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Effect of source of supplemental selenium on uterine health and embryo quality in high-producing dairy cows

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Abstract

Lactating Holstein cows ($n = 135$) were randomly assigned to one of the two sources of supplemental selenium (Se), sodium selenite (SS) or Se yeast (SY), fed at 0.3 mg/kg diet dry matter from 25 d before calving to 70 d in milk (DIM), in diets not suboptimal in basal Se concentrations. Cows were evaluated for health daily in the first 10 DIM, and uterine cytology of the previously gravid uterine horn was assessed at 30 DIM. The Ovsynch protocol was initiated at 42 DIM; ovarian responses to hormonal treatments were evaluated by ultrasonography. The uteri of cows were flushed 6 d after timed AI for collection of embryos and oocytes. Plasma concentrations of Se and progesterone were measured throughout the postpartum period and during the reproductive protocol, respectively, and plasma glutathione peroxidase activity was determined 6 d after AI. Concentrations of Se in pre- and postpartum diets ranged from 0.43 to 0.56 mg/kg of dry matter. Incidence of retained placenta, fever, ketosis, mastitis, acute puerperal metritis, clinical endometritis, and subclinical endometritis were not significantly different between treatments. There were no differences between groups in concentrations of Se and progesterone or glutathione peroxidase activity in plasma. Treatment did not influence ovarian responses to the synchronization protocol, fertilization rate, number of blastomeres and live blastomeres, or proportions of grades 1 and 2, degenerated, and degenerated-unfertilized embryos/oocytes. Odds of subclinical endometritis on Day 30 postpartum more than doubled in cows with fever of unknown origin or acute puerperal metritis in the first 10 DIM. Fertilization rate tended to be reduced in cows with subclinical endometritis. In summary, replacing SS with an organic source of Se in diets not suboptimal in basal Se concentrations did not improve Se status, uterine health, fertilization, or embryo quality in early lactation dairy cows.

Keywords: Dairy cow; Embryo; Glutathione peroxidase; Selenium; Uterus

1. Introduction

Selenium is present in several selenoproteins that participate in the antioxidant defense system of cells. The enzyme glutathione peroxidase (GSH-Px) has Se as an integral element of its structure and it is essential for the enzyme's catalytic activity. The GSH-Px is present in both the intra- and extracellular compartments and it is critical for reducing hydrogen peroxide and organic hydroperoxides to water [1], thereby minimizing cellular damage. Increased concentrations of Se in plasma or whole blood have been associated with a reduced incidence of retained placenta [2], metritis [3], mastitis [4], and improved neutrophil function [5]. Plasma Se concentration also had a strong positive correlation with GSH-Px activity [6]; therefore, strategies that increase plasma concentrations of Se have the potential to

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improve the efficiency of the cell antioxidant system, thereby impacting disease resistance and fertility.

Organic Se in the form of Se yeast increased plasma and milk concentrations of Se compared with an inorganic source [7], which indicates improved Se absorption and retention. Few studies, however, have been conducted to determine whether an increase in systemic concentrations of Se could modulate GSH-Px activity, with effects in the prevalence of early postpartum diseases, uterine health, oocyte fertilization, and embryo quality in lactating dairy cows. Reactive oxygen species are formed as a byproduct of the normal cell metabolism and, when in excess, they can impair fertilization and early embryonic development. Bovine embryos in the 4- to 6-cell stage cultured *in vitro* under a high oxygen tension or in the presence of a free radical generator [8] were less likely to develop to the blastocyst stage. However, there was an improvement in the percentage of live cells, blastocyst formation and hatched blastocysts in murine embryos under heat stress that were cultured with an inducer of gluthatione synthesis [9]. This benefit of glutathione to blastocysts was possibly caused by improved removal of free radicals generated during heat shock [10]. Uhm et al. [11] demonstrated that *in vitro* produced porcine partenotes cultured in media containing Se had increased development to blastocyst, cell number and GSH-Px expression. Furthermore, they also reported that Se reduced the number of apoptotic cells, caspase 3 activity, and expression of apoptotic genes, suggesting that Se is pivotal to prevent oxidative damage and apoptosis in the early stages of embryo development.

The hypotheses were that an organic source of dietary Se, in the form of Se yeast during late gestation and early lactation, could increase the concentrations of Se and GSH-Px activity in plasma of Holstein cows, with concurrent improvements in uterine and systemic health. It was also hypothesized that improving concentration of Se in plasma by feeding Se yeast would enhance fertilization and embryo quality, possibly related to removal of free radicals through increased GSH-Px activity. The objectives were, therefore, to determine the effects of two sources of supplemental Se (organic versus inorganic) during the transition period on concentrations of Se and GSH-Px activity in plasma, incidence of postpartum disorders, uterine health, fertilization, and early embryo development.

2. Materials and methods

The University of California at Davis (Davis, CA, USA) Institutional Animal Care and Use Committee approved all procedures involving cows in this study.

2.1. Cows and housing

One hundred and thirty-five lactating Holstein dairy cows were used in this study. The 3.5% fat-corrected milk rolling herd average during the year of the study was 11,630 kg. Primiparous and multiparous cows were housed together prepartum in open lots with shade, headlocks, and waterers. Postpartum, primiparous and multiparous cows were also housed together in a free stall barn equipped with fans and sprinklers, which were automatically activated once the temperature reached 26.7 °C.

2.2. Treatments

All cows were fed the same diet, except for the source of supplemental Se, as a total mixed ration, once daily during the prepartum period and twice daily postpartum, to meet or exceed the dietary requirements [12] for a non-lactating cow of 670 kg, consuming 12 kg of dry matter, and carrying a last trimester pregnancy during the prepartum period, and for a lactating cow weighing 630 kg, consuming 24 kg of dry matter, and producing 45 kg/d of milk containing 3.5% fat and 3.1% true protein in the first 70 DIM during the postpartum period. Diets were formulated using the CPM-Dairy cattle ration analyzer (Cornell-Penn-Miner Ver. 3.0.8; Miner Institute, Chazy, NY, USA). Ingredient composition of diets is published elsewhere [13]. Description of the nutrient composition of the total mixed ration is shown (Table 1).

