnRH, double ovulation to the final GnRH, and size of ovulatory follicle at PGF_{2α} and final GnRH of the Ovsynch, but did not influence ovulation at the final GnRH of the Ovsynch. Concentrations of estradiol during the Ovsynch increased with follicle diameter and were greater for cows receiving CIDR-PS than Presynch, but they were not influenced by source of Se. Pregnancy per AI on d 28 (32.7%), 42 (28.5%), and 56 (25.9%) after AI, and pregnancy loss (20.5%) from 28 to 56 d were not influenced by source of Se or method of presynchronization. Although cows

receiving CIDR-PS had an increased incidence of ovulation to the first GnRH (73.2 vs. 57.8%) and double ovulation to the final GnRH of the Ovsynch (18.7 vs. 9.0%), both of which enhanced pregnancy, the CIDR-PS protocol did not improve pregnancy per AI or reduce pregnancy loss compared with presynchronization with PGF_{2α} alone.

Key words: dairy cow, presynchronization, reproduction, selenium

INTRODUCTION

The Ovsynch (d 0 GnRH, d 7 PGF_{2α}, d 9 GnRH) timed AI (TAI) protocol is commonly used for reproductive management of lactating dairy cows (Caraviello et al., 2006). The use of this regimen has resulted in acceptable pregnancy per AI with maximum submission to insemination compared with programs aimed at synchronizing estrus rather than controlling ovulation (Pursley et al., 1997). The first GnRH injection of the Ovsynch protocol given at random days of the estrous cycle caused ovulation in 64% of the cows, and this varied according to the stage of the estrous cycle (Vasconcelos et al., 1999). Incidences of ovulation were greater in cows receiving the first GnRH of the Ovsynch between d 5 and 9, and d 17 and 21 of the estrous cycle, than cows between d 1 and 4, and d 10 and 16 (Vasconcelos et al., 1999). Also, ovulation to the final GnRH injection was not affected by day of the estrous cycle when the Ovsynch protocol was initiated, but it was increased when cows ovulated to the initial GnRH (Vasconcelos et al., 1999). In addition to changes in ovulatory response, day of the cycle when Ovsynch is initiated might also influence the proportion of cows that undergo premature luteolysis, as cows receiving the first GnRH of the Ovsynch after d 10 had increased incidence of premature luteolysis (Vasconcelos et al., 1999). Lack of ovulation to the first GnRH of the Ovsynch protocol increased the period of dominance of the ovulatory follicle and compromised embryo quality (Cerri et al., 2005), which reduced pregnancy per AI (Chebel et al., 2006).

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To increase ovulation to the first GnRH and limit premature luteolysis during the Ovsynch, a presynchronization regimen using PGF_{2α} was developed that optimized reproductive responses to TAI (Moreira et al., 2001); however, response to the presynchronization with PGF_{2α} depended upon the cyclic status of the cow. Progesterone inserts induced cyclicity in anovular cows (Chebel et al., 2006) and, when combined with PGF_{2α}, optimized ovulation to the first GnRH of the Ovsynch protocol (Cerri et al., 2006).

Selenium is a component of several important selenoproteins and enzymes required for antioxidant defense and conversion of inactive thyroid hormone thyroxine (T₄) to its active form, triiodothyronine (T₃). Inadequate Se status was associated with increased incidence of uterine problems in cows (Trinder et al., 1973; Harrison et al., 1986), possibly because of impaired neutrophil function (Cebra et al., 2003), which might compromise reproductive performance in dairy cows. Supplementation with selenized yeast (Se-yeast) to cattle increased the concentration of Se in blood compared with sodium selenite (Ortman and Pehrson, 1999) or sodium selenate (Ortman and Pehrson, 1999; Weiss and Hogan, 2005), possibly because of absorption of Se as seleno amino acids such as methionine and cysteine.

It was hypothesized that presynchronization with an intravaginal insert containing progesterone would increase ovulation to the first GnRH of the Ovsynch protocol, and improve establishment and maintenance of pregnancy. It was also hypothesized that replacement of sodium selenite with Se-yeast would improve uterine health and reproductive performance of lactating dairy cows. Therefore, objectives of this study were to evaluate method of presynchronization and source of supplemental Se on uterine health and reproductive performance of lactating dairy cows in California where concentrations of Se in soil and plants are considered adequate.

MATERIALS AND METHODS

Animals and Housing

Five hundred eighty-one Holstein cows calving from January to October 2005 were initially enrolled in the study. Cows were housed in 8 pens: 4 in the prepartum and 4 in the postpartum periods. Individual pens were identical in design, size, and number of animals housed. Of the 581 cows initially enrolled, 15 were removed from the study because they remained less than 11 d in the prepartum diet ($n = 11$) or aborted ($n = 4$). In addition, 54 cows did not contribute to the analyses because they left the study or were designated to not

be inseminated (Rutigliano et al., 2006). Therefore, 512 cows were utilized for statistical analyses.

The lactating herd size during the study was approximately 890 cows. Cows were milked twice daily and the 3.5% FCM rolling herd average was 11,630 kg/cow. For cows enrolled in the study, the mean ($\pm$ SD) and median were, respectively, 2.3 ± 1.6 and 2.0 lactations, BCS of 3.57 ± 0.41 and 3.50 at study enrollment, and 71.3 ± 2.9 and 71.0 DIM at first postpartum AI, and none of them differed ($P > 0.40$) among treatments.

Study Design and Treatments

Cows were assigned to treatments as a randomized block design in a 2×2 factorial arrangement of treatments at approximately 25 ± 3 d before the expected date of calving (study d 0 = day of calving). Animals were blocked by parity (primiparous and multiparous) and by expected calving date and, within each block, randomly assigned to 1 of the 4 treatments. Treatments were 2 methods of presynchronization and 2 sources of dietary Se. Cows enrolled in the presynchronization with PGF_{2α} (Moreira et al., 2001) received 2 s.c. injections of 25 mg of PGF_{2α} (dinoprost tromethamine; Lutalyse sterile solution, Pfizer Animal Health, New York, NY), on study d 37 and 51 (**Presynch**; 164 multiparous and 96 primiparous cows). Cows in the progesterone insert presynchronization (**CIDR-PS**; 165 multiparous and 87 primiparous cows) received a controlled internal drug releasing device (Eazi-Breed CIDR; Pfizer Animal Health) containing 1.38 g of progesterone from study d 53 to 60. At removal of the CIDR, cows received an s.c. injection of PGF_{2α} (Figure 1). All cows received TAI on study d 72 following the Ovsynch protocol (Pursley et al., 1997) starting at 12 and 3 d after the Presynch and CIDR-PS protocols, respectively. For the Ovsynch protocol, cows received an i.m. injection of 100 μ g of GnRH (gonadorelin diacetate tetrahydrate, Cystorelin; Merial Ltd., Iselin, NJ), followed 7 d later by an s.c. injection of 25 mg of PGF_{2α}, a second i.m. injection of 100 μ g of GnRH 48 h later, and AI 12 h after the final injection of GnRH.

