Effect of bST and Reproductive Management on Reproductive Performance of Holstein Dairy Cows*

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ABSTRACT

The objective was to determine the effects of bovine somatotropin (bST) and two artificial insemination (AI) protocols on reproductive performance of Holstein cows. Lactating cows (n = 840) were assigned at 37 d in milk (DIM) to one of four treatments in a 2×2 factorial arrangement. Treatments consisted of either bST (500 mg/14 d) starting at 63 ± 3 DIM or no bST (control), with cows either submitted for timed AI following a synchronized ovulation (Ovsynch) protocol or assigned to receive AI based on estrus detection (ED). Two injections of $PGF_{2\alpha}$ at 37 ± 3 and 51 ± 3 DIM were used to presynchronize estrous cycles. Cows then received an injection of GnRH at 63 ± 3 DIM, followed 7.5 d later by $PGF_{2\alpha}$. Cows assigned to ED treatments were inseminated after observed estrus during a 7-d period. Cows in Ovsynch treatments received a second GnRH injection 48 h after the last $PGF_{2\alpha}$ and received timed AI 16 to 18 h later. Pregnancy was diagnosed by ultrasound at 31 d after AI and confirmed 14 d later. Frequency of anovulation (18.5%) at 63 DIM was similar across treatments, but proportions of anovulatory cows decreased quadratically as body condition at 70 DIM increased from 2.25 to 3.75. Estrus detection rate after $PGF_{2\alpha}$ tended to be lower in multiparous cows receiving bST, and bST reduced returns to estrus in nonpregnant cows. Conception rates were higher in cows receiving AI after ED and bST improved conception rates to first AI in cyclic cows by reducing embryonic mortality. Pregnancy loss was similar for cows inseminated following ED or the Ovsynch protocol. There was a positive impact of bST on fertility of cyclic cows inseminated at fixed time or at detected estrus, but effective resynchronization protocols are needed to optimize reinsemination of nonpregnant bST-treated cows.
(**Key words:** bovine somatotropin, reproduction, timed AI, dairy cow)

Abbreviation key: CL = corpus luteum, **ED** = group assigned to be inseminated after detection of estrus, **GH** = growth hormone, **Ovsynch** = synchronized ovulation using sequential injections of GnRH and $PGF_{2\alpha}$, **TAI** = timed artificial insemination.

INTRODUCTION

Bovine somatotropin is used to improve lactation performance and treatment with 500 mg of bST at 14-d intervals has increased milk production by an average of 3 to 5 kg/d (Hartnell et al., 1991; Bauman et al., 1999). However, some studies have suggested negative effects of bST treatment on reproduction in dairy cows (Morbeck et al., 1991; Cole et al., 1992; Lefbreve and Block, 1992; Kirby et al., 1997b). In those studies, the negative effect of bST was related to dose, time of initiation of bST treatment, breeding, nutritional factors, and milk production. Others have suggested that the possible negative effects of bST on reproductive efficiency in dairy cows might be mediated by attenuation of expression of estrus, with consequent decrease in rates of detected estrus and insemination (Morbeck et al., 1991; Cole et al., 1992; Lefbreve and Block, 1992; Kirby et al., 1997b).

Recently, a comprehensive study involving over 1200 cows in 28 dairy farms throughout the United States indicated that bST treatment did not affect days open, percentage of cows pregnant at the end of a lactation, twin births, incidence of cystic ovaries, and calving rates (Collier et al., 2001). In fact, experiments with

large numbers of lactating cows have demonstrated that treatment with bST improves fertility of cyclic lactating dairy cows inseminated at a fixed time (Moreira et al., 2000; Moreira et al., 2001) and of cows considered to be subfertile (Morales-Roura et al., 2001). These effects of bST on fertility of lactating cows are substantiated by the positive effects of growth hormone (GH) and IGF-I on fertilization and embryonic development (Moreira et al., 2002a, 2002b). Furthermore, bST and IGF-I influence follicle development, luteal function, and endometrial secretion of $PGF_{2\alpha}$. Treatment with bST altered follicle development and the interval between follicular waves with earlier emergence of the second wave of follicle growth (Lucy et al., 1994; Kirby et al., 1997a). Luteal cells express GH receptors (Hull and Harvey, 2001), and mRNA for GH receptors has been detected in luteal cells (Lucy et al., 1998; Lucy, 2000). Progesterone concentrations in blood of heifers increased with bST treatment (Lucy et al., 1994), and higher concentrations of progesterone in peripheral blood are associated with enhanced embryo development (Mann and Lamming, 2001) and increased fertility (Santos et al., 2001). Incubation of bovine endometrial cells with GH-attenuated $PGF_{2\alpha}$ secretion in vitro (Badinga et al., 2002), which might favor pregnancy maintenance (Binelli et al., 2001). Therefore, GH is an important modulator of reproductive functions, and it is essential for normal reproduction in females (Hull and Harvey, 2001).

Although bST treatment has been shown to enhance fertility of cyclic dairy cows inseminated at fixed times, the effects of bST on conception and detection of estrus among lactating cows subjected to different reproductive managements in experiments with large numbers of animals are not well documented. The objectives of the present experiment were to determine the effects of bST on reproductive traits in lactating dairy cows when subjected to insemination at fixed times or following detection of estrus.

MATERIALS AND METHODS

Animals, Housing, and Feeding

Of 840 lactating Holstein cows, 415 primiparous and 425 multiparous from a commercial dairy farm in the Central Valley of California were assigned to each of 4 treatments. The experiment was conducted from October 2001 to July 2002. During the entire study period, primiparous and multiparous cows were housed separately in 2 free-stall barns. Each free-stall barn consisted of 2 pens with capacity to house 200 cows each.

All cows received the same TMR consisting of corn silage, alfalfa hay, alfalfa silage, steam-flaked corn, whole cottonseed, pelleted soybean hulls, soybean meal, shredded beet pulp, dried corn distiller's grains, blood meal, animal fat, calcium salts of palm fatty acids, minerals, and vitamins. Cows were fed as a group, and the 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). Throughout the study, diets contained at least 18.1% CP, 6.0% ether extract, 30.5% NDF, and an NE$_L$ of 1.67 Mcal/kg after adjusting for 24 kg of DMI. Diets were fed twice daily, with an expected 3% refusal of the total amount offered daily.