Treatments consisted of sodium selenite (SS; $n = 70$) or Se yeast (SY; Sel-Plex, Alltech Biotechnology Inc., Lexington, KY, USA; $n = 65$). These two supplemental sources of Se were fed at 0.3 mg/kg, from 25 d before expected calving to 70 DIM, in order to achieve intakes of 3.6 and 7.2 mg/cow/d during the pre- and postpartum periods, respectively. Pre- and postpartum diets were sampled weekly from the manger in each of the study pens and then composited for 2-mo periods. Weekly, 0.5 kg of ration was sampled and dried at 55 °C for 48 h in an air circulating oven and moisture loss recorded. Dried samples were then ground to pass a 1 mm screen. Samples were then composited for 2-mo periods and analyzed for their contents of dry matter at 105 °C, organic matter by ashing, fat by ether extraction, fiber by digestion with neutral detergent, nitrogen (N) using a N analyzer (FP-528 Nitrogen Determinator, LECO Corporation, St. Joseph, MI, USA), with crude protein calculated as N percentage multiplied by 6.25. Concentration of Se in rations was analyzed according to the method of vapor generation inductively coupled

Table 1
Mean (± SD) chemical composition of diets fed to dairy cows.

	Treatment[a]			
	SS		SY	
	Prepartum	Postpartum	Prepartum	Postpartum
Dry matter (%)	47.3 ± 2.3	51.8 ± 3.7	48.3 ± 3.7	51.0 ± 4.2
		Dry matter basis		
NE_L[b] (Mcal/kg)	1.60	1.66	1.60	1.66
Organic matter (%)	91.0 ± 0.4	90.0 ± 0.1	91.0 ± 0.6	90.0 ± 0.6
Crude protein (%)	17.5 ± 0.4	20.8 ± 1.0	17.8 ± 0.2	21.2 ± 0.9
Neutral detergent fiber (%)	37.6 ± 2.4	37.8 ± 3.4	35.0 ± 6.2	38.8 ± 3.6
Fat (%)	3.5 ± 0.3	5.3 ± 0.2	4.5 ± 0.6	5.1 ± 0.6
Ca (%)	1.13 ± 0.09	1.01 ± 0.03	1.05 ± 0.08	0.99 ± 0.02
P (%)	0.36 ± 0.02	0.45 ± 0.01	0.36 ± 0.03	0.44 ± 0.03
Mg (%)	0.41 ± 0.04	0.41 ± 0.02	0.40 ± 0.02	0.41 ± 0.02
Se (mg/kg)	0.44 ± 0.12	0.43 ± 0.07	0.56 ± 0.09	0.54 ± 0.11

[a] SS = sodium selenite; SY = selenium yeast.
[b] NE_L = net energy for lactation, calculated according to NRC [12].

plasma emission spectrometer [14], with a minimum detection of 0.05 mg Se/kg. Other minerals were analyzed at the Dairyland Laboratory (Arcadia, WI, USA), using an inductively coupled plasma emission spectrometer (Thermo Garrell Ash, Franklin, MA, USA).

2.3. Body condition score

The body condition score (BCS) [15] of all cows was determined by the same person on Days −25, 0, and 58 relative to calving. Cows were classified according to the BCS measured at 58 ± 3 DIM as low if BCS ≤2.75 or moderate if BCS >2.75. The impact of treatment on BCS, as well the effect of BCS on the outcomes evaluated, was analyzed.

2.4. Monitoring early postpartum cows

Cows were observed daily by the research team for signs of diseases during the postpartum period. Episodes of retained placenta, acute puerperal metritis, fever, clinical ketosis, and mastitis were recorded. Retained placenta was characterized by presence of fetal membranes 24 h after calving. Rectal temperature was measured daily, during the first 10 d postpartum and episodes of fever were defined as rectal temperature ≥39.5 °C without any signs of other diseases. In cows with a fever, the uterus was assessed by transrectal palpation. When fever was accompanied by an enlarged flaccid uterus containing fetid material detected as vaginal discharge of uterine origin, cows were then classified as having puerperal metritis. Clinical ketosis was defined by the lack of appetite and presence of ketonuria using test strips (Ketostix, Bayer Diagnostics, Tarrytown, NY, USA). Clinical mastitis was the only disease evaluated by herd personnel; a clinical case was described by either the presence of abnormal milk or by signs of inflammation in one or more quarters, or by both. Cows with abnormal behavior, anorectic, weak or with rectal temperature ≥39.5 °C were examined to determine the origin of the abnormality and treated according to the protocol established by the herd veterinarian. Cows with retained placenta received no treatment. Cows with fever accompanied or not by metritis received 2.2 mg/kg of ceftiofur hydrochloride (Excenel RTU EZ sterile suspension; Pfizer Animal Health, New York, NY, USA) daily for 5 d. Cows with ketosis received 500 mL IV of a solution of 50% dextrose and 300 mL of propylene glycol orally once daily for 3 d. Cows with clinical mastitis were treated with an intramammary infusion of 200 mg of cephapirin sodium (Today; Fort Dodge Animal Health, Fort Dodge, IA, USA) in the first two milkings after the diagnosis. Cows were evaluated 24 h after the second intramammary infusion of cephapirin and those with mastitis that had not receded received treatment with 50 mg of pirlimycin hydrochloride (Pirsue Sterile Solution; Pfizer Animal Health) once daily for 2 d.

2.5. Evaluation of uterine health

Cytological examination of uterine fluid after flushing with saline was performed at 30 ± 3 DIM. The vulva was cleaned with water and soap and then sprayed with a 70% alcohol solution and dried off with a

paper towel. A silicon Foley catheter (18 French, 30 mL, 56 cm; Minitube of America, Verona, WI, USA) was introduced through the cervix into the previously gravid uterine horn after the cow received an epidural anesthesia with 4 mL of 2% lidocaine (Lidocaine HCl with epinephrine injection; Watson Laboratories, Inc., Corona, CA, USA). The balloon cuff was placed approximately 3 cm past the intercornual ligament and inflated with air to a volume consistent with the diameter of the uterine horn. Exactly 20 mL of sterile saline was infused into the uterine horn and aspirated back using a syringe with a connecting tube. 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 μL was pipetted onto a glass slide and smeared into two slides per flush. Smears were air dried and stained using a Romanowsky stain (Diff-Quick; Fisher Diagnostics, Middletown, VA, USA). Slides were examined under a microscope and number of total leukocytes (mononuclear and polymorphonuclear neutrophils) and epithelial endometrial cells were counted to complete 100 cells per slide. Subclinical endometritis was defined when the proportion of neutrophils was ≥18% in relation to the total number of endometrial cells and leukocytes [16]. Clinical endometritis was characterized when a mucopurulent or purulent flush was recovered from the uterus.