In addition, half of the cows in each presynchronization treatment were supplemented with Se in the pre- and postpartum diets at 0.3 mg/kg of diet DM as sodium selenite (**SS**; 164 multiparous and 92 primiparous cows) or as Se-yeast (**SY**; Sel-Plex, Alltech Biotechnology, Nicholasville, KY; 165 multiparous and 91 primiparous cows). The experimental design resulted in 4 treatments in a 2×2 factorial arrangement with the following number of cows: SS-Presynch = 126; SS-CIDR-PS = 130; SY-Presynch = 134; and SY-CIDR-PS = 122.

Figure 1. Diagram of presynchronization treatments. Cows in the Presynch treatment received 2 injections of 25 mg of PGF_{2α} on study d 37 and 51. Cows in the CIDR-PS received a controlled internal drug releasing insert (CIDR) containing 1.38 g of progesterone for 7 d and an injection of PGF_{2α} on the day of insert removal. All cows received AI at a fixed time on study d 72 after completion of the Ovsynch protocol starting at 12 or 3 d after the presynchronization with the Presynch and CIDR-PS protocols, respectively. Ovsynch = injection of 100 µg of GnRH, followed 7 d later by an injection of 25 mg of PGF_{2α}, and a second injection of 100 µg of GnRH 48 h later; PGF = 25 mg of PGF_{2α}; TAI = timed AI 12 h after the second GnRH injection of the Ovsynch protocol; US = ultrasound examination of the ovaries.

Dietary Treatments

Prepartum cows were fed once daily at 1200 h and lactating cows twice daily immediately after milking at 0600 and 1600 h. Prepartum and lactation diets were formulated using the CPM-Dairy cattle ration analyzer (Cornell-Penn-Miner, Ver. 3.0.8) to meet or exceed the nutrient requirements set forth by the NRC (2001) for prepartum Holstein cows weighing 670 kg and consuming 12 kg of DM in the last 3 wk of gestation, and for lactating Holsteins weighing 630 kg, consuming 24 kg of DM, and producing 45 kg/d of milk containing 3.5% fat and 3.1% true protein in the first 80 d of lactation. Diets were formulated to differ only in the source of supplemental Se, which was the only ingredient incorporated separately into the SS and SY TMR (Table 1). Detailed description of the dietary ingredients and nutrient composition of TMR and ingredients is provided elsewhere (Rutigliano et al., 2006). Dietary treatments

and cows were switched among pens such that all pens received both SS and SY to avoid pen effects on treatment responses and to allow cow as the experimental unit.

The expected daily intake of supplemental Se was approximately 4 mg prepartum and 7 mg postpartum. Dietary treatments lasted from 25 ± 3 d before expected calving (study day -25) to 80 d postpartum (study d 80). After that, all cows were fed the SS ration.

Evaluation of Subclinical Endometritis

Cows on study d 35 ± 3 were evaluated for uterine health based on cytologic examination of uterine flush. Briefly, a Foley catheter was introduced into the previously pregnant uterine horn and 20 mL of sterile saline solution was infused and aspirated. The aspirated fluid was placed in ice, transported to the laboratory, and centrifuged at 750 × g for 10 min and the supernatant

discarded. The pellet was resuspended with 2 mL of saline, and an aliquot of 20 μ L was pipetted onto a glass slide and smeared in 2 slides per flush. Smears were air-dried and stained using a Diff-Quick (Fisher Diagnostics, Middletown, VA) stain. Slides were examined and number of total leukocytes, epithelial endometrial cells, and neutrophils were counted to complete 100 cells per slide, and the percentage of neutrophils was calculated. Subclinical endometritis was defined when the proportion of neutrophils $\geq 18\%$ (Kasimanickam et al., 2004).

Ovarian Ultrasonography, Classification of Anovular Cows, and Ovulatory Responses

Transrectal ultrasonography of the ovaries was performed using a portable ultrasound equipped with a 7.5-MHz linear probe (Sonovet 2000, Universal Medical System, Bedford Hills, NY) on study d 51 and 63, and on study d 48 and 60 in the Presynch and CIDR-PS treatments, respectively, for determination of cyclic status. Cows with at least one ovary bearing a corpus luteum (CL) in any of the 2 ultrasonographic examina-

Table 1. Ingredient composition of diets and mixture of grains and nutrient content of diets

Item	Diet ¹			
	Prepartum		Postpartum	
	SS	SY	SS	SY
Ingredient	% DM basis			
Corn silage	40.0	40.0	17.8	17.8
Alfalfa hay, dry cow	26.0	26.0	—	—
Alfalfa hay, lactating cow	—	—	16.0	16.0
Alfalfa silage	—	—	16.0	16.0
Orange pulp	8.4	8.4	7.2	7.2
Grain mixture	25.6	25.6	43.0	43.0
Composition of grain mixtures				
Corn distillers grains	39.1	39.1	23.2	23.2
Steam-rolled corn, 400 g/L	46.9	46.9	46.9	46.9
Whole cottonseed	—	—	11.6	11.6
Ca salts of fatty acids ²	—	—	1.7	1.7
Prepartum mineral ³	7.0	7.0	—	—
Postpartum mineral ⁴	—	—	13.7	13.7
SS supplement ⁵	7.0	—	2.9	—
Se-yeast supplement ⁶	—	7.0	—	2.9
Nutrient content of diets				
NE _L , ⁷ Mcal/kg	1.60	1.60	1.66	1.66
Organic matter, %	91.0 $\pm$ 0.4	91.0 $\pm$ 0.6	90.7 $\pm$ 0.1	90.6 $\pm$ 0.6
CP, %	16.5 $\pm$ 0.4	16.8 $\pm$ 0.2	18.8 $\pm$ 1.0	19.2 $\pm$ 0.9
NDF, %	38.6 $\pm$ 2.4	36.0 $\pm$ 6.2	32.8 $\pm$ 3.4	33.8 $\pm$ 3.6
Fat, %	3.5 $\pm$ 0.3	4.5 $\pm$ 0.6	5.3 $\pm$ 0.2	5.1 $\pm$ 0.6
Se, mg/kg	0.44 $\pm$ 0.12	0.56 $\pm$ 0.09	0.43 $\pm$ 0.07	0.54 $\pm$ 0.11

¹SS = sodium selenite; SY = selenium yeast. Prepartum diets were fed during the last 25 d of gestation; postpartum diets were fed during the first 80 d of lactation.