Cows were milked twice daily, starting at 0500 and 1700 h. Milk yields were recorded for individual cows once monthly during the official California DHIA test. Individual milk samples were also collected during DHIA testing from consecutive milkings (a.m. and p.m.), composited, and analyzed for SCC, fat, and true protein concentrations (Foss 303 Milk-O-Scan; Foss Foods, Inc.; Eden Prairie, MN) at the DHIA Laboratory in Fresno, CA.

Treatments

Cows were randomly assigned to each of 4 treatments consisting of 2 AI protocols with or without bST treatment. At enrollment, cows were palpated per rectum to eliminate animals with ovarian or uterine adhesions, pyometra, or any other genital-tract abnormality that was considered to compromise future fertility. Only healthy cows with a BCS equal to or greater than 2.5 (Ferguson et al., 1994) were assigned to the study. Of 840 cows initially enrolled in the study, 5 were excluded from the statistical analyses because they either died before the beginning of treatments and data collection, or because the correct protocols established for the respective treatment of the animals were not followed. Treatments were bST (POSILAC, 500 mg, Monsanto Co., St. Louis, MO), given subcutaneously in the space between the ischium and tailhead at 14-d intervals starting at 63 ± 3 DIM or no bST (control), with cows submitted to 2 insemination protocols, a timed artificial insemination (TAI) following a synchronized ovulation (**Ovsynch**) program (Pursley et al., 1997a), or at detected estrus (**ED**). Therefore, the four treatments were: ED with bST (ED-bST), ED with no bST (ED-control), Ovsynch with bST (Ovsynch-bST), and Ovsynch with no bST (Ovsynch-control).

A diagram of activities is displayed in Figure 1. Cows were subjected to a presynchronization treatment with i.m. injections of 25 mg of $PGF_{2\alpha}$ (Dinoprost Tromethamine, Lutalyse, Pharmacia Animal Health, Kalamazoo, MI) at 37 ± 3 and 51 ± 3 d postpartum (Moreira et al., 2001). Twelve days after the second $PGF_{2\alpha}$ (63 ± 3 DIM), all cows received an i.m. injection of 100 μg of GnRH
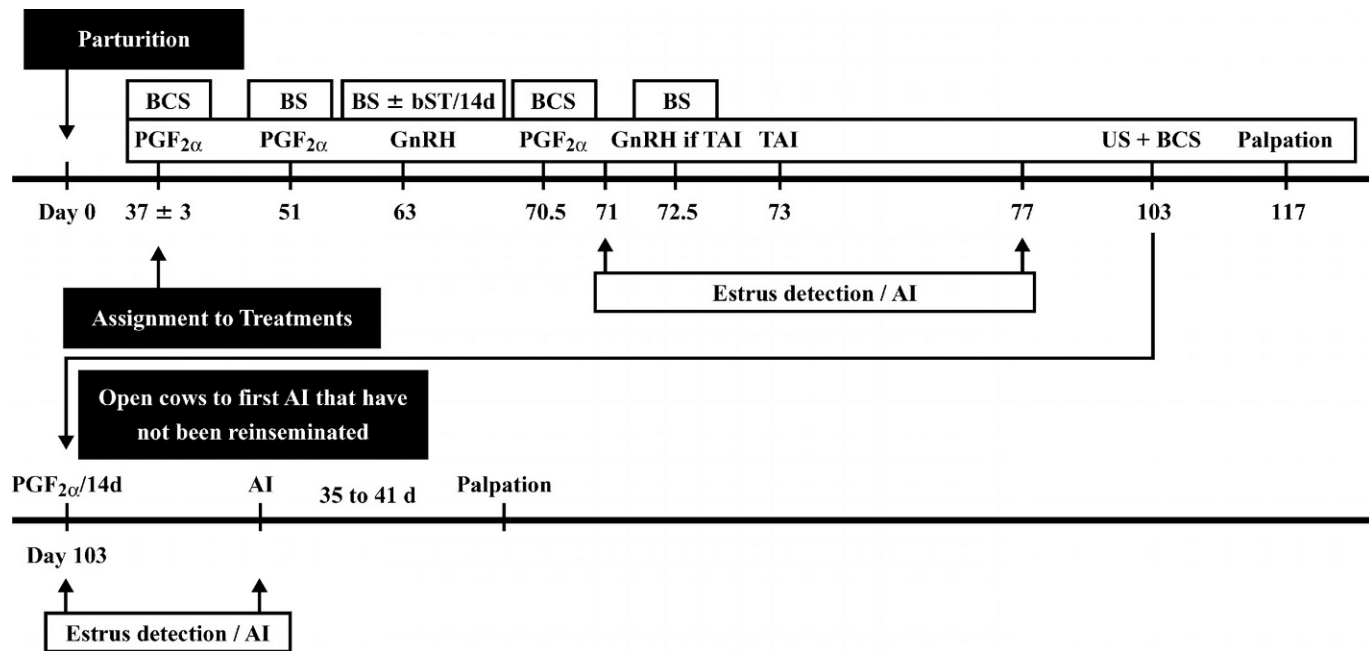
Figure 1. Diagram of activities and treatments during the study. BS = Blood sample, TAI = timed artificial insemination, US = ultrasound.

(Gonadorelin Hydrochloride, Factrel, Fort Dodge Animal Health, Fort Dodge, IA). At 7.5 d after the GnRH injection, cows received a third injection of $PGF_{2\alpha}$. Cows enrolled in the ED-bST and ED-control treatments were observed for signs of estrus once every morning by tail chalking (Macmillan et al., 1988) using paintsticks (All-Weather Paintstik, LA-CO Industries, Chicago, IL). Cows detected in estrus in the 7 d following the last $PGF_{2\alpha}$ injection were inseminated that same morning, and those not observed in estrus were considered nonresponsive to the protocol. Cows in the Ovsynch treatments received a second injection of GnRH 48 h after $PGF_{2\alpha}$ and were timed inseminated 16 to 18 h later. The same technician artificially inseminated all cows throughout the study. After the initial AI, cows were observed for signs of estrus once in the morning by tail chalking (Macmillan et al., 1988) using paintsticks and were inseminated that same morning.

Pregnancy was diagnosed by ultrasonography when cows were 31 ± 2 d after AI. Four cows were examined for pregnancy at 28 d after AI. Observation of embryonic fluid, appearance of the embryo, and embryonic heartbeat were used as determinants of pregnancy. A real-time ultrasound scanner (Sonovet 2000, Universal Medical System, Bedford Hills, NY) equipped with a 7.5-MHz rectal probe was used. Those cows diagnosed as pregnant on d 31 had their pregnancies reconfirmed 14 d later by palpation per rectum of an embryonic vesicle. Cows diagnosed as nonpregnant on d 31 and that had not been reinseminated received an injection

of $PGF_{2\alpha}$ at 14-d intervals until observed in estrus and inseminated. Pregnancy in the second AI was diagnosed by palpation 35 to 41 d after insemination.

