Effect of bovine somatotropin (500 mg) administered at ten-day intervals on ovulatory responses, expression of estrus, and fertility in dairy cows

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ABSTRACT

The objectives of this study were to evaluate the effect of administering 500 mg of recombinant bovine somatotropin (bST) every 10 d on ovulatory responses, estrous behavior, and fertility of lactating Holstein cows. Lactating dairy cows were assigned to 1 of 2 treatments: a control with no administration of bST (73 primiparous and 120 multiparous cows) or 6 consecutive administrations of 500 mg of bST (83 primiparous and 123 multiparous cows) given subcutaneously at 10-d intervals starting 61 ± 3 d postpartum (study d 0), concurrent with the initiation of the timed artificial insemination (AI). Blood samples were collected thrice weekly from 61 ± 3 to 124 ± 3 d in milk (DIM), and plasma samples were analyzed for concentrations of estradiol, glucose, insulin, insulin-like growth factor 1, and progesterone. The estrous cycle of cows was presynchronized with 2 injections of PGF_{2α} at 37 ± 3 and 51 ± 3 DIM, and the Ovsynch timed AI protocol was initiated at 61 ± 3 DIM. Ovaries were scanned to determine ovulatory responses during the Ovsynch protocol. Pregnancy was diagnosed at 33 and 66 d after AI. Body condition was scored on study d 0, 10, 42, and 76. Sixty-four cows were fitted with a pressure mounting sensor with radiotelemetric transmitters to monitor estrous behavior. Treatment of lactating dairy cows with 500 mg of bST at 10-d intervals increased yields of milk and milk components in the first 2 mo after treatment. Body condition of bST-treated cows remained unaltered, whereas control cows gained BCS. Treatment with bST increased concentrations of insulin-like growth factor 1 chronically, but concentrations of insulin and glucose increased only transiently in the first 7 d after the first injection of bST. Concentrations of progesterone during and after the Ovsynch protocol remained unaltered after treatment with bST; likewise, ovulatory responses during the Ovsynch protocol were

mostly unaltered by treatment. Concentration of estradiol tended to be greater for bST cows than for control cows immediately before induction of ovulation in the Ovsynch protocol. Similarly, the mean and the peak concentrations of estradiol were greater for bST cows than for control cows when monitored during spontaneous estrus. Nevertheless, duration of estrus and the median number of standing events were less for bST cows than for control cows. Pregnancies per AI after the first and second postpartum inseminations were not affected by bST treatment. Treatment of lactating dairy cows with 500 mg of bST every 10 d improved lactation performance, but it did not affect pregnancies per AI and it reduced expression of estrus.

Key words: bovine somatotropin, dairy cow, estrous behavior, reproduction

INTRODUCTION

Bovine somatotropin is commonly used to improve lactation performance of dairy cows starting at the ninth or tenth week of lactation. Treatment with a sustained release formulation containing 500 mg of recombinant bST at 14-d intervals has increased milk production by an average of 3 to 5 kg/d (Hartnell et al., 1991; Collier et al., 2001). Because of the increased milk yield and partition of nutrients favoring the mammary gland and not body reserve, reproduction of cows treated with bST has been a concern. Some studies suggested that bST negatively affects reproduction of dairy cows because of negative effects on detection of estrus (Morbeck et al., 1991; Lefebvre and Block, 1992; Kirby et al., 1997a) and risk of pregnancy (Dohoo et al., 2003).

As part of the postapproval monitoring program, a comprehensive study involving more than 1,200 cows on 28 dairy farms throughout the United States indicated that administration of 500 mg of bST every 14 d did not affect measures of reproductive performance in lactating dairy cows (Collier et al., 2001). Because administration of bST increases blood concentrations of growth hormone (GH) and IGF-1, both of which have effects on reproductive tissues (Lucy, 2000; Bilby

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et al., 2004, 2006), it is expected that it might influence reproduction of dairy cows. When administered at 500 mg every 14 d concurrent with synchronization of the estrous cycle, bST improved pregnancy per AI (P/AI) in lactating dairy cows (Moreira et al., 2000, 2001; Santos et al., 2004). Furthermore, administration of 500 mg of bST on the day of AI increased P/AI in cows considered to be subfertile (Morales-Roura et al., 2001) or not (Starbuck et al., 2006). Nevertheless, treatment with bST reduced expression of estrus in estradiol-treated ovariectomized heifers (Lefebvre and Block, 1992) and detection of estrus in lactating dairy cows (Morbeck et al., 1991; Kirby et al., 1997a; Santos et al., 2004).

Although bST has been shown to positively affect P/AI in dairy cows at a dosage of 500 mg at 14-d intervals, lactation response to the hormone is suggested to be linear up to 750 mg every 14 d (Hartnell et al., 1991). The label use of bST is 500 mg at 14-d intervals starting between the ninth and tenth week of lactation, but it is not uncommon for producers to reduce the treatment interval from 14 to 11 or 10 d to maximize milk yield. Treatment with bST increases GH, IGF-1, and insulin in blood of cows (Bilby et al., 2004, 2006), and these responses are dose-dependent (Bilby et al., 1999). In a dose-titration study, P/AI for inseminations performed between 50 and 100 d of treatment decreased when cows were administered 700 mg of bST every 14 d compared with untreated controls (Downer et al., 1993), although the study used a limited number of cows. When lactating cows received 2 treatments of 500 mg of bST at 11-d intervals, P/AI on d 17 of gestation increased (Bilby et al., 2006); however, when nonlactating cows received the same treatment regimen, P/AI decreased with bST (Bilby et al., 2004). The authors suggested that bST may have excessively increased plasma IGF-1 and insulin to cause asynchrony between conceptus and uterus that was detrimental to pregnancy (Bilby et al., 2004). Therefore, it is possible that bST given at doses >500 mg every 14 d might not benefit fertility of lactating dairy cows.

It was hypothesized that administration of 500 mg of bST at 10-d intervals would not benefit fertility of dairy cows as it has been demonstrated with 500 mg of bST at 14-d intervals (Moreira et al., 2000, 2001; Santos et al., 2004). Cows treated with bST were expected to have increased concentrations of plasma IGF-1 and insulin and reduced estrous behavior. Therefore, the objective of the current study was to examine the effects of 500 mg of exogenous bST administered every 10 d on ovulatory responses, estrous behavior, and fertility when cows are subjected to a timed AI protocol for first postpartum AI and then reinsemination after detection of estrus.