²Ener GI Transition Formula (Virtus Nutrition LLC, Fairlawn, OH).

³Contains (DM basis) 32.1% anhydrous calcium chloride, 32.1% anhydrous magnesium sulfate, 4.9% magnesium oxide, 4.4% Zinpro 4-Plex (mixture of Zn methionine, Mn methionine, Cu lysine, and Co glucoheptonate; Zinpro Co., Eden Prairie, MN), 17.3% Diamond V XP (culture of *Saccharomyces cerevisiae*; Diamond V Mills, Cedar Rapids, IA), and a mixture of iodine and vitamins. Supplement contained (DM basis): 13.6% Ca, 0.1% P, 8.2% Mg, 0.2% K, 8.1% S, 0.04% Na, 23.71% Cl; and (per kg) 1,240 mg of Zn, 430 mg of Cu, 690 mg of Mn, 86 mg of Co, 5.5 mg of I, 210,000 IU of vitamin A, 40,000 IU of vitamin D, and 12,000 IU of vitamin E.

⁴Contains (DM basis) 23.8% Pro-Lak (blend of marine and animal by-products; H. J. Baker & Bro. Inc., Stamford, CT), 52.2% Amino Plus (lignosulfonate treated soybean meal; Ag Processing Inc., Emmetsburg, IA), 0.36% Alimet (hydroxy methyl-thio butanoic acid; Novus International Inc., St. Louis, MO), 0.36% Smartamine M (polymer-protected D,L-methionine, Adisseo Inc., Alpharetta, GA), 2.85% Diamond V XP, 13.1% sodium bicarbonate, 2.61% dicalcium phosphate, 4.00% magnesium oxide, 0.71% Zinpro 4-Plex, and 0.024% zinc sulfate, and a mixture of iodine and vitamins. Supplement contained (DM basis): 44% CP, 2.45% Ca, 1.66% P, 3.00% Mg, 3.18% K, 0.76% S, 3.70% Na, 0.21% Cl; and (per kg) 515 mg of Zn, 116 mg of Cu, 525 mg of Mn, 12 mg of Co, 9.5 mg of I, 50,000 IU of vitamin A, 11,000 IU of vitamin D, and 500 IU of vitamin E.

⁵Contains (DM basis) 1,350 mg/kg of sodium monensin and 19 mg/kg of Se from sodium selenite with corn germ meal as carrier.

⁶Contains (DM basis) 1,350 mg/kg of sodium monensin and 19 mg/kg of Se from Sel-Plex (selenized yeast; Alltech Biotechnology Inc., Nicholasville, KY) with corn germ meal as carrier.

⁷NE_L calculated from NRC (2001) based on nutrient composition of ingredients and the average DMI in the prepartum (12 kg/d) and lactation (24 kg/d) periods (Rutigliano et al., 2006).

tions were classified as cyclic, whereas those without a CL in both examinations were classified as anovular.

Ovaries also were examined during all injections of the Ovsynch protocol and again 48 h after the final injection of GnRH. Maps of the ovaries were drawn, and size and location of follicles >8 mm and CL were recorded. Cows were classified as ovulating to the first GnRH of the Ovsynch protocol when they had a follicle ≥ 10 mm in diameter on the day of GnRH and a new CL was detected the day the PGF_{2 α} was administered. Ovulation to the final GnRH of the Ovsynch protocol was determined when a follicle ≥ 10 mm in diameter detected at the time of the injection was not observed 48 h later.

Blood Sampling

Blood was sampled by puncture of the median coccygeal vein or artery using evacuated tubes (Becton Dickinson, Franklin Lakes, NJ) with K₂ EDTA for plasma separation. Samples were immediately placed in ice and transported to the laboratory within 5 h of collection. Blood tubes were centrifuged at $2,000 \times g$ for 15 min, and plasma was frozen at -25°C until analyses.

Concentrations of Se and Estradiol in Plasma and Glutathione Peroxidase Activity in Plasma

Concentrations of Se in plasma collected from 30 cows fed SS and 25 fed SY were measured on study days -45 , 0 , 21 , 42 , and 63 . Analysis of Se in blood plasma was performed by a fluorometric method (Phyllis and Ullrey, 1978). Glutathione peroxidase (GPx) activity was determined in plasma on study d 0 and 42 with a GPx assay kit (Cayman Chemical Company, Ann Arbor, MI).

Concentrations of estradiol were measured in plasma collected from 50 cows, 18 primiparous and 32 multiparous, with the following distribution per treatment: 11 SS-Presynch, 12 SY-Presynch, 14 SS-CIDR-PS, and 13 SY-CIDR-PS. Plasma samples were collected on the day of PGF_{2 α} and 48 h later at final GnRH of the Ovsynch protocol and analyzed by RIA according to Kirby et al. (1997). Samples were analyzed in triplicate in a single assay and the CV was 9.2%. Mean sensitivity of the assay, calculated as 2 SD below the mean cpm at maximum binding, was 0.51 pg/mL.

Pregnancy Diagnosis and Calculation of Reproductive Outcomes

Pregnancy was diagnosed at 28 d after TAI by ultrasonography of the uterus and its contents and was characterized by visualization of an embryo. Cows

diagnosed as pregnant on d 28 were re-examined by palpation per rectum of the uterine contents at 42 and 56 d after AI. Pregnancy per AI at first and second postpartum AI were calculated as the number of cows that became pregnant divided by the number of inseminated cows for each postpartum AI. Pregnancy loss after first postpartum AI was calculated as the number of cows that lost pregnancy from 28 to 56 d of gestation divided by the number of pregnant cows on d 28 after TAI.