Body condition of all cows was scored (Ferguson et al., 1994) at the beginning of the study (37 ± 3 DIM), on the day of the $PGF_{2\alpha}$ (70 DIM) just before first postpartum AI, and at pregnancy diagnosis (103 DIM).

Blood-Sample Collection and Analyses

Blood samples (10 mL) were collected from every cow by puncture of the coccygeal vein or artery into evacuated tubes containing sodium EDTA (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) at 51 and 63 DIM, which coincided with the second injection of $PGF_{2\alpha}$ and the first of GnRH during the synchronization protocol. A third blood sample was collected 48 h after the last $PGF_{2\alpha}$. Samples were placed immediately on ice and arrived at the laboratory within 5 h of collection. Blood tubes were centrifuged at $1500 \times g$ for 15 min in a refrigerated centrifuge at 10°C for plasma separation. Plasma was frozen at −25°C and later analyzed for progesterone by a solid-phase radioimmunoassay using a commercial kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). The assay sensitivity was 0.10 ng/mL and the intra- and interassay CV were 6.9 and 10.1%, respectively. Progesterone concentrations in the first 2 samples were used to determine whether cows were cycling or not during the first 63 DIM. Cows with plasma progesterone ≥ 1.0 ng/mL in at

least one of the two samples were considered cycling, and those with both plasma samples containing <1.0 ng/mL were considered as anovulatory/anestrous. The third plasma sample was used to determine whether corpus luteum (**CL**) regression occurred 48 h after the last $PGF_{2\alpha}$ just before AI. Regression of the CL was considered if plasma progesterone was <1.0 ng/mL.

Experimental Design and Statistical Analyses

The experimental design was a randomized complete block design (Kuehl, 1994). Weekly, a cohort of 30 to 60 cows with a BCS ≥2.50 and displaying no signs of clinical illness were blocked according to parity and milk production during the first month of lactation. Within each block, cows were randomly assigned to each of 4 treatments in a 2 × 2 factorial arrangement of treatments, with bST and insemination protocol as the main factors.

Lactation performance and BCS were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS (Littell et al., 1998) with observed mean, block, bST (bST vs. control), AI protocol (Ovsynch vs. ED), period (test date month), the interaction between bST and period, parity, BCS at 70 DIM, BCS change from 37 to 70 DIM, the pretreatment covariate, higher order interactions, and cow nested within treatment as the random error. For analyses of yields of milk and milk components, individual cow deviation from the mean production of primiparous and multiparous cows before bST treatment was used as covariate. For analyses of BCS, the initial BCS at 37 DIM was used as a covariate. To determine whether an effect of bST on SCS was caused by bST treatment or by changes in milk yield, in addition to the mean SCS in the first 2 mo in lactation, the average monthly milk production after bST treatment and the interaction between bST and average milk production after bST treatment were also used as covariates. The covariance structure (unstructured, compound symmetry, toeplitz, and autoregressive) for the repeated measures in the MIXED models was tested (Littell et al., 2000), and the autoregressive was chosen as the one that best fit the data.

Binomially distributed data, such as percentages of cows detected in estrus, conception rate, pregnancy rate, and pregnancy loss, were analyzed by logistic regression (Allison, 1999) by the LOGISTIC procedure of SAS (2001) using a backward stepwise logistic model that included: the effects of bST, AI protocol, interaction between bST and AI protocol, parity, BCS at AI, BCS change from d 37 to 70 postpartum, cyclicity, CL regression 48 h after the last $PGF_{2\alpha}$ before AI, milk production during the first 90 DIM, and higher order interactions. Variables were continuously removed from the model

by the Wald statistic criterion, if the significance was greater than 0.20.

Conception rate was defined as the number of pregnant cows at any given time (31 or 45 d after AI) divided by the total number of cows inseminated within each treatment group. Pregnancy rate was defined as the number of pregnant cows at any given time (31 or 45 d after AI) divided by the total number of cows in each treatment.

The interval between the first and second postpartum AI in cows diagnosed as nonpregnant at the ultrasound examination was analyzed by ANOVA (Littell et al., 2002) using the GLM procedure of SAS (2001) with a model that included the effects of bST, AI protocol, interaction between bST and AI protocol, parity, BCS at AI, BCS change from d 37 to 70 postpartum, cyclicity, CL regression, milk production, and higher-order interactions. Similarly, the interval between the first bST injection and the diagnosis of the first clinical mastitis case was analyzed by ANOVA (Littell et al., 2002) using the GLM procedure of SAS (2001) with a model that included bST, AI protocol, interaction between bST and AI protocol, parity, mastitis incidence before bST treatment, SCS before bST treatment, and higher-order interactions.

Incidence of clinical mastitis after the initial bST treatment injection was analyzed by logistic regression (Allison, 1999) by the LOGISTIC procedure of SAS (2001) using a backward stepwise logistic procedure, as described previously, based on a mathematical model that included bST, AI protocol, interaction between bST and AI protocol, parity, mastitis before the initial bST treatment, SCS before the initial bST treatment, and higher-order interactions. Number of clinical mastitis cases per cow after the initial bST treatment was analyzed by the GENMOD procedure, using a Poisson distribution and log transformation function (Allison, 1999) with the SAS (2001) program.

Regression analyses were performed to determine the best fitted line plot between BCS at AI and the frequency of anovulation before the first AI using the regression procedure of MINITAB (2000). Cows were grouped based on their BCS at AI, which ranged from 2.25 to 3.75 and, within each group, the frequency of anovulation was calculated. The number of cows within each BCS group ranged from 53 to 170 animals. Similarly, milk response to bST (difference between control and bST-treated cows) was plotted against test-day DIM to determine the best fitted line between milk response and test-day DIM during the first 171 d in lactation for primiparous and multiparous cows.