2.6. Concentrations of Se and progesterone, and activity of GSH-Px in blood plasma

Approximately 7 mL of blood was sampled from the coccygeal vein or artery into evacuated tubes containing 17.55 mg of K_2 EDTA (Vacutainer®, Becton Dickinson, Franklin Lakes, NJ, USA). Plasma was harvested after blood was centrifuged at 3000 × *g* for 15 min in a refrigerated centrifuge at 5 °C. Plasma was then stored at −25 °C until further analyses.

Concentration of Se was measured in plasma harvested from blood collected on Days 0, 21, 42 and 63 postpartum using a fluorometric method [17]. The activity of GHS-Px was determined in plasma at 58 ± 3 DIM, the day of uterine flush for embryo–oocyte collection. Activity was measured using a commercially available GSH-Px assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). The GSH-Px activity was expressed in nmol/min/mL which is the amount of enzyme necessary to oxidize 1.0 nmol of NADPH to $NADP^+$ per minute per milliliter of plasma at 25 °C.

Blood samples collected on Days 49, 51, and 58 postpartum were used for analysis of concentrations of progesterone in plasma. These days coincided with the days of $PGF_{2\alpha}$ of the Ovsynch, final GnRH of Ovsynch, and uterine flush, respectively. Plasma samples were analyzed in duplicates for concentrations of progesterone by a validated ELISA [18]. The intra- and interassay CV were, respectively, 6.4% and 9.2%. Individual samples or microplates with a CV >15% were reanalyzed.

2.7. Ovulation synchronization protocol and artificial insemination (AI)

Cows in both treatments were subjected to the same reproductive protocol for synchronization of ovulation (Fig. 1). The estrous cycle was presynchronized starting at 33 ± 3 DIM, with a protocol consisting of 100 μg of GnRH im (gonadorelin diacetate tetrahydrate, Cystorelin; Merial Ltd., Iselin, NJ, USA) and placement of a controlled internal drug-releasing (CIDR; EAZI Breed, Pfizer Animal Health) containing 1.38 g of progesterone. Seven days later, 25 mg of $PGF_{2\alpha}$ (dinoprost tromethamine, Lutalyse Sterile Solution; Pfizer Animal Health) was given concomitantly with the removal of the CIDR insert. The Ovsynch protocol started 2 d after the injection of $PGF_{2\alpha}$ and removal of the CIDR of the presynchronization. In the Ovsynch, cows received 100 μg of GnRH followed by 25 mg of $PGF_{2\alpha}$ 7 d later. A final injection of GnRH was given 48 h after the $PGF_{2\alpha}$, and fixed-time AI was performed 12 h later. The same person inseminated all cows with semen from a single sire of proven fertility (based on pregnancy in

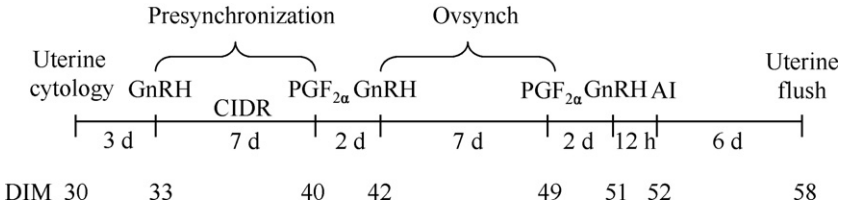

Fig. 1. Diagram of the reproductive protocol in dairy cows. AI = artificial insemination; CIDR = controlled internal drug-releasing containing 1.38 g of progesterone; DIM = days in milk.

lactating cows from several thousand inseminations on commercial farms).

2.8. Ultrasound evaluation of the ovarian follicles and corpus luteum

Ovaries were examined with ultrasongraphy (7.5 MHz transrectal linear-array transducer; Sonovet 2000; Alliance Medical, Bedford Hills, NY, USA) for determination of cyclic status before initiation of Ovsynch. Ultrasonography was used to measure the diameter of ovarian follicles and corpus luteum (CL), and ovulatory responses to hormonal treatments. Maps of the ovaries were drawn for each individual cow, and size and position of follicles $\geq$5 mm in diameter and CL were recorded. Ultrasonographic images were taken concurrent with the injections of GnRH and $PGF_{2\alpha}$ of the presynchronization for determination the cyclic status. Cows were considered cyclic if a CL was observed in at least one of the two ultrasound examinations, and they were classified as anovular if no CL was detected in both examinations. Ovaries were scanned concurrent with hormonal treatments of the Ovsynch protocol and again 48 h after each injection of GnRH to determine size and location of ovarian follicles and CL, and if ovulation occurred. An additional ultrasound scanning 6 d after AI evaluated the diameter of the CL. Occurrence of ovulation within 48 h after each GnRH treatment was characterized by the disappearance of a previously recorded follicle $\geq$10 mm in diameter.