MATERIALS AND METHODS

Animals, Housing, and Feeding

Lactating Holstein cows (156 primiparous, 243 multiparous) from a commercial dairy farm in California were enrolled in the study. Throughout the study period, primiparous and multiparous cows were housed separately in free-stall barns. All cows received the same TMR fed twice daily, immediately after the a.m. and p.m. milkings, consisting of corn silage, alfalfa hay, alfalfa silage, steam-flaked corn, whole cottonseed, citrus pulp, soybean meal, dried corn distillers grains, an animal-marine protein blend, calcium salts of palm fatty acids, minerals, and vitamins. Diet was formulated to meet the nutrient requirements for lactating Holstein cows weighing 650 kg and producing 45 kg of 3.5% FCM (NRC, 2001). Chemical composition of the diet during the study averaged 17.1% CP, 30.1% NDF, 23.6% starch, 5.1% ether extract, and 43% NFC and contained an estimated 1.71 Mcal/kg of NE_L after adjusting for 24 kg of DMI.

Treatments

Twenty weekly cohorts of cows at 37 ± 3 DIM were blocked by parity and, within each block, randomly assigned to 1 of the 2 treatments. Cows with ovarian or uterine adhesions or pyometra were not enrolled in the study. Treatments were control (no administration of bST; 73 primiparous and 120 multiparous cows) or 6 consecutive subcutaneous injections of 500 mg of bST (Posilac, Elanco Animal Health, Indianapolis, IN) given at 10-d intervals starting on d 61 ± 3 postpartum (83 primiparous and 123 multiparous cows), concurrent with the initiation of the ovulation synchronization protocol. Day 61 ± 3 postpartum was considered study d 0.

Reproductive Program

Cows were assigned to a presynchronized timed AI protocol (d 0 GnRH, d 7 PGF_{2α}, 48 h GnRH, and 12 h timed AI; Moreira et al., 2001), which is depicted in Figure 1. Two technicians inseminated all cows throughout the study. After the initial AI, cows were observed for signs of estrus once daily in the afternoon based on removal of tail chalk that was applied daily. Cows observed in estrus based on removal of the tail chalk were reinseminated on the same afternoon.

Ultrasonography of Ovaries

Ovaries were examined by transrectal ultrasonography (Sonovet 2000, Universal Medical System, Bedford Hills, NY) to determine the diameter of the dominant

Figure 1. Diagram of activities during the study. Cows either remained as untreated control or were treated with 6 consecutive injections of 500 mg of recombinant bST every 10 d starting on the day of the first GnRH of the timed AI protocol. Blood samples (BS) were collected 3 times weekly throughout the study from 64 cows. GnRH = injection of 100 μ g of gonadorelin; PD = pregnancy diagnosis; PGF_{2 α} = injection of 25 mg of dinoprost; US = ultrasonography of the ovaries.

follicle and corpus luteum (CL) and ovulatory responses to the timed AI protocol. Cows were scanned during the Ovsynch protocol on the days of the first GnRH, the PGF_{2 α} , and the second GnRH injections of the protocol. Two additional examinations were performed on d 2 and 7 after AI (Figure 1). Ovarian maps were drawn at each examination, and ovulation was determined based on the disappearance of 1 or more follicles >8 mm in diameter and confirmed by the appearance of 1 or more CL.

Blood Sampling and Analyses of Plasma

Blood (~8 mL) was sampled immediately after the morning milking from a subset of 64 cows (31 control and 33 bST) by puncture of the coccygeal vessels into tubes containing K₂ EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) 3 times weekly for 9 wk. Sampling started on study d 0, at 61 $\pm$ 3 DIM, which corresponded to the first bST injection in bST-treated cows, and finished at 124 $\pm$ 3 DIM. Samples were refrigerated and centrifuged at 3,000 $\times$ *g* for 15 min, and plasma was frozen at -25°C for later analyses.

Concentrations of progesterone were analyzed in all samples from 64 cows by a validated ELISA assay (Cerri et al., 2004). The intra- and interassay coefficients of variation were determined for each 96-well plate using 2 plasma samples with known concentrations of progesterone (low = 1.5 ng/mL; high = 2.5 ng/mL). The sensitivity of the assay was 0.10 ng/mL, and the intraassay coefficient of variation was 9.4%. The interassay coefficients of variation for the low and high progesterone samples were 12.7 and 7.2%, respectively. Individual samples and microplates with coefficients of variation >15% were reanalyzed.

Estradiol was analyzed by a double extraction RIA according to Kirby et al. (1997b). Addition of known amounts of estradiol-17 β (1, 5, and 10 pg/mL) to charcoal-stripped calf serum was used to determine recovery. Intra- and interassay coefficients of variation for estradiol assays were 3.4 and 14.1%, respectively, and assay sensitivity was 0.5 pg/mL calculated at 2 standard deviation below the mean counts per minute at maximum binding. Estradiol was analyzed in samples collected immediately before the PGF_{2 α} and the second GnRH of Ovsynch protocol and 24 h after timed AI.

Estradiol was also analyzed in plasma samples collected immediately before cows were detected in estrus after the first timed AI and when progesterone decreased to <1 ng/mL.

Concentrations of total IGF-1 in plasma were analyzed on d 0, 2, 4, 7, 9, 11, 14, 16, and 18 relative to the first GnRH of the timed AI protocol. Analysis was performed by a nonextraction ELISA assay according to Obese et al. (2008) using a commercial kit (Diagnostic Systems Laboratories Inc., Webster, TX). Samples were analyzed in duplicate and those with coefficients of variation $>15\%$ were repeated. Two known samples, 1 with low concentration (140 ± 40 ng/mL) and 1 with high concentration (340 ± 40 ng/mL) provided in the kit, were used as quality controls and for calculations of interassay coefficient of variation. Samples in microplates whose low and high known samples resulted in more than 15% coefficient of variation relative to their values were reanalyzed. Intra- and interassay coefficients of variation were 3.9 and 4.9%, respectively.

Concentrations of insulin were analyzed on the same days as those for IGF-1 using a solid-phase RIA with a commercial kit (Immulin, Siemens Healthcare Diagnostics, Flanders, NJ). The sensitivity of the assay was 0.40 μ IU/mL calculated at 2 standard deviation below the mean counts per minute at maximum binding, and the intra- and interassay coefficients of variation were 4.0 and 6.9%, respectively.

Concentrations of glucose in plasma were analyzed on the same days as those for IGF-1 by direct measurement using the YSI Model 2700 Select Biochemistry Analyzer (Yellow Springs Instrument Co. Inc., Yellow Springs, OH). The coefficient of variation between duplicate analyses averaged ($\pm$ SD) $4.6 \pm 1.1\%$.