Treatment differences with $P ≤ 0.05$ were considered significant and $0.05 < P ≤ 0.10$ were considered as a tendency.
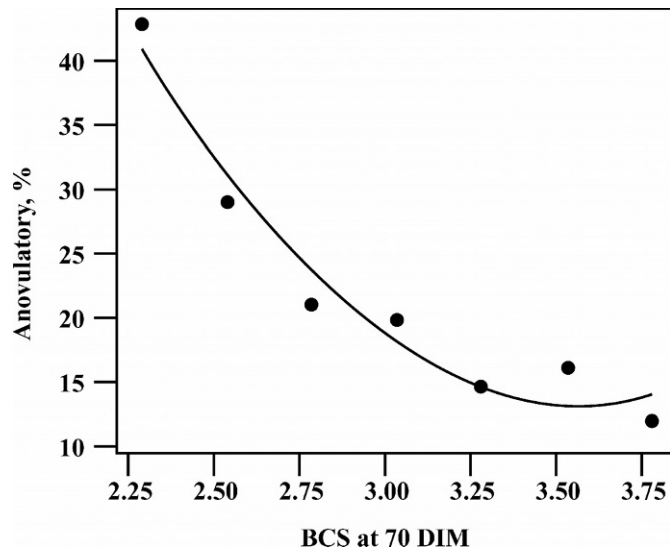
Figure 2. Relationship between the frequency of cows classified as anovulatory/anestrous based on plasma progesterone (<1.0 ng/mL) on d 51 and 63 and BCS at 70 DIM in lactating dairy cows. Frequency of anovulation = 223.114 − 118.914 X + 16.8457 X^2, where X is the BCS at 70 DIM ($P < 0.02$).

RESULTS

The DIM and lactation number at the beginning of the study were similar ($P > 0.40$) for ED-bST, ED-control, Ovsynch-bST, and Ovsynch-control, and they averaged 37.0 and 2.0, 36.7 and 2.0, 36.8 and 2.0, and 37.0 and 2.0, respectively. Forty-one cows were not enrolled in the study at 37 ± 3 DIM because of uterine or ovarian adhesions (12 cows), lame cows moved to a dirt lot with bulls (19 cows), cows with poor udder conformation that were designated for noninsemination (2 cows), sick cows with BCS < 2.5 (4 cows), and healthy cows with BCS < 2.50 (4 cows).

Cyclicity

Incidence of cyclicity before first AI was similar ($P = 0.21$) among all treatments, and it was 80.1, 80.8, 79.2, and 85.4% for ED-bST, ED-control, Ovsynch-bST, and Ovsynch-control, respectively. More multiparous cows were cycling than primiparous cows in the first 63 DIM (85.9 vs. 77.1%; $P < 0.001$). Milk production in the first 3 mo of lactation had no effect on cyclicity ($P = 0.21$). However, changes in BCS from 37 to 70 d postpartum, as well as the BCS at AI (70 d postpartum) were related to cyclicity in the first 63 DIM. In cows that maintained and gained BCS, and in cows that lost BCS from 37 to 70 d postpartum, incidence of cyclicity was 83.6 and 78.1%, respectively ($P = 0.05$). Furthermore, a greater proportion of cows with a BCS at AI equal to or greater than 3.0 were cyclic, compared with those with a BCS equal to or less than 2.75 (83.9 vs. 75.5; $P < 0.001$). In fact, when regression analysis was performed between BCS at AI and frequency of cows classified as anovulatory (Figure 2), a quadratic relationship was established. It became clear that frequency of anovulation decreased as BCS at AI increased ($P = 0.02$). Interestingly, an interaction between BCS changes and BCS at AI was detected for incidence of cyclicity ($P = 0.05$). In cows with a BCS equal to, or greater than, 3.0 on d 70, incidence of cyclicity in cows that maintained or gained BCS (83.5%) was similar to those that lost BCS (85.0%) from 37 to 70 DIM. However, in cows with low BCS on d 70, incidence of cyclicity in cows that maintained or gained BCS was 84.6% compared with 72.8% in those that lost BCS from 37 to 70 DIM. These data indicate that changes in BCS from 37 to 70 DIM affected cyclicity in the first 63 DIM only among the lactating cows with a low BCS on d 70 postpartum.

Detection of Estrus in ED Cows and Reproductive Performance of All Cows at First Insemination

An interaction between bST and parity was detected for the rate of detected estrus in the 7 d following the PGF$_{2\alpha}$ injection before the first postpartum AI (Table 1; $P = 0.10$). In primiparous cows, bST had no effect on detection of estrus (73.1%). However, in multiparous cows, bST tended to reduce the percentage detected in

Table 1. Effects of bovine somatotropin (bST) on estrus detection rate (EDR) in estrus detected (ED) cows and interinsemination interval for all cows.

| | Treatment | | | | | $P <$ | | |
| | Control % (no.) | | bST % (no.) | | | | | |
Item	Primiparous	Multiparous	Primiparous	Multiparous	SEM	bST	Parity	bST × Parity
EDR for ED treatments	72.4 (105)	79.6 (108)	73.8 (103)	64.7 (99)	. . .	0.11	0.54	0.10
Open cows rebred by d 21	30.1 (103)	21.5 (107)	17.9 (84)	15.0 (100)	. . .	0.02	0.23	0.49
Open cows rebred by d 31	67.0 (103)	59.8 (107)	48.8 (84)	56.0 (100)	. . .	0.03	0.40	0.08
Inter-AI interval by d 31, d	21.3	21.1	21.9	22.9	0.89	0.06	0.56	0.36
Inter-AI interval, d	31.0	31.4	35.4	34.2	1.76	0.02	0.78	0.59

Table 2. Effect of bST and insemination protocol (IP) on reproductive performance of all lactating cows in the first postpartum AI.

| | Treatment[1] | | | | $P <$ | | |
| | Control | | bST | | | | |
Item	ED	Ovsynch	ED	Ovsynch	bST	IP	bST × IP
Number of cows	212	212	202	209	. . .	. . .	. . .
Service rate, %	75.5	100.0	68.3	100.0	0.15	0.01	. . .
Conception rate, %							
Day 31	45.6	40.6	52.9	39.2	0.51	0.02	0.41
Day 45	38.8	34.5	50.4	34.5	0.13	0.01	0.18
Pregnancy rate, %							
Day 31	34.4	40.6	36.1	39.2	0.99	0.23	0.88
Day 45	29.3	34.5	34.3	34.5	0.45	0.52	0.58
Pregnancy loss, %	15.1	13.3	4.2	12.2	0.06	0.20	0.12

[1]ED = AI based on detection of estrus after GnRH and $PGF_{2\alpha}$; Ovsynch = synchronized ovulation and followed by timed AI.

estrus (64.7 vs. 79.6%; $P = 0.10$). After first AI, bST treatment reduced ($P = 0.02$) the proportion of nonpregnant cows reinseminated by the end of the first 21-d estrous cycle. Similarly, the proportion of nonpregnant cows that was reinseminated before the pregnancy diagnosis on d 31 after the initial AI was reduced ($P = 0.03$) by bST treatment. This decrease in reinsemination rates caused by bST tended ($P = 0.08$) to be greater for primiparous than multiparous cows. The reduced reinsemination rates of nonpregnant cows treated with bST resulted in a tendency ($P = 0.06$) to increase the interval between AI for those cows reinseminated before the pregnancy diagnosis on d 31. Similarly, the interval between the first and second postpartum inseminations was longer for bST-treated cows ($P = 0.02$).