2.9. Embryo–oocyte collection and evaluation

Cows were flushed on Day 6 after AI by a transcervical procedure using a silicon Foley catheter (18 French, 30 mL, 56 cm; Minitube of America, Verona, WI, USA). The balloon cuff of the catheter was placed approximately 3 cm past the intercornual ligament of the uterine horn ipsilateral to the CL and inflated with air to a volume consistent with the diameter of the uterine horn. Approximately 300 mL of a flushing solution (ViGro® complete flush solution; Bioniche Life Sciences Inc., Belleville, ON, Canada) was used in the uterine horn in 20 cycles of infusion/recovery of 15 mL each. For cows with CL in both ovaries, both horns were flushed as described previously. Recovered embryos–oocytes were evaluated for fertilization and grade quality (1 = excellent and good, 2 = fair, 3 = poor, and 4 = degenerated) according to the guidelines by the International Embryo Transfer Society [19]. Embryos were stained with 5 μg/mL propidium iodide (Sigma, St. Louis, MO, USA) to determine the number of non-viable blastomeres and then with 5 μg/mL Hoechst 33342 (Molecular Probes Inc., Eugene, OR, USA) to determine the number of accessory spermatozoa using epifluorescence microscopy (365 nm excitation, >400 nm emission). The zona pellucida was then dissolved with 0.02 N HCl in 0.1% Tween-20 (Sigma). The embryo was again stained with 5 μg/mL Hoechst 33342 and the blastomeres spread in a glass slide and counted using epifluorescence microscopy.

2.10. Experimental design and statistical analyses

The experimental design was a randomized block design. Cows were blocked according to parity (1st vs. >1st lactation) and BCS at enrollment and, within each block, randomly assigned to one of the two treatments.

Binary data were analyzed by logistic regression utilizing the LOGISTIC procedure of the SAS program (SAS/STAT, SAS Inst., Inc., Cary, NC, USA) and all the models included the effects of treatment, parity, cyclic status and BCS at 58 DIM. Count data such as number of accessory spermatozoa and number of blastomeres were analyzed by the GENMOD procedure using a Poisson distribution correcting for over-dispersion of the data with the SAS program. The model included the effects of treatment, parity, cyclic status and BCS at 58 DIM.

Continuous data with a single measurement per cow were analyzed by ANOVA using the GLM procedure of the SAS program. The models included the effects of treatment, parity, cyclic status, and BCS at 58 DIM. Progesterone and Se concentrations were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS. The covariance structure with the smallest Akaike's information criterion was selected. The models included the fixed effects of treatment, day of measurement, and interaction between treatment and day of measurement, and the random effect of cow nested within treatment.

Treatment differences with $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ were designated as a tendency.

3. Results

The number of days (mean ± SEM) the cows remained in the prepartum diets did not differ between treatments and averaged 23.7 ± 0.6 and 24.9 ± 0.6 for the SS and SY, respectively. Similarly the mean (± SD)

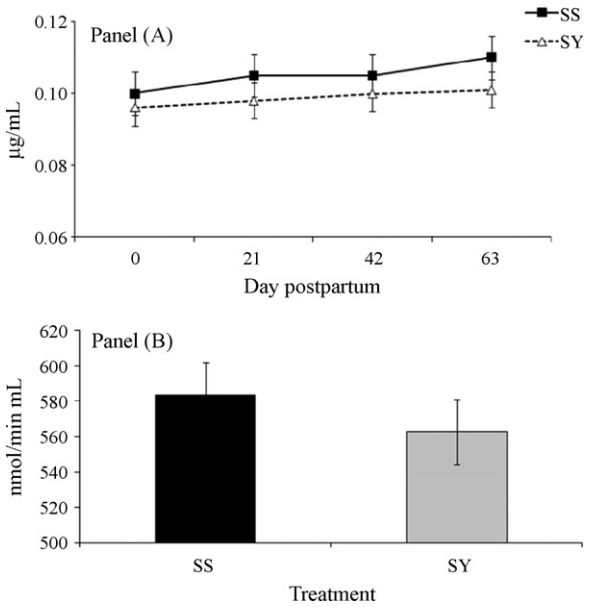

Fig. 2. Concentrations of Se in plasma during the postpartum period (Panel A) and glutathione peroxidase (GSH-Px; Panel B) activity in plasma on Day 58 ± 3 postpartum in dairy cows. There were no effects of treatment ($P = 0.38$), day postpartum ($P = 0.19$), and interaction between treatment and day postpartum ($P = 0.93$) on concentrations of Se in plasma. The GSH-Px activity was not different ($P = 0.42$) between treatments. The values are LSM ± SEM. SS = sodium selenite ($n = 70$); SY = Se yeast ($n = 65$).

and median lactation number did not differ between diets and were 2.4 ± 1.4 and 2.0, respectively. The chemical compositions of the pre- and postpartum diets are in Table 1.

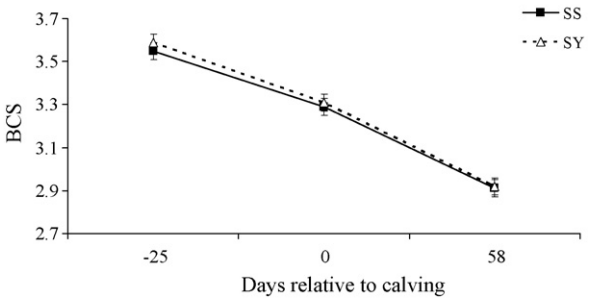

Fig. 3. Body condition score (BCS) of cows fed sodium selenite (SS; $n = 70$) or Se yeast (SY; $n = 65$). The BCS did not differ ($P > 0.50$) between treatments. Cows lost ($P < 0.001$) BCS during the study, with no interaction ($P = 0.93$) between treatment and day. The values are LSM ± SEM.

3.1. Concentration of Se and GSH-Px activity in plasma and body condition score

The mean concentration of Se in plasma (Fig. 2, Panel A) was not different between treatments and averaged (±SEM) 0.104 ± 0.005 µg/mL. Similarly, the GSH-Px activity in plasma collected at the day of embryo–oocyte collection did not differ between treatments (Fig. 2, Panel B) and averaged 572.3 ± 18.5 nmol/min/mL.

The BCS of cows (Fig. 3) did not differ between treatments throughout the study. Cows lost ($P < 0.001$) BCS from −25 d to 58 d relative to calving, with no interaction between treatment and day postpartum. Multiparous cows had a smaller ($P < 0.001$) BCS than primiparous cows and they averaged 3.14 ± 0.02 and 3.38 ± 0.02, respectively.

Table 2
Effect of selenium treatment on incidence of postpartum diseases in dairy cows.