Characterization of Estrous Behavior

The same 64 cows from which blood was sampled were also monitored for estrous behavior. At 61 ± 3 DIM, cows were fitted with a pressure mounting sensor with radiotelemetric transmitters (HeatWatch, CowChips LLC; Manalapan, NJ). Transmitters were kept in place until the cow was diagnosed pregnant on d 33 after the first timed AI or until a cow was reinseminated following the nonpregnancy diagnosis.

Onset of estrus was identified by the first activation of the transmitter. Duration of estrus was defined as the interval from the first to the last activation of the transmitter recorded during a period of estrus. A single activation of the transmitter was not considered estrus.

For cows displaying estrous behavior, the progesterone concentration in plasma collected on the day of estrus ± 1 d was identified. Only 1 cow had activated

heat mount detector when progesterone concentrations in the same week were always >1 ng/mL, and the data from that event were removed from analyses. For calculation of estrus detection in cows fitted with pressure mounting sensors, each time progesterone decreased <1 ng/mL (luteolysis) and then increased >1 ng/mL in the subsequent days (ovulation) was considered a period for display of estrus. Cows that displayed estrus during those periods were considered to have experienced the event, whereas those not displaying estrus were considered failure to display estrus. Concentrations of estradiol in the samples collected on the day of estrus ± 1 d were evaluated. In cows not displaying estrus, the concentration in the 2 plasma samples collected after luteolysis (progesterone <1 ng/mL) were also analyzed. The peak and the mean concentrations were used for analyses of estradiol in cows after the first timed AI. Concentrations of estradiol on the days of injections of PGF_{2 α} , GnRH, and 12 h after AI in the Ovsynch protocol were also analyzed.

Pregnancy Diagnoses and Reinsemination of Nonpregnant Cows

Pregnancy was diagnosed by ultrasonography 33 d after timed AI. Pregnant cows on d 33 were reexamined for pregnancy 33 d later. Cows diagnosed as nonpregnant on d 33 and that had not been reinseminated received an injection of PGF_{2 α} and were observed for estrus in the following 7 d. Pregnancy in the second AI was diagnosed by palpation per rectum 35 to 41 d after insemination. Pregnancy loss between 33 and 66 d of gestation was calculated.

Body Condition Scoring and Yields of Milk and Milk Components

Body condition of all cows was scored (Ferguson et al., 1994) on the day of study enrollment at 37 ± 3 DIM, on the day of the PGF_{2 α} of the timed AI at 68 ± 3 DIM, and at 33 and 66 d after timed AI, corresponding to 105 ± 3 and 138 ± 3 DIM, respectively.

Cows were milked twice daily, and milk yield was recorded once monthly during the official DHIA test. Individual milk samples were collected during DHIA test from 2 consecutive milkings, composited, and analyzed for SCC, fat, and true protein concentrations (Foss 303 MilkoScan, Foss Foods Inc., Eden Prairie, MN). Yields of milk and milk components were monitored for the first 60 DIM, corresponding to the period before initiation of treatments, to be used as covariates for analyses, and for the 60 d following the initiation of treatments.

Experimental Design and Statistical Analyses

The study design was a randomized complete with blocks. Weekly, a cohort of cows was blocked by parity and, within each block, randomly assigned to 1 of 2 treatments. The baseline P/AI in this herd at the beginning of the study was 38%. To have at least a 95% chance of detecting a 10 percentage unit difference in P/AI with 80% protection against a Type II error, it was calculated that 170 cows were needed. The sample size was based on a potential 10 percentage units increase in P/AI with the use of bST (Moreira et al., 2001; Santos et al., 2004).

Binary responses such as ovulation to the first and second GnRH of the timed AI protocol, P/AI, pregnancy loss, and detection of estrus before pregnancy diagnosis on d 33 after the timed AI were analyzed by logistic regression using the LOGISTIC procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). All models included the effects of treatment, parity, BCS on the week of AI, BCS change from enrollment to AI, and milk yield. Covariates that were not significant ($P > 0.10$) were dropped from the final model. Treatment was forced in the final logistic regression model.

Measures of estrous behavior and diameters of the ovulatory follicles and CL were analyzed by ANOVA using the GLM procedure of SAS. The statistical model included the effects of treatment, parity, interaction between treatment and parity, and other covariates such as BCS, milk yield, hormone concentrations, and ovulatory response as found appropriate. Detection of estrus in cows fitted with pressure mounting sensors was analyzed using the GLIMMIX procedure of SAS with random effect of cow in the model. Median number of standing events per estrus was analyzed using the Kruskal-Wallis nonparametric test in SAS.

Yields of milk and milk components and concentrations of hormones and glucose in plasma were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS; the model included the effects of treatment, parity, day of measurement, interaction between treatment and parity, interaction between treatment and day of measurement, and cow nested within treatment as the random error. For concentrations of progesterone during the Ovsynch protocol, concentration of progesterone on study d 0 was used as covariate. For yields of milk and milk components, data from the first 60 DIM were used as covariate. The covariance structure that best fitted the data was selected based on the smallest Bayesian information criterion fit statistics. Treatment differences with $P \leq 0.05$ were considered significant and $0.05 < P \leq 0.10$ were considered a tendency.

Figure 2. Concentrations of IGF-1 in plasma of cows treated or not treated with 500 mg of recombinant bST at 10-d intervals. Day 0 represents the day of first bST treatment. Effect of treatment ($P < 0.001$), day ($P < 0.001$), and interaction between treatment and day ($P < 0.001$).

RESULTS

Concentrations of IGF-1, Glucose, and Insulin

On study d 0, concentrations of IGF-1 were similar between treatments (Figure 2). In the first 2 d after treatment, plasma IGF-1 began to increase ($P < 0.001$) in bST-treated cows, whereas in control cows it remained unaltered. After the first injection of bST, concentrations of IGF-1 seemed to have reached a plateau on study d 9, but it increased again starting on study d 11 after the second injection of bST. Concentrations of IGF-1 were always greater ($P < 0.001$) for bST cows than for control cows. Neither parity nor the interaction of treatment and parity influenced the concentrations of IGF-1.

Concentrations of glucose in plasma in the first 18 d of study tended ($P = 0.10$) to be greater for bST cows than for control cows (63.4 ± 0.7 vs. 61.7 ± 0.7 mg/dL; Figure 3). Although no treatment and day interaction was observed for plasma glucose, the tendency for increased concentrations was observed primarily after the first bST treatment. Glucose was not affected by parity or the interaction of treatment and parity. Concentrations of insulin in plasma followed a similar pattern as those of glucose. An interaction ($P < 0.001$) between treatment and study day was detected, and cows receiving bST had greater ($P < 0.05$) concentrations of insulin in the first 7 d of study (Figure 4). After study d 7, concentrations of insulin decreased ($P = 0.008$) in cows treated with bST, whereas in control cows they remained mostly unaltered. Parity or the interaction of

Figure 3. Concentrations of glucose in plasma of cows treated or not treated with 500 mg of recombinant bST at 10-d intervals. Day 0 represents the day of first bST treatment. Effect of treatment ($P = 0.10$), day ($P = 0.02$), and interaction between treatment and day ($P = 0.23$). Within a day, asterisk indicates $P = 0.02$.

treatment and parity did not affect the concentrations of glucose in plasma.