Resynchronization of Cows and Reinsemination of Nonpregnant Cows After First AI and Pregnancy Diagnosis

After the first AI, estrus was observed once daily based on removal of tail chalk (All-Weather Paintstik, LA-CO Industries, Chicago, IL), and cows were reinseminated upon detection of estrus in the morning. Cows not reinseminated received an injection of 100 μg of GnRH 21 d after the first TAI and, 7 d later, those cows found nonpregnant during the ultrasonography received an injection of 25 mg of PGF_{2 α} and the Ovsynch protocol was completed. After the second postpartum AI, pregnancy was diagnosed by palpation per rectum of the uterine contents at 42 ± 3 d after AI. Of the cows reinseminated after the first postpartum AI, 126 (37.5%) were inseminated based on detection of spontaneous estrus and 210 (62.5%) based on TAI, and all cows were included in the analysis of pregnancy to second AI.

Body Condition Scoring

Body condition of all cows was scored at enrollment (study day -25 ± 3), at calving, and study d 42 ± 3 , 72 ± 3 , and 100 ± 3 . Cows were scored for body condition on a scale of 1 (emaciated) to 5 (obese), with 0.25-unit increments as described by Ferguson et al. (1994).

Temperature and Humidity Recording and Heat Stress

Ambient temperature and relative humidity (RH) were recorded hourly by data recorders (Hobo H8 Pro Series, Onset Computer Corp., Bourne, MA), operated by a computer software program (BoxCar Pro 4.0 Starter Kit, Onset Computer Corp.). Temperature and RH accuracy were within $\pm 0.2^{\circ}\text{C}$ and $\pm 2\%$, respectively. Two recorders were placed in each study pen at a height of 1.9 m from the floor and placed under the central shades in the prepartum pens and immediately above the stalls in the lactation pens. The probes recorded data from February to November 2005. The temperature-humidity index (THI) was calculated as:

THI = $td - (0.55 - 0.55 \text{ RH}/100) (td - 58)$, in which td is the temperature (in °F) and RH is expressed as a percentage (NOAA, 1976). For each 24-h period, average daily mean and maximum temperature, RH, and THI were determined.

Statistical Analyses

Binary data such as cyclic status, subclinical endometritis, ovulation, double ovulation, pregnancy per AI, and pregnancy loss were analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS Inst. Inc., Cary, NC). A backward stepwise regression was utilized, and all models included the effects of method of presynchronization, source of Se, interaction between method of presynchronization and source of Se, parity, heat stress, interaction between source of Se and parity, interaction between source of Se and heat stress, interaction between method of presynchronization and parity, interaction between method of presynchronization and heat stress, and other explanatory variables as appropriate. Variables were sequentially removed from the model by the Wald's statistic criterion if $P > 0.10$; however, method of presynchronization and source of Se were forced in the final model. Adjusted odds ratio (AOR) and the 95% confidence interval (CI) were calculated. For second postpartum AI, type of insemination, either at spontaneous estrus or TAI was also included in the model.

Diameters of the ovulatory follicle at the injection of PGF_{2α} and at final GnRH of the Ovsynch protocol were analyzed by ANOVA using the GLM procedure (SAS Inst. Inc.). The model included the effects of method of presynchronization, source of Se, interaction between method of presynchronization and source of Se, parity, and other explanatory variables as appropriate. Concentrations of Se and estradiol in plasma were analyzed by ANOVA for repeated measures with the MIXED procedure (SAS Inst. Inc.). For plasma Se, the model included the effects of source of Se, day in the study, and interaction between source of Se and day in the study, the initial Se concentration on study d -45 as covariate. Cow nested within source of Se was the random experimental error. Covariance structure was tested and that with the smallest Schwarz Bayesian criterion was chosen for each statistical model. For concentrations of estradiol, the statistical model included the effects of method of presynchronization, source of Se, interaction between method of presynchronization and source of Se, hour of blood collection, interaction between method of presynchronization and hour of blood collection, interaction between source of Se and hour of blood collection, interaction between method of presynchronization and source of Se and hour of blood

collection, parity, and cyclic status. Cow nested within treatment was the random error for the model. The covariance with the smallest Schwarz Bayesian criterion was selected for the repeated statement of the model. Treatment differences with $P \leq 0.05$ were considered significant and differences at $0.05 < P \leq 0.10$ were designated as tendency.

RESULTS

Because none of the variables analyzed presented an interaction between method of presynchronization and source of Se, results are presented separately for the effects of method of presynchronization and source of Se.

The mean ($\pm$ SEM) and median days cows were fed the prepartum diets were, respectively, 24.7 ± 0.3 and 24 for SS and 24.9 ± 0.4 and 25 d for SY treatment. Concentrations of Se in plasma were similar ($P = 0.38$) for cows fed SS ($0.101 \pm 0.005 \mu\text{g/mL}$) and SY ($0.107 \pm 0.005 \mu\text{g/mL}$). Concentrations of Se increased ($P = 0.01$) with days in the study, but no interaction between dietary treatment and day in the study was observed. Similarly, GPx activity in plasma did not differ ($P = 0.59$) between sources of Se and averaged 594.0 ± 18.5 and $604.2 \pm 20.0 \text{ nmol/mL per min}$ for SS and SY, respectively.

Weekly mean and maximum temperatures, relative humidity, and THI were all similar among pre- and postpartum pens. Based on the mean daily THI ≥ 72 (Armstrong, 1994), cows were subjected to the greatest heat stress in July and August (Figure 2). When the average maximum daily THI was considered, cows began to be exposed to some heat stress in May and continued to be exposed through September.

BCS

The mean BCS during the study was not different between presynchronization protocols. Source of Se did not influence ($P = 0.45$) BCS in the first 100 DIM; BCS averaged 3.07 ± 0.02 and 3.08 ± 0.02 in cows fed SS and SY, respectively. Cows lost BCS in the first 48 to 70 d postpartum and started to regain at 100 d postpartum, but no interaction between source of Se and day postpartum on BCS was observed. Multiparous cows had a lesser ($P = 0.02$) mean BCS during the study period compared with primiparous cows (3.05 ± 0.01 vs. 3.10 ± 0.02).