	Treatment[a] (% (no./no.))		AOR[b]	95% CI[c]	*P*
	SS	SY			
Retained placenta[d]	10.0 (7/70)	3.0 (2/65)	0.2	0.1–1.2	0.12
Fever[e]	25.7 (18/70)	16.9 (11/65)	0.5	0.2–1.4	0.21
Acute puerperal metritis[f]	25.7 (18/70)	16.9 (11/65)	0.6	0.2–1.4	0.48
Clinical endometritis[g]	13.6 (9/66)	9.4 (6/64)	0.8	0.3–2.2	0.66
Subclinical endometritis[h]	40.9 (27/66)	43.7 (28/64)	1.2	0.6–2.5	0.44
Ketosis[i]	12.8 (9/70)	12.3 (8/65)	1.0	0.3–2.9	0.98
Mastitis[j]	35.7 (25/70)	26.1 (17/65)	0.6	0.3–1.3	0.18

[a] SS = sodium selenite; SY = selenium yeast.
[b] AOR = adjusted odds ratio (the SS treatment was the reference for comparison).
[c] CI = confidence interval.
[d] Placenta retained >24 h after calving.
[e] Rectal temperature ≥39.5 °C without clinical signs of other diseases.
[f] Fetid vaginal discharge of uterine origin concurrent with fever.
[g] Mucopurulent or purulent uterine flush.
[h] Uterine cytology ≥18% of cells as neutrophils [16].
[i] Lack of appetite and ketonuria using test strips.
[j] Abnormal milk or signs of inflammation in one or more mammary glands.

Table 3
Factors associated with increased risk for subclinical endometritis in dairy cows.

	Subclinical endometritis (% (no./no.))	AOR[a]	95% CI[b]	*P*
Fever[c]				
Yes	57.1 (16/28)	2.4	1.1–5.5	0.03
No	38.2 (39/102)	Referent		
Acute puerperal metritis[d]				
Yes	53.9 (14/26)	2.1	0.9–5.1	0.10
No	39.4 (41/104)	Referent		

[a] AOR = adjusted odds ratio.
[b] CI = confidence interval.
[c] Rectal temperature ≥39.5 °C without clinical signs of other diseases.
[d] Fetid vaginal discharge of uterine origin concurrent with fever.

3.2. Postpartum diseases

The proportion of cows observed with retained placenta, fever not associated with puerperal metritis during the first 10 DIM, acute puerperal metritis, ketosis, and mastitis did not differ between treatments (Table 2). Parity, however, influenced the risk of diseases; multiparous cows tended ($P = 0.10$) to have increased prevalence of retained placenta (9.8% vs. 1.9%) and also to be more affected ($P = 0.04$) by clinical mastitis (37.8% vs. 20.7%) compared with primiparous cows. However, multiparous cows were less susceptible ($P < 0.01$) to develop acute puerperal metritis (15.8% vs. 30.2%) and clinical ketosis (4.9% vs. 24.5%) than primiparous cows. There was no difference between multiparous and primiparous cows for incidence of fever not associated with puerperal metritis.

3.3. Clinical and subclinical endometritis

Prevalence of clinical and subclinical endometritis at 30 DIM did not differ between treatments (Table 2). Subclinical endometritis was not different between multiparous (39.2%) and primiparous cows (47.0%). Retained placenta, clinical ketosis and mastitis did not influence the prevalence of subclinical endometritis, but cows with fever of undiagnosed cause in the first 10 DIM had 2.4 times greater odds of developing subclinical endometritis than cows without fever (Table 3). Similarly, acute puerperal metritis tended ($P = 0.10$) to increase the prevalence of subclinical endometritis at 30 DIM.

3.4. Ovarian structures and responses to the synchronization protocol, and plasma progesterone concentration

Proportion of cyclic cows before initiation of the Ovsynch protocol (Table 4) did not differ between treatments (average, 82.1%). Multiparous cows, however, were more likely ($P = 0.04$) to be cyclic than primiparous cows (87.6% vs. 73.5%). Ovulation to the first and second GnRH injections, as well as luteolysis after administration of $PGF_{2\alpha}$ during the Ovsynch

Table 4
Effect of selenium treatment on ovarian responses of dairy cows to the Ovsynch protocol.

	Treatment[a] (% (no./no.))		AOR[b]	95% CI[c]	*P*
	SS	SY			
Cyclic cows	85.5 (59/69)	78.5 (51/65)	0.6	0.2–1.5	0.26
Ovulation to 1st GnRH	80.9 (55/68)	85.5 (53/62)	1.6	0.6–4.2	0.46
Corpus luteum regression	86.8 (59/68)	90.5 (57/63)	1.2	0.4–3.7	0.74
Ovulation to 2nd GnRH	89.5 (60/67)	93.6 (59/63)	1.7	0.5–6.3	0.82
Double ovulation to 2nd GnRH					
Ovulatory cows	13.3 (8/60)	11.9 (7/59)	0.7	0.2–2.5	0.68
All cows	11.9 (8/67)	11.1 (7/63)	0.8	0.3–2.4	0.79
Synchronization[d]	83.6 (56/67)	87.3 (55/63)	1.3	0.5–3.5	0.27
Diameter (mm)					
Dominant follicle at AI[e]	18.6 ± 0.4	18.2 ± 0.4	–	–	0.49
Corpus luteum 6 d after AI	21.2 ± 0.4	21.7 ± 0.4	–	–	0.76

[a] SS = sodium selenite; SY = selenium yeast.
[b] AOR = adjusted odds ratio (the SS treatment was the reference for comparison).
[c] CI = confidence interval.
[d] Cows with corpus luteum regression after $PGF_{2\alpha}$ and ovulation within 48 after the final GnRH.
[e] AI = artificial insemination.