Lactation Performance and BCS

Milk yield in the first 2 mo after the initial treatment with bST increased ($P < 0.001$) 3.5 kg/d (45.4 ± 0.5 vs. 41.9 ± 0.5 kg/d). A greater increase in 3.5% FCM was observed, and cows treated with bST produced 6.1 kg more FCM compared with control cows (46.6 ± 1.6 vs. 40.5 ± 1.7 kg/d; $P < 0.01$). Milk fat content did not change with bST treatment and were $3.65\% \pm 0.20$ and $3.30\% \pm 0.21$ for control and bST-treated cows, respectively, whereas milk true protein content was greater ($P = 0.01$) for control cows ($2.86 \pm 0.01\%$) than for bST-treated cows ($2.81 \pm 0.01\%$). Nevertheless, yields of both fat (1.67 ± 0.10 vs. 1.37 ± 0.10 kg/d; $P = 0.04$) and true protein (1.27 ± 0.01 vs. 1.19 ± 0.01 kg/d; $P < 0.001$) were greater for cows treated with bST than for control cows. The SCS did not differ between treatments and averaged 1.57 ± 0.10 .

As expected, the mean BCS of control and bST-treated cows were similar on study d 0 (control = 2.97 ± 0.02 , bST = 2.98 ± 0.02) and 10 (control = 2.90 ± 0.02 , bST = 2.88 ± 0.02), after which they differed ($P < 0.001$). An interaction ($P < 0.001$) between treatment and study day was observed because control cows gained BCS after study d 10, whereas cows receiving bST maintained their BCS in the same period. On study d 42 (control = 2.98 ± 0.02 , bST = 2.88 ± 0.02) and 76 (control = 3.07 ± 0.02 , bST = 2.81 ± 0.02), bST-treated cows had smaller ($P < 0.001$) BCS than did control cows. The mean BCS during the study period were 3.00 ± 0.01 and 2.88 ± 0.01 for control and bST-treated cows, respectively.

Ovarian Responses and Steroid Hormone Concentrations in the Ovsynch

The proportion of cyclic cows at the beginning of the study was similar between treatments and it averaged 70.0%. Multiparous cows were more likely to be cyclic than primiparous cows [adjusted odds ratio (AOR) = 1.83; 95% CI = 1.16–2.87]. Cows receiving bST had greater ($P = 0.02$) ovulation to the first GnRH compared with controls (66.5 vs. 56.5%; AOR = 1.67; 95% CI = 1.10–2.56). Despite the changes in ovulatory response, the proportion of cows with at least 1 CL at the injection of PGF_{2α} did not differ ($P = 0.16$) between treatments (bST = 94.2, control = 90.7%; AOR = 1.77; 95% CI = 0.80–3.90). Ovulation to the final GnRH injection of the Ovsynch was not affected ($P = 0.51$) by treatment and were 91.8 and 92.2% for bST-treated and control cows, respectively (AOR = 0.78; 95% CI = 0.37–1.65). Approximately 6.5% of the cows ovulated before the final GnRH injection. Double ovulation after the final GnRH of the Ovsynch did not differ ($P = 0.79$) with bST treatment; they were 18.0 and 20.2% for bST-treated and control cows, respectively (AOR = 0.93; 95% CI = 0.56–1.57).

The diameters of the ovulatory follicles on the day of PGF_{2α} of the Ovsynch did not differ ($P = 0.33$) between bST (16.1 ± 0.29 mm) and control (16.4 ± 0.28 mm) cows. Similarly for cows having double ovulation, the diameter of the codominant ovulatory follicle was similar ($P = 0.60$) between treatments and averaged 13.7 ± 0.52 and 13.3 ± 0.45 mm for bST-treated and control cows, respectively. Between the injections of PGF_{2α} and the final GnRH, the ovulatory follicles grew to similar diameters for both the largest ovulatory follicle (bST =

Figure 4. Concentrations of insulin in plasma of cows treated or not with 500 mg of recombinant bST at 10-d intervals. Day 0 represents the day of first bST treatment. Effect of treatment ($P = 0.62$), day ($P = 0.008$), and interaction between treatment and day ($P = 0.02$). Within a day, asterisk indicates $P < 0.05$.

18.4 $\pm$ 0.28 mm, control = 18.7 $\pm$ 0.28 mm; P = 0.45) and the second largest ovulatory follicle (bST = 16.2 $\pm$ 0.57 mm, control = 15.1 $\pm$ 0.46 mm; P = 0.14) in cows with double ovulation. Similar to the diameter of the ovulatory follicle, the diameter of the largest CL 7 d after timed AI did not differ (P = 0.22) between bST-treated cows (26.8 $\pm$ 0.37 mm) and control cows (26.3 $\pm$ 0.36 mm). For cows with double ovulation, the diameter of the second CL was not affected (P = 0.64) by treatment and were 21.3 $\pm$ 0.83 and 21.8 $\pm$ 0.66 mm for bST-treated and control cows, respectively.

Concentrations of progesterone during the Ovsynch protocol did not differ with treatment. On the day of timed AI, concentration of progesterone was similar between treatments and averaged 0.61 ng/mL. Regression of the CL after the PGF_{2 α} of the Ovsynch, based on progesterone <1.0 ng/mL, was similar between treatments and were 90.3 and 91.0% for control and bST-treated cows, respectively. Cyclic cows had greater (P < 0.001) concentrations of progesterone than anovular cows during the Ovsynch protocol (3.86 $\pm$ 0.15 vs. 1.49 $\pm$ 0.33 ng/mL). After the timed AI, concentrations of progesterone did not differ with treatment and averaged 3.40 $\pm$ 0.28 ng/mL in the first 18 d after insemination. Parity did not influence progesterone concentrations during and after the Ovsynch protocol.

The concentrations of estradiol on the day of the PGF_{2 α} injection of the Ovsynch did not differ between bST-treated cows (5.06 $\pm$ 0.25 pg/mL) and control cows (4.62 $\pm$ 0.25 pg/mL). Concentrations increased on the day of the final GnRH and tended (P = 0.10) to be greater for bST-treated cows than control cows (6.71 $\pm$ 0.32 vs. 5.97 $\pm$ 0.33 pg/mL). Concentrations sharply decreased in the 24 h following AI and were 3.55 $\pm$ 0.19 and 3.52 $\pm$ 0.19 for bST-treated and control cows, respectively (P = 0.90). Parity did not influence estradiol concentrations during or 24 h after the Ovsynch protocol.