As expected, service rates for cows in the Ovsynch treatments, which were subjected to TAI, were higher than those in cows in the ED treatments (Table 2).

Conception rate on d 31 after the first AI was not affected by bST, but a greater proportion of cows inseminated at detected estrus was pregnant on d 31 compared with those subjected to TAI (49.0 vs. 39.9%; $P = 0.02$). Primiparous cows tended to have higher conception rates on d 31 after AI compared with multiparous cows (45.9 vs. 41.5%; $P = 0.09$). Furthermore, cows that were cyclic before first AI had higher conception on d 31 than anovulatory cows (Table 3), and cows with plasma progesterone < 1 ng/mL 48 h after the $PGF_{2\alpha}$ treatment immediately before AI also had higher conception on d 31 than those with progesterone ≥ 1 ng/mL.

Conception rate on d 45 after AI was numerically higher for bST-treated cows, but not statistically different (40.8 vs. 36.3%; $P = 0.13$). However, cows inseminated following detected estrus had higher fertility than those inseminated following TAI (44.1 vs. 34.5%; $P = 0.01$). Cows that were cyclic before first AI had

Table 3. Effect of cyclicity at 63 DIM and progesterone concentrations 48 h after $PGF_{2\alpha}$ on reproductive performance of all lactating cows in the first postpartum AI.

| | Subgroup categories[1] | | | | $P <$ | |
| | Cyclic | | Progesterone, ng/mL | | | |
Item	Yes	No	< 1.0	≥ 1.0	Cyclicity	Progesterone
Number of cows	685	155	774	61	. . .	. . .
Detection of estrus in ED[2] cows, %	84.1	15.9	74.6	54.1	0.002	0.001
Conception rate, %						
Day 31	45.8	33.1	45.1	22.7	0.02	0.01
Day 45	40.7	27.3	40.0	15.3	0.02	0.01
Pregnancy rate, %						
Day 31	40.2	26.0	39.3	15.4	0.001	0.001
Day 45	35.8	21.4	34.8	11.6	0.001	0.001
Pregnancy loss, %	10.4	17.5	11.0	22.2	0.43	0.27

[1]Cyclic = Based on one or both plasma progesterone concentrations at ≥ 1 ng/mL for samples at 51 and 63 DIM; progesterone = plasma progesterone either < 1 ng/mL or ≥ 1 ng/mL 48 h after the $PGF_{2\alpha}$ before insemination.

[2]ED = Cows to receive AI based on detection of estrus after GnRH and $PGF_{2\alpha}$.

Table 4. Effects of bST on estrus detection rate (EDR) in cyclic estrus detected (ED) cows and interinsemination interval for all cyclic cows.

Item	Control % (no.)		bST % (no.)		SEM	P <		
	Primiparous	Multiparous	Primiparous	Multiparous		bST	Parity	bST × Parity
EDR for ED treatments	77.6 (76)	80.2 (96)	79.5 (78)	65.9 (85)	. . .	0.17	0.19	0.10
Open cows rebred by d 21	27.7	23.1	17.5	13.4	. . .	0.03	0.36	0.96
Open cows rebred by d 31	68.7	62.6	55.6	59.8	. . .	0.13	0.96	0.23
Inter-AI interval by d 31, d	21.8	21.1	22.4	23.3	0.85	0.05	0.95	0.25
Inter-AI interval, d	29.1	28.1	33.2	31.1	1.83	0.03	0.35	0.72

higher conception on d 45 than anovulatory cows, and cows with progesterone <1.0 ng/mL after the $PGF_{2\alpha}$ treatment had higher conception than cows with progesterone ≥1.0 ng/mL (Table 3).

Pregnancy rates at 31 and 45 d after first AI were not affected by either bST or AI protocol (Table 2). Similar to conception rates, cyclicity before insemination and plasma progesterone after the $PGF_{2\alpha}$ treatment also influenced pregnancy rates (Table 3). Pregnancy rates on d 31 and 45 after the first postpartum AI tended to be affected by parity, with primiparous cows having higher pregnancy rates than multiparous cows at both 31 (39.5 vs. 35.8%; $P = 0.07$) and 45 d (35.1 vs. 31.1%; $P = 0.06$) after AI.

Losses of pregnancy between 31 and 45 d after AI were not affected by AI protocol, but bST treatment tended to reduce embryonic mortality (8.4 vs. 14.1%; $P = 0.06$). This positive trend of bST on pregnancy maintenance was observed regardless of AI protocol and parity, with no significant interaction observed between bST and AI protocol ($P = 0.20$) and bST and parity ($P = 0.11$). Cows with low BCS (≤ 2.75) at AI experienced higher pregnancy losses than cows with a moderate BCS (≥ 3.00) at AI (15.2 vs. 10.0%; $P = 0.02$).

Detection of Estrus in Cyclic ED Cows and Reproductive Performance of Cyclic Cows at First Insemination

Because cyclicity has a major influence on reproductive outcomes and bST improves conception rates in cycling cows inseminated at fixed time, additional analyses were performed with only animals that were cyclic in the first 63 DIM. A total of 685 cows were cyclic before the first postpartum AI, 365 multiparous and 320 primiparous.

An interaction between bST and parity was detected for percentages of cows detected in estrus in the 7 d following the $PGF_{2\alpha}$ injection before the first postpartum AI (Table 4; $P = 0.10$). In primiparous cows, bST had no effect on detection of estrus, and it averaged 78.6%. However, in multiparous cows, bST tended to reduce the percentage detected in estrus (65.9 vs. 80.2%; $P = 0.10$). Plasma progesterone 48 h after the $PGF_{2\alpha}$ treatment influenced percentages detected in estrus as more cows with progesterone <1.0 ng/mL were observed in estrus during the 7-d observation period than cows with progesterone >1.0 ng/mL (82.0 vs. 59.1%; $P < 0.001$).

Table 5. Effect of bST and insemination protocol (IP) on reproductive performance of cyclic lactating cows in the first postpartum AI.