Subclinical Endometritis

Source of Se had no effect on the prevalence of subclinical endometritis at 35 DIM, and it affected 18.1

Figure 2. Temperature-humidity index (THI; NOAA, 1976) during the different months of the study. Mean THI post = mean daily THI in the postpartum pens; Mean THI pre = mean daily THI in the prepartum pens; Max THI post = maximum daily THI in the postpartum pens; Max THI pre = maximum daily THI in the prepartum pens. Mean and maximum daily THI differed ($P < 0.01$) among weeks of the study for the pre- and postpartum periods, but not among pens ($P > 0.05$). Cows were exposed to heat stress during the months of July and August based on mean daily THI ≥ 72 (dashed line indicates THI = 72).

and 18.3% of SS and SY cows. Prevalence of subclinical endometritis did not differ between presynchronization protocols (Presynch = 18.0 vs. CIDR-PS = 18.4%) and parity (multiparous = 18.0 vs. primiparous = 18.5%). Risk of subclinical endometritis tended to increase (AOR = 1.95, 95% CI, 0.98 to 3.89; $P = 0.06$) in cows that had retained placenta [31.1 (14/45) vs. 17.0% (80/472)], which was defined as presence of fetal membranes 24 h after calving (Rutigliano et al., 2006). Similarly, risk of subclinical endometritis more than doubled (AOR = 2.12; 95% CI, 1.25 to 3.59; $P = 0.005$) in cows with acute puerperal metritis compared with those without puerperal metritis, with the former characterized by foul watery uterine discharge of brownish or reddish color concurrent with rectal temperature $>39.5^{\circ}\text{C}$ (Rutigliano et al., 2006).

Source of Se and Reproductive Responses

Source of Se had no effect on cyclic status of the cows (Table 2) or any of the ovarian responses evaluated during the Ovsynch protocol (data not shown). Pregnancy per AI evaluated at 28, 42, and 56 d after first postpartum AI were similar for cows fed SS and SY. Similarly, source of Se had no effect on pregnancy loss after the first AI and proportion pregnant at second postpartum AI (Table 2).

Cyclic Status, Ovarian Responses to the Ovsynch Protocol, and Estradiol Concentrations

A greater ($P = 0.02$) proportion of cows that received Presynch were cyclic than those receiving CIDR-PS based on the presence of a CL during ultrasound at differing times during the 2 presynchronization treatments (Table 3). Cyclic status was not affected by occurrence of puerperal metritis, retained placenta, and changes in BCS from calving to AI, but multiparous cows were 2.19 times more likely ($P = 0.002$) to be cyclic than primiparous cows (multiparous = 85.1% vs. primiparous = 76.5%), and cows with greater BCS at AI were also more likely ($P = 0.002$) to be cyclic. As BCS at AI increased from <2.75 to between 2.75 and 3.25 and finally to >3.25 , the proportion of cyclic cows also increased (76.5 vs. 82.1 vs. 95.8%).

The proportion of cows with a CL at the time of the PGF_{2a} treatment of the Ovsynch protocol was similar between methods of presynchronization (Table 3). Cyclic cows were 3.15 times more likely ($P < 0.001$) to have a CL at time of PGF_{2a} of the Ovsynch protocol. The diameters of the ovulatory follicle at the moment of the PGF_{2a} and final GnRH injections of the Ovsynch protocol were larger ($P = 0.006$) for cows in the CIDR-PS compared with those in the Presynch protocol. Cows in the CIDR-PS had greater incidences of ovulation to

Table 2. Effect of source of Se on reproductive responses of dairy cows

Item	Source of Se ¹		AOR ²	95% CI ³	P-value
	SS	SY			
Cows, n	256	256	—	—	—
Cyclic, %	79.7	84.4	0.81	0.50, 1.30	0.38
Pregnant, %					
Day 28	34.5	30.9	1.2	0.8, 1.8	0.40
Day 42	30.2	26.8	1.2	0.8, 1.9	0.35
Day 56	27.8	24.0	1.3	0.8, 2.0	0.30
Pregnancy loss, %					
Day 28 to 56	18.8	22.4	0.7	0.3, 1.6	0.41
Pregnant at 2nd AI, ⁴ %	30.3	26.4	1.1	0.7, 1.9	0.65

¹SS = sodium selenite; SY = selenium yeast.²AOR = adjusted odds ratio; SY as the reference value.³CI = confidence interval.⁴Includes all reinseminated cows.

the first GnRH ($P < 0.001$) and double ovulation to the final GnRH ($P = 0.005$) of the Ovsynch than Presynch cows, but overall ovulation to the final GnRH did not differ between methods of presynchronization.

Ovulation to the final GnRH of the Ovsynch protocol was influenced by BCS at AI and ovulation to the first GnRH (Table 4). Cows that ovulated to the first GnRH

injection of the Ovsynch protocol were 2.56 times more likely ($P < 0.001$) to ovulate to the final GnRH, whereas cows with a BCS < 2.75 at AI were less likely ($P = 0.04$) to ovulate to the final GnRH than those with BCS ≥ 2.75 . In addition to presynchronization method, multiparous cows were 2.66 times more likely ($P = 0.006$) to have a double ovulation than primiparous cows.

Table 3. Effect of method of presynchronization on ovarian responses to the Ovsynch protocol and reproductive responses

Item	Presynchronization ¹		AOR ²	95% CI ³	P-value
	Presynch	CIDR-PS			
Cows, number	260	252	—	—	—
Cyclic, %	85.8	78.2	0.57	0.35, 0.92	0.02
Presence of CL ⁴ at PGF _{2α} , %	87.6	87.4	1.04	0.60, 1.79	0.90
Ovulatory follicle, mm					
PGF _{2α}	15.3 ± 0.3	16.2 ± 0.3	—	—	0.006
Final GnRH	18.2 ± 0.3	19.1 ± 0.3	—	—	0.006
Ovulation during Ovsynch					
First GnRH, %	57.8	73.2	2.03	1.39, 2.95	< 0.001
Final GnRH, %	84.9	83.3	0.95	0.55, 1.63	0.85
Double ovulation, ⁵ %	9.0	18.7	2.29	1.30, 4.12	0.005
Estradiol, ⁶ pg/mL					
PGF _{2α}	2.71 ± 0.23	3.11 ± 0.23	—	—	0.007
GnRH	5.16 ± 0.23	5.98 ± 0.24	—	—	0.007
Pregnant, %					
Day 28	32.6	32.9	0.97	0.63, 1.49	0.90
Day 42	27.8	30.4	1.16	0.75, 1.81	0.50
Day 56	25.6	26.3	1.03	0.65, 1.62	0.90
Pregnancy loss, %					
Day 28 to 56	20.7	20.3	0.78	0.32, 1.92	0.59
Pregnant at 2nd AI, ⁷ %	28.1	28.5	0.94	0.57, 1.55	0.81

¹Presynch = 2 injections of 25 mg of PGF_{2α} at 37 and 51 d postpartum and the Ovsynch program started 12 d later; CIDR-PS = cows received a controlled internal drug releasing device containing 1.38 g of progesterone from 53 to 60 d postpartum with an injection of PGF_{2α} at insert removal and the Ovsynch protocol started 3 d later.

²AOR = adjusted odds ratio; Presynch as the reference value.