Estrous Behavior and Estradiol Concentrations After AI

Detection of estrus in cows monitored by radiotelemetry did not differ statistically between treatments (Table 1). Duration of estrus was less (P = 0.04) for bST-treated cows than for control cows. Cows were in estrus for 7.6 h when not treated with bST, whereas bST-treated cows were in estrus only 5.7 h. The median number of standing events was reduced (P = 0.04) when cows were treated with bST; however, time standing per each standing event and total standing time per estrus were not different between treatments. Similar to estradiol concentrations during the Ovsynch protocol, cows treated with bST had greater mean (P

= 0.01) and peak (P = 0.03) concentrations of estradiol when they were observed in estrus. Similar to cows monitored for estrous behavior, the detection of estrus and reinsemination of nonpregnant cows after the first postpartum AI was not statistically different between bST-treated cows and control cows (Table 2). Similarly, multiparous cows had a reduced (P < 0.05) mean (5.0 $\pm$ 1.3 vs. 9.6 $\pm$ 1.2) and median (4 vs. 7) number of mounts per estrous event compared with primiparous cows, which resulted in estrus with less mounting time for multiparous than primiparous cows (10.6 $\pm$ 2.3 vs. 18.3 $\pm$ 2.1 s). Because of that, duration of estrus was less (P < 0.001) for multiparous cows than for primiparous cows (321.2 $\pm$ 34.6 vs. 475.4 $\pm$ 29.0 h).

Short Cycling, P/AI, and Pregnancy Loss

Treatment with bST did not affect the proportion of cows that ovulated after the final GnRH of the Ovsynch protocol but had a short estrous cycle, and short cycling affected 12.1% of the cows that ovulated to the final GnRH of the Ovsynch protocol. Pregnancy to the first postpartum timed AI did not differ between treatments at either 33 or 66 d after insemination (Table 2). Similarly, pregnancy loss between d 33 and 66 after the first AI and P/AI after the second postpartum insemination were not influenced by bST treatment. Parity did not influence short cycling or pregnancy responses after the first and second postpartum AI.

DISCUSSION

Use of bST increases yields of milk and milk components in lactating dairy cows (Hartnell et al., 1991; Collier et al., 2001), and lactation response to prolonged-release injectable formulation of bST is linear up to 750 mg every 14 d (Hartnell et al., 1991). The use of 500 mg of bST at 10-d intervals would result in a similar amount of bST administered when compared with 750 mg at 14-d intervals.

Cows treated with bST starting at 61 DIM maintained BCS in the following 76 d, whereas control cows gained BCS. On study d 76, control cows had 0.20 units greater BCS than bST-treated cows. This difference in BCS was expected because of the increase in yields of milk and 3.5% FCM observed in response to bST treatment. Energy balance of dairy cows is reduced in the first weeks of bST treatment because of the immediate increase in milk yield with no concurrent increase in DMI until 4 to 8 wk later (Bauman et al., 1985, 1988). To some extent, the improvements in lactation performance observed in the first weeks of bST treatment are supported in detriment of replenishment of body energy stores as a consequence of nutrient repartitioning (Bau-

Table 1. Effect of 500 mg of recombinant bST administered at 10-d intervals on estrous behavior of dairy cows

Item	Treatment		AOR (95% CI) ¹	P-value
	Control	bST		
Estrus, ² % (n/n)	44.8 (26/58)	33.3 (19/57)	0.62 (0.28–1.33)	0.21
Duration of estrus, min	457.7 ± 30.2	339.0 ± 46.4	—	0.04
Standing events, n				
Mean	7.8 ± 1.2	6.7 ± 1.3	—	0.55
Median	6.0	4.0	—	0.04
Standing time, s				
Per standing event	2.1 ± 0.2	2.0 ± 0.3	—	0.95
Total	16.6 ± 2.0	12.3 ± 2.3	—	0.17
Estradiol, ³ pg/mL				
Mean	4.17 ± 0.20	4.97 ± 0.21	—	0.01
Peak	4.96 ± 0.30	5.98 ± 0.33	—	0.03

¹AOR = adjusted odds ratio (control was the reference for comparison).

²Activation of pressure sensors with radiotelemetric transmitters (HeatWatch, CowChips LLC, Manalapan, NJ). Number of potential estruses based on a decrease in progesterone <1 ng/mL followed by a subsequent increase >1 ng/mL. A single mount was not considered estrus.

³Mean = mean concentration of estradiol in 2 samples analyzed on the day of estrus ± 1 for cows in estrus and in 2 samples collected after a decrease in progesterone <1 ng/mL. Peak = sample with the greatest concentration of estradiol from those analyzed on the day of estrus ± 1 for cows in estrus, or from the 2 samples collected in the days after progesterone <1 ng/mL.

man et al., 1988). Nevertheless, as in cows receiving 500 mg of bST every 14 d, the difference in BCS on study d 76 following 6 consecutive treatments with 500 mg administered every 10 d was small.

Administration of bST to lactating and nonlactating dairy cows increased the blood concentrations of GH, IGF-1, and insulin (Bilby et al., 2004, 2006), and the response was dose-dependent (Bilby et al., 1999). In the current study, bST increased IGF-1 chronically, but both glucose and insulin were only transiently increased. Bovine somatotropin increases hepatic gluconeogenesis (Knapp et al., 1992), and acute changes in gluconeogenesis would be expected to increase plasma glucose and insulin concentrations; however, if no additional nutrient intake follows, the effect on glucose and insulin would be of short duration. Treatment with bST did not alter abundance of hepatic mRNA encoding pyruvate carboxylase and phosphoenolpyruvate carboxykinase in dairy calves (Hammon et al., 2005) and cows (Per-

shing et al., 2002), or activity of those gluconeogenic enzymes (Hammon et al., 2005). Therefore, changes in gluconeogenesis might occur by increased efficiency of gluconeogenesis (Knapp et al., 1992) or increased availability of substrate by mobilization of hepatic glycogen and adipose tissue glycerol until dietary substrates increase. Furthermore, increases in glucose and insulin concentrations in plasma might result from decreased peripheral tissue glucose oxidation (Bauman et al., 1988) and reduced insulin sensitivity by adipose tissue after bST treatment. In the current study, treatment with bST resulted in a marked increase in milk yield, which likely reduced energy balance (Bauman et al., 1985) and would be expected to negate any further increases in plasma glucose and insulin until DMI and energy status improved.