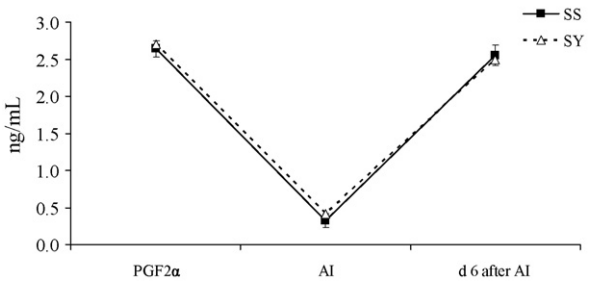

Fig. 4. Plasma concentrations of progesterone in dairy cows. There was no effect ($P = 0.75$) of treatment. Day influenced ($P < 0.001$) progesterone concentrations, but no interaction ($P = 0.66$) between treatment and day was detected. The values are LSM ± SEM. AI = artificial insemination; $PGF_{2\alpha}$ = injection of $PGF_{2\alpha}$ in the Ovsynch protocol; SS = sodium selenite ($n = 70$); SY = Se yeast ($n = 65$).

protocol, were not different between SS and SY. Synchronization of ovulation, which included CL regression and ovulation within 48 h after the second GnRH of the Ovsynch protocol, did not differ between treatments. Proportion of cows with double ovulation was not different between treatments, both when considering all cows or only cows that ovulated to the second GnRH of the Ovsynch. Multiparous cows tended ($P < 0.10$) to have more double ovulation after the second GnRH of the Ovsynch than primiparous cows when considering all cows (15.6% vs. 5.7%) or only ovulatory cows (17.4% vs. 6.0%). Diameter of the ovulatory follicle on the day of AI averaged (± SEM) 18.4 ± 0.4 mm and that of the CL on Day 6 after AI averaged 21.4 ± 0.5 mm; neither differed between treatments. Concentrations of progesterone throughout the synchronization protocol and on Day 6 after AI were not different between treatments (Fig. 4).

3.5. Embryo–oocyte evaluation

Following uterine flush on Day 6 after AI, proportion of recovered embryos–oocytes did not differ between treatments (average, 63.9%; Table 5). Of the recovered embryos–oocytes, fertilization averaged 78.8% and it was not affected by source of dietary Se. Cows previously diagnosed with subclinical endometritis tended ($P = 0.10$) to have less fertilization compared with cows not diagnosed with subclinical endometritis (70.1% vs. 83.7%). Relative to embryo–oocyte, the proportion of embryos graded as 1 and 2, as well as the proportion of degenerated embryos were not influenced by treatment. Source of Se had no impact on the proportion of cows with degenerated embryos and unfertilized oocytes. In relation to embryos only, treatment did not influence any of the outcomes evaluated. The mean and median numbers and the proportion of live blastomeres did not differ between

Table 5
Effect of selenium treatment on recovery, fertilization and embryo quality of embryos/oocytes collected from dairy cows.

	Treatment[a] (% (no./no.))		AOR[b]	95% CI[c]	*P*
	SS	SY			
Recovery	65.7 (44/67)	62.1 (41/66)	0.8	0.4–1.6	0.50
Embryo–oocyte					
Fertilization	84.1 (37/44)	73.2 (30/41)	0.4	0.1–1.3	0.17
Grades 1 and 2	50.0 (22/44)	53.6 (22/41)	1.2	0.5–3.0	0.70
Degenerated	18.2 (8/44)	9.8 (4/41)	0.4	0.1–1.8	0.27
Degenerated-unfertilized	36.4 (16/44)	36.6 (15/41)	1.2	0.4–3.1	0.76
Embryo					
Grades 1 and 2	59.5 (22/37)	73.3 (22/30)	2.5	0.8–7.8	0.23
Degenerated	21.6 (8/37)	13.3 (4/30)	0.5	0.1–2.4	0.40
Blastomeres					
Mean (*n*)	40.6 ± 3.0	43.7 ± 3.6	–	–	0.49
Median (*n*)	45	49	–	–	0.39
Live (%)	97.7 ± 0.8	98.5 ± 0.9	–	–	0.46
Accessory spermatozoa					
Mean (*n*)	36.1 ± 7.4	16.5 ± 7.8	–	–	0.07
Median (*n*)	14.5	5.0	–	–	0.10
Embryo–oocyte with ≥1	88.6 (39/44)	78.0 (32/41)	0.4	0.1–1.2	0.10

[a] SS = sodium selenite; SY = selenium yeast.
[b] AOR = adjusted odds ratio (the SS treatment was the reference for comparison).
[c] CI = confidence interval.

treatments. The mean and median numbers of accessory spermatozoa and embryos–oocytes with at least one accessory spermatozoon tended ($P < 0.10$) to be greater for cows fed SS than SY.

4. Discussion

The objectives of the present study were to determine if source of supplemental Se fed from late gestation to early lactation would influence Se status and, consequently, impact uterine health, fertilization, and early embryo development. The transition period is unique; it is the time during lactation when cows are most susceptible to diseases that influence survival, production and reproduction. It is known that the increased risk for certain diseases is related to immunosuppression in the peripartum, which is in part linked with increased nutrient needs for lactation and inadequate intake of nutrients. Parturition and associated uterine disorders are known as pro-inflammatory processes [20], and inflammation might result in increased oxidative stress in tissues with subsequent impacts on reproduction.

Despite some indications that Se yeast was more bioavailable than inorganic sources of Se in dairy cattle [7,21], replacing SS with SY in the current study did not improve concentrations of Se and activity of GSH-Px in plasma. The lack of effects of source of dietary Se on Se status apparently accounted for the inability of treatment to affect uterine health, fertilization, and embryo quality in high-producing Holstein dairy cows.

The supplementation of Se at 0.3 mg/kg is the typical and maximum legal amount added to complete diets of cattle in the United States, whereas in the European Union, cattle can be fed diets with a maximum of 0.57 mg/kg of total dietary Se (dry matter basis). Although diets were supplemented with 0.3 mg of Se/kg, the concentrations of Se in total mixed rations ranged from 0.4 to 0.5 mg/kg, because of the somewhat high basal concentrations of Se in the diets [22]. Typically, Se contained in forages and grains is mostly in the organic form. When cows were fed the same diets as in the current experiment, concentrations of Se and GSH-Px activity in plasma, and neutrophil phagocytic and killing activities remained, unaltered regardless of source of Se [22]. Conversely, when transition cows fed diets with basal ingredients marginal in Se, supplementation with Se yeast improved plasma Se concentrations and neutrophil function [21].