³CI = confidence interval.

⁴CL = corpus luteum.

⁵Percentage of cows responding to the final GnRH injection of the Ovsynch protocol that ovulated 2 follicles within 48 h of treatment.

⁶Concentrations of estradiol in plasma of 50 cows at the injections of PGF_{2α} and final GnRH of the Ovsynch protocol.

⁷Includes all reinseminated cows.

Table 4. Risk factors for ovulation within 48 h of the final GnRH of the Ovsynch protocol

Item	Ovulation, % (n/n)	AOR ¹	95% CI ²	P-value
BCS at AI				
>3.25	91.7 (66/72)	1.60	0.64, 3.97	0.04
2.75 to 3.25	88.0 (279/317)	1.00	—	
<2.75	79.8 (91/114)	0.54	0.30, 0.96	
Ovulation to first GnRH				
Yes	90.6 (298/329)	2.56	1.51, 4.33	<0.001
No	79.3 (138/174)	1.00	—	

¹AOR = adjusted odds ratio.²CI = confidence interval.

Concentrations of estradiol in plasma were not affected by source of Se and averaged 4.3 ± 0.17 and 4.17 ± 0.21 pg/mL during the Ovsynch for SS and SY, respectively; however, cows receiving CIDR-PS had greater ($P = 0.007$) concentrations of estradiol at the injections of PGF_{2α} (Table 3). Concentrations of estradiol increased ($P < 0.001$) from PGF_{2α} to 48 h later (2.91 ± 0.19 vs. 5.57 ± 0.19 pg/mL), but no interaction ($P > 0.25$) was observed between method of presynchronization and source of Se, method of presynchronization and hour after PGF_{2α}, and between source of Se and hour after PGF_{2α}. Parity and cyclic status influenced plasma estradiol concentrations; primiparous cows had greater ($P = 0.006$) concentrations than multiparous cows (4.56 ± 0.20 vs. 3.92 ± 0.19 pg/mL), and concentrations were

greater ($P = 0.02$) in cyclic than anovular cows (4.62 ± 0.13 vs. 3.86 ± 0.28 pg/mL). Plasma estradiol concentrations were related ($R^2 = 40.2\%$; $P < 0.001$) to ovulatory follicle diameter in both Presynch and CIDR-PS treatments (Figure 3).

Pregnancy at First and Second Postpartum AI and Pregnancy Loss

Method of presynchronization did not affect pregnancy after first and second postpartum inseminations or pregnancy loss after first postpartum TAI (Table 3). Subclinical endometritis at 35 d postpartum negatively affected ($P < 0.01$) pregnancy per AI at all times. On d 28 after AI, cows diagnosed with subclinical endometri-

Figure 3. Relationship between ovulatory follicle diameter and estradiol concentrations at the final GnRH of the Ovsynch protocol in cows presynchronized with 2 injections of PGF_{2α} 14 d apart, with the second injection given 12 d before the Ovsynch (Presynch), or with a controlled internal drug releasing insert (CIDR) for 7 d and PGF_{2α} at CIDR removal, with the Ovsynch starting 3 d after CIDR removal (CIDR-PS). Estradiol, pg/mL = $0.7029 + 0.2591 \times \text{follicle diameter}$, $r^2 = 40.2\%$ ($P < 0.001$).

Table 5. Risk factors for pregnancy 28 d after first postpartum AI

Item	Pregnant, % (n/n)	AOR ¹	95% CI ²	P-value
Subclinical endometritis				
Yes	18.3 (15/82)	0.37	0.20, 0.69	0.002
No	35.7 (140/392)	1.00	—	
Cyclic				
Yes	34.9 (144/413)	1.84	1.01, 3.32	0.04
No	22.0 (18/82)	1.00	—	
Ovulation to first GnRH				
Yes	37.4 (122/326)	1.73	1.09, 2.74	0.02
No	23.7 (40/169)	1.00	—	
Ovulation to final GnRH				
Yes	35.4 (152/430)	2.45	1.17, 5.15	0.02
No	15.4 (10/65)	1.00	—	
Double ovulation ³				
Yes	50.0 (29/58)	2.27	1.24, 4.15	0.01
No	33.1 (123/372)	1.00	—	
Heat stress ⁴				
Yes	8.6 (9/105)	0.17	0.08, 0.35	<0.001
No	39.2 (153/390)	1.00	—	

¹AOR = adjusted odds ratio.²CI = confidence interval.³Double ovulation in cows that ovulated to the final GnRH of the Ovsynch protocol.⁴Heat stress = cows inseminated in the weeks when the daily mean temperature-humidity index ≥ 72 .

tis were less likely ($P = 0.002$) to be pregnant (Table 5). Cyclic cows had greater ($P = 0.005$) pregnancy per AI than anovular cows. Similarly, cows that ovulated either to the first or to the final GnRH of the Ovsynch protocol had increased pregnancy per AI than those that did not ovulate to GnRH. Of the cows that ovulated within 48 h of the final GnRH, those experiencing double ovulation were 2.27 times more likely ($P = 0.01$) to become pregnant than single ovulating cows. On the other hand, cows exposed to mean THI ≥ 72 had a lesser pregnancy per AI. Pregnant cows at 28 d after first AI ovulated larger ($P = 0.003$) follicles than nonpregnant cows (19.4 ± 0.3 vs. 18.4 ± 0.2 mm).

Pregnancy loss between 28 and 56 d of gestation was 20.5%. Cows inseminated with a BCS between

2.75 and 3.25 were less likely ($P = 0.04$) to lose the pregnancy than those inseminated with a BCS < 2.75 , but pregnancy loss did not differ between cows with BCS > 3.25 and other categories of BCS (Table 6). In addition, cows that ovulated within 48 h of the final GnRH of the Ovsynch had less ($P = 0.05$) pregnancy loss, whereas cows exposed to heat stress were 7.42 times more likely ($P = 0.007$) to lose their pregnancy than cows not exposed to heat stress at AI.