Cows receiving bST had increased ovulation to the first GnRH of the Ovsynch protocol, but treatment did not alter ovulation or double ovulation to the final

Table 2. Effect of 500 mg of recombinant bST administered at 10-d intervals on fertility responses of dairy cows

Item	Treatment, % (n/n)		AOR ¹	95% CI	P-value
	Control	bST			
Estrus before d 33 ²	50.4 (62/123)	41.5 (56/135)	0.70	0.43–1.15	0.15
Pregnant					
33 d	36.3 (70/193)	34.5 (71/206)	0.90	0.58–1.38	0.62
66 d	32.1 (62/193)	28.6 (59/206)	0.81	0.52–1.27	0.36
Pregnancy loss	11.4 (8/70)	16.9 (12/71)	1.63	0.61–4.38	0.33
Pregnant second AI	28.5 (35/123)	27.4 (37/135)	0.97	0.56–1.71	0.93

¹AOR = adjusted odds ratio (control was the reference for comparison).

²Day 33 was the day of pregnancy diagnosis and treatment with PGF_{2α} in nonpregnant cows.

GnRH of the Ovsynch protocol. Such an acute effect of bST on ovulatory response to GnRH was unexpected given that bST was administered concurrently with GnRH and ovulation usually occurs 24 to 30 h later. It is known that IGF-1 is a potent stimulator of ovarian steroidogenesis and gonadotropin function (Lucy, 2000; Ginther et al., 2004; Velazquez et al., 2008), and it influences ovarian follicular development and CL lifespan (Lucy et al., 1994). It is possible that bST might have potentiated the effects of a GnRH-induced LH surge at the beginning of the Ovsynch protocol to increase ovulatory response. Nevertheless, treatment with bST did not increase the incidence of double ovulations during the course of the Ovsynch protocol. Administration of 500 mg of bST at 14-d intervals did not change the risk of twinning (Collier et al., 2001; Dohoo et al., 2003). Collectively, these data suggest that bST administered at either 10- or 14-d intervals does not change the risk of double ovulation and twinning in lactating dairy cows.

Treatment with bST increased the concentrations of estradiol in cows in the periovulatory period either during the Ovsynch protocol or during spontaneous estrus. The changes during the Ovsynch protocol were observed despite the lack of difference in diameter of the ovulatory follicle. Because treatment with bST increased the concentrations of IGF-1, and the latter has been shown to stimulate ovarian steroidogenesis (Lucy, 2000; Ginther et al., 2004; Velazquez et al., 2008) and be critical for follicle selection in cattle (Beg and Ginther, 2006), it is possible that increases in circulating concentrations of estradiol might have been mediated by changes in IGF-1. Gong et al. (1993) demonstrated that IGF-1, but not bST, stimulated the *in vitro* proliferation of granulosa cells from bovine ovarian follicles of different follicular diameters. Furthermore, intrafollicular injection of IGF-1 in the second largest follicle of a follicular wave, when the largest follicle reached 8.5 mm in diameter, increased concentrations of estradiol and androstenedione in the follicular fluid (Ginther et al., 2004). Therefore, it is plausible to suggest that treatment with bST and the increase in IGF-1 might have mediated changes in ovarian steroidogenesis to increase follicular estradiol synthesis and plasma concentrations of estradiol.

Because bST increased IGF-1 concentrations, it was thought that it could enhance luteal progesterone synthesis and plasma concentrations. Nevertheless, systemic concentrations of progesterone remained unaltered by treatment. Progesterone concentrations in blood of heifers increased with bST treatment (Lucy et al., 1994); however, these responses have not been consistent in dairy cows (Kirby et al., 1997a; Bilby et al., 2006). In some studies, bST has been reported to

reduce the concentrations of progesterone in lactating dairy cows (Bilby et al., 2006), and such response might be an indirect effect of bST stimulating milk yield and subsequent DMI, which is known to increase progesterone catabolism (Wiltbank et al., 2006). Because in the current study bST did not influence the diameter of the ovulatory follicle, ovulation and double ovulation to the final GnRH of the Ovsynch protocol, and diameter of the resulting CL, it is concluded that treatment with 500 mg of bST at 10-d intervals does not enhance progesterone concentrations in blood of lactating dairy cows. It is possible, however, that if luteal synthesis of progesterone is enhanced by bST treatment, catabolism might also be increased by changes in milk yield and DMI (Wiltbank et al., 2006), offsetting increased steroidogenesis and resulting in unaltered plasma progesterone concentration.

Despite increases in plasma estradiol, cows treated with bST had a reduction in measures of expression of estrus. Although detection of estrus was only numerically reduced, the duration of estrus and the median number of mounts per estrus decreased with bST. Santos et al. (2004) observed an interaction between bST and parity for detection of estrus in the 7 d following a synchronized estrus. Detection of estrus in primiparous cows was unaffected by bST, but multiparous cows receiving 500 mg of bST every 14 d tended to have reduced estrous detection. In the same study, treatment with bST also reduced the proportion of nonpregnant cows that was observed in estrus and reinseminated before the pregnancy diagnosis on d 31 after the initial AI (Santos et al., 2004). These negative effects of bST on expression or detection of estrus have been observed by others. Treatment with 500 mg of bST every 14 d reduced visual observation of estrus in lactating dairy cows (Kirby et al., 1997a). Treatment with 16.5 mg of bST/d starting at 5 wk postpartum extended the interval from calving to first observed estrus and reduced detection of estrus (Morbeck et al., 1991). Ovariectomized heifers primed with estradiol had estruses with fewer mounts when treated with bST (Lefebvre and Block, 1992). At 500 mg every 10 d, treatment with bST also reduced duration of estrus and mounts per estrus event in the current study, which might explain the reduced detection of estrus observed by others.

The exact mechanism by which bST influences estrous behavior is unknown. Increased milk production has been shown to influence duration and intensity of estrus in lactating dairy cows (Wiltbank et al., 2006). This effect has been suggested to be mediated by changes in steroid metabolism in higher producing cows (Wiltbank et al., 2006). However, although bST increased milk yield and reduced expression of estrus, estradiol concentrations were generally greater for bST-treated

cows than for control cows. Therefore, it is unlikely that the depressive effect of bST on expression of estrus is mediated by changes in DMI and steroid catabolism. Others have suggested that bST might influence estrous behavior by affecting the neural pathways that control estrus in cattle (Lefebvre and Block, 1992). Whether bST has a direct effect on the hypothalamus and affects its sensitivity to estradiol to express signs of estrus remains to be clarified.