Item	Treatment[1]				P <		
	Control		bST				
	ED	Ovsynch	ED	Ovsynch	bST	IP	bST × IP
Number of cows	171	181	163	166	. . .	. . .	. . .
Service rate, %	78.4	100.0	71.8	100.0	0.09	0.01	. . .
Conception rate, %							
Day 31	48.5	40.9	54.7	42.8	0.29	0.02	0.67
Day 45	40.3	35.4	52.6	38.6	0.05	0.03	0.30
Pregnancy rate, %							
Day 31	38.0	40.9	39.3	42.8	0.55	0.58	0.93
Day 45	31.6	35.4	37.7	38.6	0.14	0.49	0.63
Pregnancy loss, %	16.9	11.3	3.2	9.9	0.03	0.41	0.11

[1]ED = AI based on detection of estrus after GnRH and $PGF_{2\alpha}$; Ovsynch = synchronized ovulation followed by timed AI.

Table 6. Effect of bST and insemination protocol (IP) at the first postpartum AI on the second postpartum AI conception rate of all cows and only cyclic cows.[1]

	Control		bST		$P <$		
Item	ED	Ovsynch	ED	Ovsynch	bST	IP	bST $\times$ IP
All cows, N	133	118	123	120			
Conception rate, %	36.8	38.1	32.5	30	0.14	0.84	0.84
Cycling cows, N	102	101	94	94			
Conception rate, %	37.3	40.6	31.9	34.0	0.25	0.50	0.90

[1]ED = AI based on detection of estrus after GnRH and $PGF_{2\alpha}$; Ovsynch = synchronized ovulation followed by timed AI.

After the first postpartum AI, the proportion of non-pregnant cows that were observed in estrus and reinseminated before d 21 after AI was reduced by bST treatment (15.2 vs. 25.3%; $P = 0.03$), but the same effect was not observed by d 31 after AI ($P = 0.13$). The reduced reinsemination rates for nonpregnant cows treated with bST resulted in an increase in the interval between inseminations for those cows reinseminated before the pregnancy diagnosis on d 31 ($P = 0.05$). Similarly, the interval between the first and second postpartum inseminations was longer for bST-treated cows ($P = 0.03$).

Conception rate on d 31 after the first AI was not affected by bST (Table 5), but a greater proportion of cows inseminated at detected estrus was pregnant on d 31 compared to those timed inseminated (51.4 vs. 41.8%; $P = 0.02$). On d 45 after AI, conception rate was increased by cows inseminated following detected estrus (46.0 vs. 36.9%; $P = 0.03$) and by treatment with bST (44.3 vs. 37.5%; $P = 0.05$).

The positive effects of bST and AI protocol of cyclic cows on conception rate were not observed for pregnancy rates at 31 and 45 d after AI. However, a tendency for interaction between bST and parity was observed for pregnancy rates on d 31 ($P = 0.10$), and it was significant for d 45 pregnancy rate ($P = 0.05$). For multiparous cows, pregnancy rates on d 45 after AI were similar between bST-treated cows and controls (31.8 vs. 34.6%), but primiparous cows treated with bST had higher pregnancy rates than primiparous control cows (45.2 vs. 32.3%).

Conception rate was greater for cows with progesterone <1.0 ng/mL, compared with those with progesterone ≥1.0 ng/ml 48 h after the $PGF_{2\alpha}$ injection at 31 (47.3 vs. 23.5%; $P < 0.01$) and 45 d after AI (42.3 vs. 15.2%; $P < 0.01$). Similarly, pregnancy rates were higher for cows with progesterone <1.0 ng/mL than for those with progesterone ≥1.0 ng/mL 48 h after the $PGF_{2\alpha}$ injection at 45 d after AI (37.8 vs. 10.4%; $P < 0.001$).

Losses of pregnancy between 31 and 45 d after AI were not affected by AI protocol in cyclic cows ($P = 0.41$), but bST treatment reduced embryonic mortality (6.7 vs. 14.0%; $P = 0.03$). This positive effect of bST on preg-nancy maintenance was observed for cows inseminated at fixed time or following detected estrus, with no inter-action between bST and AI protocol ($P = 0.11$) and bST and parity ($P = 0.41$). Cows with progesterone <1.0 ng/mL at 48 h after $PGF_{2\alpha}$ tended to have lower pregnancy losses than those with progesterone ≥1.0 ng/mL (9.9 vs. 28.6%; $P = 0.07$).

Reproductive Responses to Second Postpartum AI

Nonpregnant cows after first AI were reinseminated, either following spontaneous return to estrus or estrus induced by $PGF_{2\alpha}$ treatment after pregnancy diagnosis on d 31. When all nonpregnant cows were included in the analyses, neither bST nor previous AI protocol affected conception rates to second postpartum AI (Table 6). Similarly, conception rate to second AI for cyclic cows before the first postpartum AI was not affected by bST or previous insemination AI protocol.

Lactation Performance and BCS

Treatment with bST increased test-day yields of milk, 3.5% FCM, milk fat, and milk true protein (Table 7). During the first 2 mo in lactation, cows in the control and bST-treated groups had similar milk production (Figure 3). However, an increase in milk yield due to bST was clearly observed after the second month in lactation. An interaction between bST and period was observed for milk production ($P < 0.0001$), which indi-cates that production declined more slowly after 81 DIM for cows treated with bST than control cows. This effect was more exacerbated in primiparous cows, as indi-cated by the interaction between bST, period, and parity on yields of milk ($P < 0.0001$). Milk responses to bST indicate a steady increase in response throughout the treatment period, and it ranged from 1 to 3.1 kg/d (Fig-ure 4). Regression analyses between test-day DIM and milk response resulted in a linear relationship for both primiparous ($r^2 = 0.76$; $P = 0.08$) and multiparous ($r^2 = 0.92$; $P = 0.02$) cows in the first 171 DIM. When re-

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Table 7. Effect of bovine somatotropin (bST) treatment on lactation performance of dairy cows between 63 and 171 DIM.

Item	Control	bST	SEM	P < bST	P < Period	P < bST × Period
Milk, kg/d	44.8	47.1	0.33	0.0001	0.0001	0.0001
3.5% FCM, kg/d	43.3	45.9	0.32	0.0001	0.0001	0.01
Milk fat						
%	3.31	3.37	0.02	0.05	0.04	0.41
g/d	1,478	1,577	12	0.0001	0.0001	0.30
Milk true protein						
%	2.89	2.89	0.01	0.72	0.0001	0.002
g/d	1,291	1,354	9	0.0001	0.0001	0.0001
BCS, mean	3.17	3.10	0.01	0.0001	0.05	0.0001
BCS change[1]	0.02	−0.11	0.015	0.0001	. . .	. . .