DISCUSSION

Previous studies demonstrated that Se deficiency was associated with increased incidence of uterine problems such as retained placenta and poor uterine

Table 6. Risk factors for pregnancy loss from gestation d 28 to 56 after first AI

Item	Pregnancy loss, % (n/n)	AOR ¹	95% CI ²	P-value
BCS at AI				
> 3.25	26.9 (7/26)	2.59	0.85, 7.92	0.04
2.75–3.25	15.0 (15/100)	1.00	—	
< 2.75	31.4 (11/35)	3.34	1.27, 8.82	
Ovulation to final GnRH ³				
Yes	18.5 (28/151)	0.25	0.06, 1.00	0.05
No	50.0 (5/10)	1.00	—	
Heat stress ⁴				
Yes	55.6 (5/9)	7.42	1.71, 32.14	0.007
No	18.4 (28/152)	1.00	—	

¹AOR = adjusted odds ratio.²CI = confidence interval.³Ovulation detected within 48 h of the final GnRH injection of the Ovsynch protocol.⁴Heat stress = cows inseminated in the weeks when the daily mean temperature-humidity index ≥ 72 .

involution (Harrison et al., 1986; Trinder et al., 1973), and Se concentrations in blood were associated with improved neutrophil function (Cebra et al., 2003). Although Se status influences uterine health presumably by affecting immune cells, altering the source of Se in the diet of transition cows did not influence the prevalence of subclinical endometritis at 35 d postpartum, resumption of cyclicity, or fertility responses of lactating dairy cows in the current study. A possible explanation for the lack of Se source effects is that cows in both dietary treatments had adequate Se concentrations in blood plasma, and feeding SY did not improve Se status throughout the transition period to ameliorate uterine health and reproductive responses of cows. In fact, GPx activity in plasma and neutrophil function were not altered by source of Se (Rutigliano et al., 2006). Because dietary ingredients had considerable concentrations of Se (Rutigliano et al., 2006), which is mostly in organic forms, and both diets had greater than expected concentrations of Se, cows in both dietary treatments had plasma Se concentrations greater than 0.08 µg/mL throughout the postpartum period indicative that Se statuses were adequate (Dargatz and Ross, 1996). It is possible that diets containing 0.5 mg/kg of Se might mask any beneficial effects of source of Se on reproduction.

When cows were fed sodium selenate or Se-yeast from 60 d prepartum to 30 DIM, immunological responses were not influenced by source of Se (Weiss and Hogan, 2005). On the other hand, replacing SS with SY in the diets containing <0.4 mg/kg of Se improved plasma Se concentrations and reduced the risk for some uterine problems postpartum (Silvestre et al., 2006). Therefore, lack of improvements in reproductive performance when cows were fed different sources of Se likely reflect the inability of Se-yeast to alter immune response and overall periparturient health when diets contained at least 0.4 mg/kg of Se and plasma Se concentrations were above those considered adequate for cattle (Dargatz and Ross, 1996).

Incidence of subclinical endometritis in the present study was less than previously reported. Kasimanickam et al. (2004) observed that approximately 45.1 and 41.4% of the cows at 20 to 33 DIM and at 34 to 47 DIM, respectively, had subclinical endometritis. Differences in the prevalence of subclinical endometritis between herds were observed by Gilbert et al. (2005), and prevalence ranged from 37 to 74% of cows. A possible reason for differences in prevalence may be the distinct techniques used, as it has been demonstrated that the cytobrush technique yields a greater proportion of polymorphonuclear cells compared with the uterine lavage technique when performed between 20 and 33 d after parturition (Kasimanickam et al., 2005).

Nevertheless, subclinical endometritis negatively influenced pregnancy per AI of dairy cows. Others have also shown marked decreases in risk of pregnancy and extended days open in cows diagnosed with subclinical endometritis anytime after 20 d postpartum (Kasimanickam et al., 2004; Gilbert et al., 2005). The exact mechanism by which subclinical endometritis influences establishment of pregnancy is unknown, but the negative effects on fertility might be related to reduced fertilization (Cerri et al., 2006), or possibly presence of an uterine environment that is less conducive for adequate embryo development.

Cows presynchronized with a CIDR insert had a larger ovulatory follicle at the PGF_{2α} and GnRH injections of the Ovsynch and were more likely to ovulate in response to the first GnRH of the protocol. The progesterone from the CIDR insert blocks the LH surge during treatment (Sirois and Fortune, 1990), which is expected to more precisely synchronize estrus and, subsequently, ovulation. Previous studies have demonstrated that addition of a CIDR to an estrous synchronization with PGF_{2α} resulted in an altered pattern of estrous expression, with tighter synchronization compared with PGF_{2α} alone (Chebel et al., 2006). Therefore, the majority of the cyclic cows in the CIDR-PS were in proestrus at the initiation of the Ovsynch protocol, which should favor ovulatory response to GnRH (Vasconcelos et al., 1999; Cerri et al., 2006). Cows in early to mid diestrus and proestrus at initiation of the Ovsynch experience increased incidence of ovulation to a GnRH injection (Vasconcelos et al., 1999).

Lactating dairy cows that did not ovulate to the first GnRH injection of the Ovsynch protocol had ovulatory follicles with an extended period of dominance, compromised embryo quality (Cerri et al., 2005), and reduced pregnancy per AI (Chebel et al., 2006). Therefore, it was expected that the increased incidence of ovulation in response to the first GnRH of the Ovsynch protocol in the CIDR-PS treatment would result in increased pregnancy per AI. Nonetheless, pregnancy per AI did not differ between Presynch and CIDR-PS. It is possible that the lack of difference in pregnancy after first postpartum TAI was a result of ovulation of follicles from different follicular waves in the Presynch and CIDR-PS. Cyclic cows in the Presynch likely ovulated a second-wave follicle at induction of ovulation to the second injection of GnRH as most of them were expected to be on estrous cycle d 6 to 10 when the first GnRH of the Ovsynch was given (Moreira et al., 2001). On the other hand, CIDR-PS cows ovulated a first-wave follicle following the second injection of GnRH of the Ovsynch protocol.