Treatment of lactating dairy cows with bST has been shown to improve P/AI when cows are treated with a single (Starbuck et al., 2006) or 2 injections of 500 mg administered 10 d apart (Morales-Roura et al., 2001), or sequential injections of 500 mg every 14 d (Moreira et al., 2000, 2001; Santos et al., 2004). Administration of 500 mg of bST at intervals shorter than 14 d, however, is expected to result in greater concentrations of GH and IGF-1 (Bilby et al., 1999), which might not benefit fertility of dairy cows. In fact, treatment with 500 mg of bST every 10 d did not improve P/AI or maintenance of pregnancy in the current study.

The stimulatory effect of bST on fertility of lactating dairy cows is thought to be mediated in part by IGF-1 (Moreira et al., 2002), and supplementation of lactating cows with bST usually increases plasma IGF-1 to concentrations similar to those observed in untreated, nonlactating cows (Bilby et al., 2004, 2006). However, it is also known that high concentrations of IGF-1 can be detrimental to fertility and hyperstimulation with IGF-1 during the preimplantation period reduced pregnancy in rats (Pinto et al., 2002). Bilby et al. (2004) administered 500 mg of bST at AI and 10 d later to nonlactating cows and observed a decrease in P/AI on d 17 after insemination. On the other hand, when 2 doses of 500 mg of bST were given to lactating dairy cows, P/AI on d 17 after insemination increased (Bilby et al., 2006). Concentrations of IGF-1 are higher in nonlactating cows than in lactating cows, and the potentially excessive IGF-1 in nonlactating cows treated with bST at 10-d intervals might have been detrimental to pregnancy. In the present study, bST was administered for 60 d, and it is possible that treatment with 500 mg at 10-d intervals caused chronic increases in GH and IGF-1 that might have been above the potential optimum concentrations to stimulate fertility, thereby negating a positive effect on P/AI. Furthermore, changes in the period of follicle dominance in cows treated with bST (Kirby et al., 1997b) could have influenced P/AI in the second AI, potentially negating a positive effect of bST on fertility.

Dairy cows in early lactation have concentrations of IGF-1 in plasma that are usually less than those observed later in lactation or in dry cows. The increase

in concentrations of IGF-1 is mediated by energy status and DMI (Lucy, 2000; Velazquez et al., 2008). Lactating beef cows treated with 167 mg of bST had increased plasma IGF-1, which was similar to the concentrations observed in untreated, nonlactating cows, but improvements in P/AI were only numerical (Bilby et al., 1999). In beef heifers treated with a single dose of bST, no benefit was observed on fertility (Bilby et al., 1999). It is plausible to suggest that effects of bST in modulating IGF-1 to enhance fertility of cattle depend on the endogenous concentrations of GH and IGF-1. In cows in which lactation-induced uncoupling of the GH-IGF system is not observed (Jiang et al., 2005), supplemental bST might not be beneficial because such cows would be able to sustain increased concentrations of IGF-1 during the transition and breeding periods. On the other hand, treatment of lactating dairy cows, which are known to have reduced IGF-1 concentrations, with 500 mg of bST as a single or 2 treatments, or sequentially every 14 d, is expected to improve fertility. Administration of higher doses of bST (i.e., sequential injections of 500 mg at 10-d intervals), however, seems not to have a fertility-enhancing effect in lactating dairy cows.

CONCLUSIONS

Treatment of lactating dairy cows with 500 mg of bST injected at 10-d intervals for 60 d increased yields of milk and milk components. In the 76 d following treatments, BCS of bST-treated cows remained unaltered, whereas control cows gained BCS. Treatment with bST increased concentrations of IGF-1 chronically, but concentrations of insulin and glucose increased only transiently in the 7 d following the first treatment. Concentrations of progesterone during the Ovsynch protocol and after timed AI and ovulatory responses during the Ovsynch protocol were mostly unaltered by treatment. Estrous behavior was depressed in cows receiving bST despite greater concentrations of estradiol. Administration of 500 mg of bST at 10-d intervals did not influence P/AI or pregnancy loss after the first 2 postpartum inseminations. Treatment of lactating dairy cows with 500 mg of bST every 10 d improved lactation performance, but it did not affect P/AI and it reduced expression of estrus.