[1]BCS change from 37 to 103 DIM.

sponses from both parities were combined, a linear response to bST was observed as test-day DIM increased from 81 to 171 ($r^2 = 0.89$; $P = 0.04$). Percentage of milk fat was higher for bST-treated cows (Table 7; $P < 0.05$), but true protein concentration in milk was not altered by treatment. Therefore, increased production of milk fat with bST treatment was a result of higher milk yield and concentration of fat in milk, whereas increased production of true protein was caused only by a greater milk output in bST-treated cows.

The mean BCS after the beginning of bST treatment (70 and 103 DIM) was lower for cows receiving bST (Table 7; $P < 0.0001$). Cows in the control groups experienced a slight increase in BCS from 37 to 103 DIM, whereas bST-treated cows lost BCS ($P < 0.0001$). In fact, both controls and bST-treated cows had similar BCS at the initial bST treatment, but BCS increased in control cows after 70 DIM, whereas bST-treated cows continued to experience BCS loss until 103 DIM, as observed by the interaction between bST and period ($P < 0.0001$). Primiparous cows had higher BCS than multiparous cows (3.19 vs. 3.08; $P < 0.0001$), but no interaction between bST and parity was observed for mean BCS ($P = 0.97$) and for BCS changes ($P = 0.48$).

Body condition score at the initial bST treatment influenced milk responses to bST ($P < 0.001$) in both primiparous and multiparous cows. An interaction between BCS at the initial bST treatment and milk yield was observed ($P < 0.01$); cows with low BCS (≤2.75) at the first bST treatment had a 1.0 kg/d increase in milk
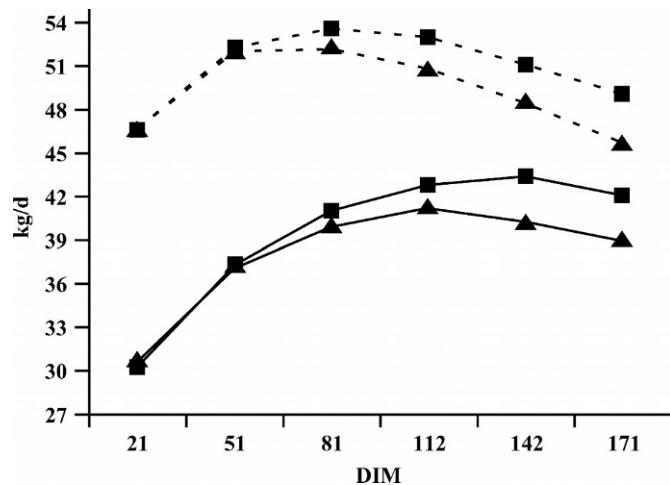
Figure 3. Least squares means of milk yield during the first 171 DIM in primiparous and multiparous dairy cows treated or not with bovine somatotropin (bST) starting at 63 DIM. Solid triangle (▲): control cows; solid squares (■): bST-treated cows; Continuous line: primiparous cows; Dashed lines: multiparous cows. Pooled SEM = 0.57. Statistical effects for data analyzed after the start of bST treatment. Effect of bST ($P < 0.0001$), parity ($P < 0.0001$), period ($P < 0.0001$), interaction between bST and period ($P < 0.0001$), interaction between bST and parity ($P < 0.89$), and interaction between bST and parity and period ($P < 0.0001$).
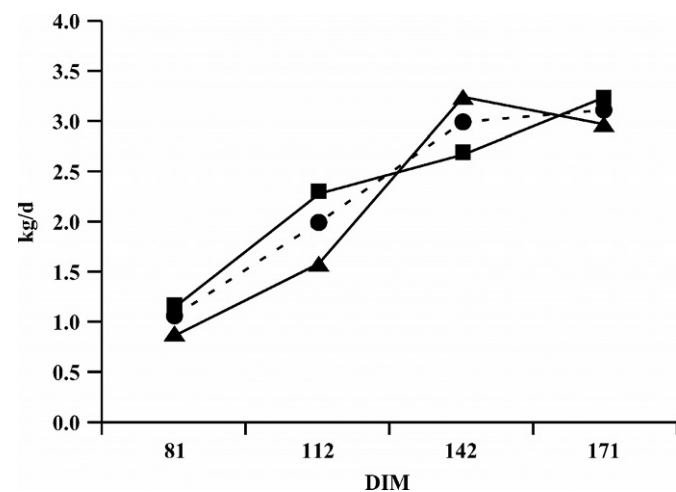
Figure 4. Milk response to bovine somatotropin (bST) treatment during the first 171 DIM for primiparous cows (▲), multiparous cows (■), and both combined (--●--). Linear effect of test day DIM on milk response to bST ($P < 0.05$).
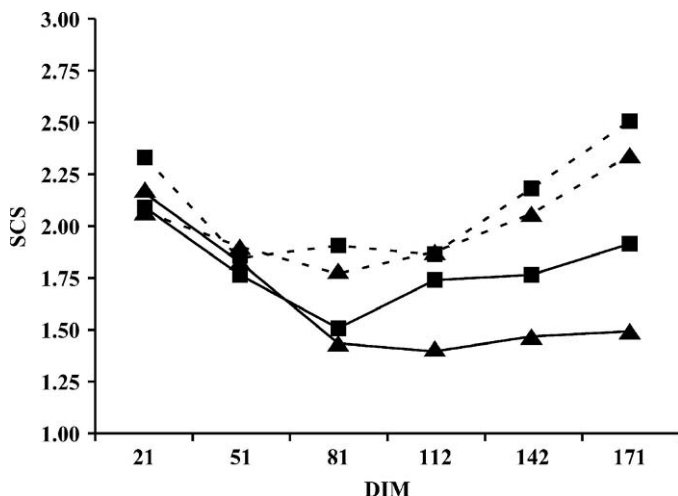
Figure 5. Least squares means of SCS during the first 171 DIM in primiparous and multiparous dairy cows treated or not with bovine somatotropin (bST) starting at 63 DIM. Solid triangle (▲): control cows; Solid squares (■): bST-treated cows; Continuous line: primiparous cows; Dashed lines: multiparous cows. Pooled SEM = 0.13. Effects: bST ($P = 0.81$); parity ($P < 0.0001$), period ($P < 0.0001$); interactions between bST and parity ($P = 0.40$) and bST and period ($P = 0.49$); and interaction between bST and parity and period ($P = 0.03$).

yield in response to bST during the subsequent 110 DIM (46.3 vs. 47.3 kg/d), whereas cows with a BCS equal to, or greater than, 3.0 increased milk yield in response to bST by 3.5 kg/d during the same period (43.3 vs. 46.8 kg/d).