Reduced concentrations of progesterone are associated with increased pulse frequency of LH (Bergfeld et

al., 1996). Because the concentrations of progesterone during the first follicular wave are lower than those during the second or third follicular waves, the concentration of LH during the first follicular wave is greater than in subsequent waves of follicle development (Kulick et al., 2001). Reduced progesterone concentrations should then favor faster follicle growth. In support of this concept, the ovulatory follicle was larger during the Ovsynch protocol in cows receiving the CIDR-PS compared with Presynch, in spite of a greater ovulatory response at initiation of the synchronization program. Furthermore, CIDR-PS cows had increased estradiol concentrations at the injections of PGF_{2α} and GnRH of the Ovsynch, which is associated with reduced concentrations of progesterone and larger follicles. Potential implications are that a first-wave follicle may not offer optimum fertility because it grows under a lower concentration of progesterone and increased pulse frequency of LH, which might compromise the oocyte. Another possibility is that the reduced concentrations of progesterone during Ovsynch in cows receiving CIDR-PS may influence the uterine environment post-AI and subsequent fertility. Recent data from our laboratory indicated that fertility is compromised when cyclic cows received TAI after induction of ovulation of a first-wave follicle (J. E. P. Santos; unpublished results). Studies with lactating dairy cows in New Zealand indicated that 2 injections of PGF_{2α} 13 d apart reduced pregnancy per AI compared with untreated cows particularly when the second PGF_{2α} was given during early (d 5 to 9) diestrus (Xu et al., 1997). In those cows, progesterone from an intravaginal insert reestablished normal fertility. Exposure to progesterone in the cycle preceding AI is important to block premature expression of the luteolytic signal (Zollers et al., 1993). Thus, it is plausible to suggest that synchronization protocols that result in ovulation to the first follicular wave, when the dominant follicle grows under low systemic concentrations of progesterone, may not be optimal for fertility and, in the current study, may have abolished the benefits of greater ovulation to the first GnRH during the Ovsynch protocol.

It is noteworthy that cows receiving CIDR-PS not only had increased incidence of ovulation to the first GnRH, but they also had greater incidence of double ovulation to the final GnRH of the Ovsynch protocol, both factors associated with increased pregnancy per AI. Kulick et al. (2001) demonstrated that codominant follicles occurred more frequently during the first follicular wave than during other waves of follicle growth, and the development of codominant follicles was preceded, on average, by a transient increase in concentrations of LH 24 h before the expected beginning of deviation. This could account for the greater

incidence of double ovulation observed for cows in the CIDR-PS compared with cows receiving Presynch. A greater proportion of multiparous cows had double ovulation, which is in agreement with previous studies that demonstrated that second-lactation cows had 6 to 7% of twin births compared with only about 1% twin births for first-lactation cows (Ryan and Boland, 1991). Because multiparous cows produced more milk than primiparous cows (Rutigliano et al., 2006), and milk yield with an associated increase in DM intake is a major risk factor for multiple ovulation (Wiltbank et al., 2006), it is expected that codominance and multiple ovulation will be more prevalent in multiparous than in primiparous cows.

Regardless of presynchronization treatment, cows that ovulated in response to the first GnRH were more likely to ovulate to the final GnRH of the Ovsynch protocol. This agrees with a previous study that demonstrated that ovulation to the final GnRH of the Ovsynch increased from 79 to 92% if cows ovulated to the initial GnRH injection (Vasconcelos et al., 1999).

The proportion of cyclic cows was less for CIDR-PS than for Presynch. Treatment with a CIDR insert results in inhibition of expression of estrus and ovulation in cows (Sirois and Fortune, 1990; Chebel et al., 2006). Cows in the CIDR-PS group received a CIDR insert for 7 d during the 12-d interval between the 2 ultrasonographic examinations to evaluate cyclicity; therefore, cows in the CIDR-PS that did not have a CL at the first evaluation had only 5 d between the 2 ultrasound examinations to ovulate and be considered cyclic, whereas cows receiving Presynch had 12 d to ovulate, which might explain the difference in proportion of cyclic cows. Another possibility is that cows in the CIDR-PS had 3 fewer days postpartum at ultrasound evaluations, and time postpartum influences resumption of ovulation. Cows at AI with BCS ≥ 2.75 were more likely to be cyclic than those with BCS < 2.75 . This relationship between increased prevalence of cyclic cows with increased BCS has been demonstrated before (Moreira et al., 2001; Chebel et al., 2006). Body condition is correlated with body reserves, in particular body fat (Ferguson et al., 1994), and increased body fatness indicates improved energy reserves that might signal for an earlier resumption of ovarian activity. Cyclic cows were more likely to be pregnant at first AI, which agrees with findings by others (Moreira et al., 2001; Chebel et al., 2006).

Lack of ovulation within 48 h of the last injection of GnRH of the Ovsynch protocol reduced the risk for pregnancy and increased that of pregnancy loss. Saacke et al. (2000) indicated that insemination early after onset of estrus reduced fertilization, which is expected to compromise fertility. Furthermore, lack of ovula-

tion within 48 h is expected to reduce pregnancy per AI particularly if cows fail to ovulate even after 48 h of the GnRH injection. Interestingly, pregnancy losses increased in cows with ovulation after 48 h of the final GnRH of the TAI protocol. It is possible that extended exposure to estradiol and LH during proestrus might have influenced subsequent embryo viability. Also, extended interval between AI and ovulation might have resulted in aged spermatozoa with DNA damage that might compromise subsequent embryo viability. In fact, when cows received AI earlier than 24 h before ovulation, embryo quality decreased compared with cows receiving AI between 12 and 24 h from AI (Roelofs et al., 2006). Therefore, the prolonged period for which the spermatozoa have to reside in the reproductive tract of cows might compromise embryo quality and increase incidence of pregnancy loss in cattle. Also, ovulation after 48 h might have influenced quality of the oocyte because of extended dominance, reducing embryo quality (Cerri et al., 2005; Roelofs et al., 2006).

In addition to ovulatory responses, exposure to heat stress in the week of TAI reduced the risk of pregnancy and increased that of pregnancy loss. Lactating dairy cows suffer from heat stress when the THI is ≥ 72 (Armstrong, 1994), although it is possible that effects on reproduction might be observed at lower values. In the current study, the average mean daily THI during the period of heat stress was always greater than 72 and it averaged 74.7, whereas during the period of thermoneutrality, it averaged 62. High temperatures have deleterious effects on steroidogenesis, oocyte quality, fertilization, and on the embryo at early stages of development (Wolfenson et al., 2000), and these effects ultimately influence establishment and maintenance of pregnancy in dairy cows.

CONCLUSIONS

Source of Se supplemented at 0.3 mg/kg of diet DM, when diets contained approximately 0.4 to 0.5 mg/kg of Se, did not influence uterine health and reproductive performance of dairy cows. Cows presynchronized with a CIDR insert and an injection of PGF_{2a} at insert removal 3 d before initiating the Ovsynch protocol had increased incidence of ovulation to the first GnRH of Ovsynch, larger ovulatory follicles with increased concentrations of estradiol, and a greater incidence of double ovulation to the final GnRH injection before TAI. Although ovulation to the first GnRH and double ovulation to the final GnRH of the Ovsynch protocol increased pregnancy rate, the CIDR-presynchronization treatment had no effect on pregnancy per AI and pregnancy loss compared with cows presynchronized with PGF_{2a} alone.

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