It is noteworthy that Se content of the diets in the current study was greater than that observed in other areas of the USA [21], which might have masked some of the potential benefits to health and fertility. Concentrations of Se in soil and feedstuffs vary among regions, and more than 50% of the feedstuffs grown in California contain Se concentrations >0.1 mg/kg [23]. In contrast, in the southeastern states of the USA, more than 42% of beef cattle were marginally or severely deficient in Se, whereas more than 90% of cattle in western states had whole blood Se concentrations >0.08 μg/mL [24]. Throughout the study, concentrations of Se in plasma of cows in both treatments were >0.08 μg/mL, a concentration considered adequate for cattle [24]. Therefore, diets containing 0.45–0.50 mg of Se/kg of dry matter can probably maintain an adequate concentration of Se in plasma, despite the dietary supplemental source of Se fed. In fact, when positive responses to organic Se supplementation were observed in dairy cattle, either the total dietary Se was <0.50 mg/kg of diet dry matter, or the background dietary concentration of Se in feedstuffs were low, generally <0.1 mg/kg [7,21].

Weiss et al. [25] observed that Se intake up to 5 mg/d was positively correlated with concentrations of Se in plasma, but no change was observed when Se intake was increased beyond 5 mg/d. Cows in the present study ingested more than 5 mg/d of Se in the postpartum period, therefore, limiting or potentially negating a possible benefit from SY on concentrations of Se in plasma and GSH-Px activity. In other reports [7,21], there was an increase in concentrations of Se in plasma when replacing an inorganic source of supplemental Se with Se yeast. In those studies [7,21], the final diet contained less than 0.4 mg of Se/kg of dry matter, which supported the observation that supplementation with an organic source of Se might not be effective in diets containing more than 0.4 mg/kg.

Prevalence of common postpartum diseases was not altered by source of Se supplementation, as initially hypothesized. However, there was a major effect of parity on the prevalence of diseases, as multiparous cows had a greater risk of mastitis, but were less likely to be affected by acute puerperal metritis and ketosis than primiparous cows. Multiparous cows are known to be more susceptible to mastitis than primiparous cows [26], in part because of increased teat end lesions and increased risk of clinical cases in cows previously diagnosed with mastitis. Conversely, primiparous cows were more likely to have puerperal metritis compared with multiparous cows, which could be caused by the greater difficulty to deliver the calf that commonly affects cows of smaller frame and weight [27]. The increased risk of ketosis in primiparous cows is likely a combination of the greater incidence of puerperal metritis, associated with increased requirements for

growth than multiparous cows. Cows diagnosed with puerperal metritis in the present study had 3.1 times the odds ($P = 0.04$) to develop ketosis than cows without puerperal metritis.

The prevalence of subclinical endometritis was 42.3% at 30 DIM, which is within the range observed by Gilbert et al. [28], although more prevalent than that reported by Kasimanickam et al. [16] and Rutigliano et al. [13]. Source of Se did not affect the prevalence of subclinical endometritis, but other risk factors such as fever of undiagnosed origin during the first 10 DIM and acute puerperal metritis were positively associated with prevalence of subclinical endometritis. In a study with larger number of cows, Rutigliano et al. [13] also reported that source of Se did not influence the risk of subclinical endometritis, but both retained placenta and puerperal metritis doubled the odds of a cow to be diagnosed with subclinical uterine disease. Collectively, these results demonstrated a carryover effect from postpartum fever and clinical uterine disorders that occurred in the first 10 DIM on subsequent health of the uterus of lactating dairy cows at 30 DIM, despite treatment with systemic antibiotics. Although there was no effect of treatment on fertilization and embryo quality, there was a negative effect of subclinical endometritis on fertilization. Cows with no apparent clinical uterine disease, but diagnosed with subclinical endometritis, had reduced oocyte fertilization, which reinforced the concept that sub-optimal uterine health influences fertility of dairy cows. It is known that subclinical endometritis has a deleterious effect on pregnancy per AI or interval from calving to pregnancy in lactating dairy cows [13,16,28]. Therefore, subclinical inflammation of the uterus not only impairs embryo development [29], but it also reduces oocyte fertilization.

Progesterone concentrations and ovarian responses to the synchronization protocol did not differ between treatments. Incidence of ovulation after GnRH injections, proportion of double ovulation, CL regression, follicle diameter at AI, and CL diameter on the day of embryo–oocyte collection were all unaffected by source of dietary Se. Similar progesterone concentrations and ovarian responses to the synchronization protocol between treatments were pivotal to ensure no bias and a proper evaluation of the effects of source of dietary Se on fertilization and embryo quality. It was expected that feeding SY would improve GSH-Px activity, which could improve the cellular mechanism of the embryo to scavenge free radicals. The presence of excessive reactive oxygen species negatively affects sperm capacitation and further acrosome reaction [30], blastocyst formation [8], and increases apoptosis in early embryos [11]. These effects of oxidative stress suggested a potentially positive impact of a more efficient antioxidant system on fertilization and early embryonic development. However, the inability of SY to alter concentrations of Se and activity of GSH-Px in plasma likely precluded any effect of treatment on the reproductive tissues. In agreement with the present study, Rutigliano et al. [13,22] observed that lactating cows supplemented with 0.3 mg/kg of Se either as SS or SY, such that the total dietary Se concentrations were between 0.4 and 0.5 mg/kg, had no improvement on measures of innate and humoral immunity, uterine health, pregnancy per AI, and embryonic survival. On the contrary, when concentrations of Se in the diet remained below 0.4 mg/kg, supplementation with 0.3 mg of Se/kg of diet improved innate and humoral immunity and second service pregnancy per AI in lactating dairy cows [21]. To explain the apparently contradictory results between studies, we inferred that diets containing low or marginally low concentrations of Se could benefit from the greater Se availability expected from an organic source of Se.