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REFERENCES

- Bauman, D. E., D. L. Hard, B. A. Crooker, M. S. Partidge, K. Garrick, L. D. Sandles, H. N. Erb, S. E. Franson, G. F. Hartnell, and R. L. Hintz. 1985. Long-term evaluation of a prolonged-release formulation of N-methionyl bovine somatotropin in lactating dairy cows. *J. Dairy Sci.* 72:642–651.
- Bauman, D. E., C. J. Peel, W. D. Steinhour, P. J. Reynolds, H. F. Tyrrell, A. C. G. Brown, and G. L. Haaland. 1988. Effect of bovine somatotropin on metabolism of lactating dairy cows: Influence on rates of irreversible loss and oxidation of glucose and nonesterified fatty acids. *J. Nutr.* 118:1031–1040.
- Beg, M. A., and O. J. Ginther. 2006. Follicle selection in cattle and horses: Role of intrafollicular factors. *Reproduction* 132:365–377.
- Bilby, C. R., J. F. Bader, B. E. Salfen, R. S. Youngquist, C. N. Murphy, H. A. Garverick, B. A. Crooker, and M. C. Lucy. 1999. Plasma GH, IGF-I, and conception rate in cattle treated with low doses of recombinant bovine GH. *Theriogenology* 51:1285–1296.
- Bilby, T. R., A. Guzeloglu, S. Kamimura, S. M. Pancarci, F. Michel, H. H. Head, and W. W. Thatcher. 2004. Pregnancy and bovine somatotropin in nonlactating dairy cows: I. Ovarian, conceptus, and insulin-like growth factor system responses. *J. Dairy Sci.* 87:3256–3267.
- Bilby, T. R., A. Sozzi, M. M. Lopez, F. T. Silvestre, A. D. Ealy, C. R. Staples, and W. W. Thatcher. 2006. Pregnancy, bovine somatotropin, and dietary n-3 fatty acids in lactating dairy cows: I. Ovarian, conceptus, and growth hormone–insulin-like growth factor system responses. *J. Dairy Sci.* 89:3360–3374.
- Cerri, R. L. A., J. E. P. Santos, S. O. Juchem, K. N. Galvão, and R. C. Chebel. 2004. Timed artificial insemination with estradiol cypionate or insemination at estrus in high-producing dairy cows. *J. Dairy Sci.* 87:3704–3715.
- Collier, R. J., J. C. Byatt, S. C. Denham, P. J. Eppard, A. C. Fabellar, R. L. Hintz, M. F. McGrath, C. L. McLaughlin, J. K. Shearer, J. J. Veenhuizen, and J. L. Vicini. 2001. Effects of sustained release bovine somatotropin (sometribove) on animal health in commercial dairy herds. *J. Dairy Sci.* 84:1098–1108.
- Dohoo, I. R., L. DesCôteaux, K. Leslie, A. Fredeen, W. Shewfelt, A. Preston, and P. Dowling. 2003. A meta-analysis review of the effects of recombinant bovine somatotropin. 2. Effects on animal health, reproductive performance, and culling. *Can. J. Vet. Res.* 67:252–264.
- Downer, J. V., D. L. Patterson, D. W. Rock, W. V. Chalupa, R. M. Cleale, J. L. Firkins, G. L. Lynch, J. H. Clark, B. O. Brodie, B. F. Jenny, and R. De Gregorio. 1993. Dose titration of sustained-release recombinant bovine somatotropin in lactating dairy cows. *J. Dairy Sci.* 76:1125–1136.
- Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci.* 77:2695–2703.
- Ginther, O. J., D. R. Bergfelt, M. A. Beg, C. Meira, and K. Kot. 2004. In vivo effects of an intrafollicular injection of insulin-like growth factor-1 on the mechanism of follicle deviation in heifers and mares. *Biol. Reprod.* 70:99–105.
- Gong, J. G., D. McBride, T. A. Bramley, and R. Webb. 1993. Effects of recombinant bovine somatotrophin, insulin-like growth factor-I and insulin on the proliferation of bovine granulosa cells in vitro. *J. Endocrinol.* 139:67–75.
- Hammon, H. M., C. Philipona, Y. Zbinden, J. W. Blum, and S. S. Donkin. 2005. Effects of dexamethasone and growth hormone treatment on hepatic gluconeogenic enzymes in calves. *J. Dairy Sci.* 88:2107–2116.
- Hartnell, G. F., S. E. Franson, D. E. Bauman, H. H. Head, J. T. Huber, R. C. Lamb, K. S. Madsen, W. J. Cole, and R. L. Hintz. 1991. Evaluation of sometribove in a prolonged-release system in lactating dairy cows—Production responses. *J. Dairy Sci.* 77:2645–2663.
- Jiang, H., M. C. Lucy, B. A. Crooker, and W. E. Beal. 2005. Expression of growth hormone receptor 1A mRNA is decreased in dairy cows but not in beef cows at parturition. *J. Dairy Sci.* 88:1370–1377.
- Kirby, C. J., M. F. Smith, D. H. Keisler, and M. C. Lucy. 1997b. Follicular function in lactating dairy cows treated with sustained-release bovine somatotropin. *J. Dairy Sci.* 80:273–285.
- Kirby, C. J., S. J. Wilson, and M. C. Lucy. 1997a. Response of dairy cows treated with bovine somatotropin to a luteolytic dose of prostaglandin F_{2α}. *J. Dairy Sci.* 80:286–294.
- Knapp, J. R., H. C. Freetly, B. L. Reis, C. C. Calvert, and R. L. Baldwin. 1992. Effects of somatotropin and substrates on patterns of liver metabolism in lactating dairy cattle. *J. Dairy Sci.* 75:1025–1035.
- Lefebvre, D. M., and E. Block. 1992. Effect of recombinant bovine somatotropin on estradiol-induced estrous behavior in ovariectomized heifers. *J. Dairy Sci.* 75:1461–1464.
- Lucy, M. C. 2000. Regulation of ovarian follicular growth by somatotropin and insulin-like growth factors in cattle. *J. Dairy Sci.* 83:1635–1647.
- Lucy, M. C., T. L. Curran, R. J. Collier, and W. J. Cole. 1994. Extended function of the corpus luteum and earlier development of the second follicular wave in heifers treated with bovine somatotropin. *Theriogenology* 41:561–572.
- Morales-Roura, J. S., L. Zarco, J. Hernández-Cerón, and G. Rodríguez. 2001. Effect of short-term treatment with bovine somatotropin at estrus on conception rate and luteal function of repeat-breeding dairy cows. *Theriogenology* 55:1831–1841.
- Morbeck, D. E., J. H. Britt, and B. T. McDaniel. 1991. Relationships among milk yield, metabolism, and reproductive performance of primiparous Holstein cows treated with somatotropin. *J. Dairy Sci.* 74:2153–2164.
- Moreira, F., C. A. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J. Dairy Sci.* 84:1646–1659.
- Moreira, F., F. F. Paula-Lopes, P. J. Hansen, L. Badinga, and W. W. Thatcher. 2002. Effects of growth hormone and insulin-like growth factor-I on development of in vitro derived bovine embryos. *Theriogenology* 57:895–907.
- Moreira, F., C. A. Risco, M. F. A. Pires, J. D. Ambrose, M. Drost, M. DeLorenzo, and W. W. Thatcher. 2000. Use of bovine somatotropin in lactating dairy cows receiving timed artificial insemination. *J. Dairy Sci.* 83:1245–1255.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Obese, F. Y., S. Humphrys, K. L. Macmillan, and A. R. Egan. 2008. Measuring concentrations of insulin-like growth factor-I with an enzyme-linked immunosorbent antibody assay in plasma samples from Holstein cows. *J. Dairy Sci.* 91:160–168.
- Pershing, R. A., S. D. Moore, A. C. Dinges, W. W. Thatcher, and L. Badinga. 2002. Short communication: Hepatic gene expression for gluconeogenic enzymes in lactating dairy cows treated with bovine somatotropin. *J. Dairy Sci.* 85:504–506.
- Pinto, A. B., A. L. Schlein, and K. H. Moley. 2002. Preimplantation exposure to high insulin-like growth factor I concentrations results in increased resorption rates in vivo. *Hum. Reprod.* 17:457–462.
- Santos, J. E. P., S. O. Juchem, R. L. A. Cerri, K. N. Galvão, R. C. Chebel, W. W. Thatcher, C. Dei, and C. Bilby. 2004. Effect of bST and reproductive management on reproductive and lactational performance of Holstein dairy cows. *J. Dairy Sci.* 87:868–881.
- Starbuck, M. J., E. K. Inskeep, and R. A. Dailey. 2006. Effect of a single growth hormone (rbST) treatment at breeding on conception rates and pregnancy retention in dairy and beef cattle. *Anim. Reprod. Sci.* 93:349–359.
- Velazquez, M. A., L. J. Spicer, and D. C. Wathes. 2008. The role of endocrine insulin-like growth factor-1 (IGF-1) in female bovine reproduction. *Domest. Anim. Endocrinol.* 35:325–342.
- Wiltbank, M., H. Lopez, R. Sartori, S. Sangsritavong, and A. Gümen. 2006. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. *Theriogenology* 65:17–29.