5. Conclusion

Replacement of SS with SY supplemented to diets with ingredients containing to some extent high concentrations of Se did not increase concentrations of Se and GSH-Px activity in plasma of dairy cows measured in early postpartum. Cows that developed fever of undiagnosed cause or acute puerperal metritis in the first 10 DIM were more likely to be diagnosed with subclinical endometritis at 30 DIM. These cows with subclinical endometritis had reduced fertilization of oocytes, suggesting that one of the deleterious effects of subclinical inflammation of the uterus on fertility is mediated by reduced sperm transport or fertilizing ability. The lack of responses to source of Se on Se concentrations and GSH-Px activity in plasma likely accounted for the inability of source of Se to influence uterine health, fertilization and early embryo development. We inferred that supplementing 0.3 mg/kg of Se either as SS or SY in the diets of transition dairy cows results in similar Se status, GSH-Px activity in plasma, risk of periparturient diseases, and embryo quality when the basal diet already contains 0.1 to 0.2 mg/kg of Se.

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References

[1] Behne D, Kyriakopoulos A. Mammalian selenium-containing proteins. Annu Rev Nutr 2001;21:453–73.
[2] Trinder N, Hall RJ, Renton CP. The relationship between the intake of selenium and vitamin E on the incidence of retained placenta in dairy cows. Vet Rec 1973;93:641–3.
[3] Harrison JH, Hancock DD, St Pierre N, Conrad HR, Harvey WR. Effect of prepartum selenium treatment on uterine involution in the dairy cow. J Dairy Sci 1986;69:1421–5.
[4] Smith KL, Harrison JH, Hancock DD, Todhunter DA, Conrad HR. Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. J Dairy Sci 1984;67:1293–300.
[5] Cebra CK, Heidel JR, Crisman RO, Stang BV. The relationship between endogenous cortisol, blood micronutrients, and neutrophil function in postparturient Holstein cows. J Vet Intern Med 2003;17:902–7.
[6] Scholz RW, Hutchinson LJ. Distribution of gluthatione peroxidase activity and selenium in the blood of dairy cows. Am J Vet Res 1979;40:245–9.
[7] Weiss WP, Hogan JS. Effect of selenium source on selenium status, neutrophil function, and response to intramammary endotoxin challenge of dairy cows. J Dairy Sci 2005;88:4366–74.
[8] Fujitani Y, Kasai K, Ohtani S, Nishimura K, Yamada M, Utsumi K. Effect of oxygen concentration and free radicals on *in vitro* development of *in vitro*-produced bovine embryos. J Anim Sci 1997;75:483–9.
[9] Aréchiga CF, Ealy AD, Hansen PJ. Evidence that glutathione is involved in thermotolerance of preimplantation murine embryos. Biol Reprod 1995;52:1296–301.
[10] Loven DP. A role for reduced oxygen species in heat induced cell killing and the induction of thermotolerance. Med Hypotheses 1988;26:39–50.
[11] Uhm SJ, Gupta MK, Yang JH, Lee S, Lee HT. Selenium improves the developmental ability and reduces the apoptosis in porcine parthenotes. Mol Reprod Dev 2007;74:1386–94.
[12] National Research Council. Nutrient requirements of dairy cattle, 7th revised edition, Washington, DC: National Academy Press; 2001.
[13] Rutigliano HM, Lima FS, Cerri RLA, Greco LF, Vilela JM, Magalhães V, et al. Effects of method of presynchronization and source of selenium on uterine health and reproduction in dairy cows. J Dairy Sci 2008;91:3323–36.
[14] Tracy ML, Moeller G. Continuous flow vapor generation for inductively coupled argon plasma spectrometric analysis. Part 1: selenium. J Assoc Off Anal Chem 1990;73:404–10.
[15] Ferguson JD, Galligan DT, Thomsen N. Principal descriptors of body condition score in Holstein cows. J Dairy Sci 1994;77:2695–703.
[16] Kasimanickam R, Duffield T, Foster R, Gartley C, Leslie K, Walton J, et al. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology 2004;62:9–23.
[17] Phyllis AW, Ullrey DE. Improved fluorometric method for determining selenium. J Assoc Off Anal Chem 1978;4:927–30.
[18] Cerri RLA, Santos JEP, Juchem SO, Galvão KN, Chebel RC. Timed artificial insemination with estradiol cypionate or insemination at estrus in high-producing dairy cows. J Dairy Sci 2004;87:3704–15.
[19] IETS. Manual of the International Embryo Transfer Society. 3rd rev. ed. Savoy, IL; 1998.
[20] Sheldon IM, Noakes DE, Rycroft A, Dobson H. Acute phase protein responses to uterine bacterial contamination in cattle after calving. Vet Rec 2001;148:172–5.
[21] Silvestre FT, Silvestre DT, Santos JEP, Risco C, Staples CR, Thatcher WW. Effects of selenium (Se) sources on dairy cows. J Anim Sci 2006;84(Suppl. 1):141 [abstr.].
[22] Rutigliano, HM, Cerri RLA, Lima FS, Vettorato LF, Araujo DB, Hillegass J, et al. 2006. Effects of source of supplemental Se on health and immune status of periparturient dairy cows. J Dairy Sci 2006;89(Suppl. 1):165 [abstr.].
[23] Kubota J, Allawayd WH, Carter DL, Gary EE, Lazar VA. Selenium in crops in 521 the United States in relation to selenium responsive diseases of animals. J Agric Food Chem 1967;15:448–53.
[24] Dargatz DA, Ross PF. Blood selenium concentrations in cows and heifers on 253 cow-calf operations in 18 states. J Anim Sci 1996;74:2891–5.
[25] Weiss WP, Hogan JS, Smith KL, Hoblet KH. Relationships among selenium, vitamin E, and mammary gland health in commercial dairy herds. J Dairy Sci 1990;73:381–90.
[26] Morse D, DeLorenzo MA, Wilcox CJ, Natzke RP, Bray DR. Occurrence and reoccurrence of clinical mastitis. J Dairy Sci 1987;70:2168–75.
[27] Hoffman PC, Funk DA. Applied dynamics of dairy replacement growth and management. J Dairy Sci 1992;75:2504–16.
[28] Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology 2005;64:1879–88.
[29] Hill J, Gilbert R. Reduced quality of bovine embryos cultured in media conditioned by exposure to an inflamed endometrium. Aust Vet J 2008;86:312–6.
[30] Hsu P, Hsu C, Guo YL. Hydrogen peroxide induces premature acrosome reaction in rat sperm and reduces their penetration of the zona pellucida. Toxycology 1999;139:93–101.