Mastitis and Somatic Cell Count in Milk

Incidence of cows with clinical mastitis was not altered by bST, but primiparous cows had lower incidence of clinical cases than multiparous cows ($P = 0.02$; Table 8). The mean number of clinical mastitis cases per cow was not affected by bST. Furthermore, the interval from the initial bST treatment to the first clinical case of mastitis, and the number of DIM at the diagnosis of the first clinical mastitis case after the initial bST treat-

ment did not differ among treatments. Treatments with bST had no overall effect on SCS, but a 3-way interaction between bST, parity, and period was observed for SCS. In multiparous cows, bST had no effect, but in primiparous cows, bST increased SCS after 81 DIM ($P = 0.03$; Figure 5). Interestingly, this effect was observed even though milk production after bST treatment and interaction between bST and milk production were included in the statistical model. In fact, mean milk production after bST treatment affected SCS ($P = 0.02$), but no interaction between bST and milk production was observed for SCS ($P = 0.57$). Similar to incidence of clinical mastitis, primiparous cows had lower SCS compared to multiparous cows (1.59 vs. 2.07; $P < 0.0001$). Somatic cell score after 51 DIM increased as DIM increased ($P < 0.001$), and this effect was independent of bST treatment in multiparous cows but was affected by bST in primiparous cows.

DISCUSSION

The energy status of dairy cows affects resumption to ovarian cycles, and BCS is an indicator of body reserves, mostly body fat, in dairy cows (Ferguson et al., 1994). Frequency of anovulation in the first 63 DIM decreased in a quadratic manner as BCS increased from 2.25 to 3.75. The relationship between BCS and cyclicity in lactating dairy cows has been previously established (Moreira et al., 2001), and BCS above 3.25 tends not to affect incidence of anovulation in the first 70 DIM. These results indicate the importance of BCS immediately before or at the first postpartum AI on cyclicity of dairy cows. Furthermore, the interaction between BCS on d 70 and BCS changes from 37 to 70 d postpartum indicates that cows with low BCS on d 70 are more likely not to be cycling if they had lost BCS from 37 to 70 DIM.

Kronfeld (1994) suggested that bST might have negative effects on fertility of lactating dairy cows and attributed some of the suggested impacts to direct effects of bST on reproductive functions. However, the body of

Table 8. Effects of bST on mastitis and SCC (LSM ± SEM).

Item[1]	Control		bST		$P <$		
	Primiparous	Multiparous	Primiparous	Multiparous	bST	Parity	bST × Parity
Mastitis incidence, % (no.)	8.1 (211)	14.4 (215)	12.3 (204)	15.2 (210)	0.31	0.02	0.30
Clinical cases per cow	0.12 (± 0.04)	0.17 (± 0.04)	0.19 (± 0.04)	0.19 (± 0.04)	0.20	0.44	0.57
Interval from bST to first clinical mastitis	58.7 (± 7.5)	54.9 (± 5.7)	53.2 (± 6.4)	57.8 (± 5.5)	0.84	0.95	0.52
DIM at first clinical mastitis	122.7 (± 7.5)	118.6 (± 5.6)	116.2 (± 6.5)	120.8 (± 5.5)	0.74	0.98	0.51
SCC, × 10^3/ml	70.8	113.5	104.8	160.3	. . .	. . .	. . .
SCS	1.45 (± 0.11)	2.01 (± 0.09)	1.73 (± 0.10)	2.12 (± 0.10)	0.81	0.0001	0.40

[1]All items were evaluated only after the beginning of bST treatments.

knowledge of the effects of GH on reproductive tissues has dramatically increased during the last 10 yr. Growth hormone has consistently been shown to be essential for normal reproduction in males and females (Hull and Harvey, 2001). In fact, beef cows with GH-receptor deficiency have reduced plasma IGF-I, impaired follicle and CL development, and reduced plasma concentrations of progesterone (Chase et al., 1998), factors known to impair fertility of cattle.

Bovine somatotropin has been shown to enhance fertility in cyclic lactating dairy cows subjected to TAI (Moreira et al., 2000; Moreira et al., 2001) and in cows considered subfertile (Morales-Roura et al., 2001). In lactating cows, the stimulatory effect of bST on fertility seems to be more pronounced during the first postpartum AI (Moreira et al., 2000; Moreira et al., 2001). Work in Florida demonstrated a positive effect of bST on fertility of cyclic cows, regardless of whether first injected 10 d before or at the moment of TAI (Moreira et al., 2001), which suggests that bST modulates reproductive processes to improve fertility in the postovulatory period. In fact, bST as well as IGF-I impacts CL differentiation (Lucy et al., 1994) and embryonic development (Moreira et al., 2002a; Moreira et al., 2002b), which might favor fertility. Increments in fertilization rates in superovulated cows can be obtained by bST treatment, and embryos from bST-treated superovulated cows increased pregnancy rates when transferred into control recipient cows (Moreira et al., 2002a).

Treatment with bST results in increased circulating concentrations of GH and IGF-I (Lucy, 2000), and both GH and IGF-I can mediate the positive effects of bST on fertility of cattle. When GH was added to the maturation medium of in vitro-derived bovine embryos, cleavage rates on d 3 were increased (Moreira et al., 2002b). Culturing bovine embryos in the presence of GH, IGF-I, or both, accelerated embryo development by d 8 postfertilization and increased the number of cells per embryo. Accelerating embryonic development during the early periods after insemination might result in more developed embryos, which are more capable of secreting interferon-t (Mann and Lamming, 2001), therefore blocking the luteolytic cascade (Binelli et al., 2001). These positive effects on early embryonic development partially explain the positive effects of bST treatment on fertility of lactating dairy cows (Moreira et al., 2000; Morales-Roura et al., 2001; Moreira et al., 2001). Furthermore, recent results by Badinga et al. (2002) indicate some potential for bST to inhibit prostanoid synthesis, which might be associated with improved embryo survival.

When data were analyzed with all cows, bST had no effect on fertility responses of lactating dairy cows, except for a tendency to reduce pregnancy loss. How-ever, we observed positive effects of bST on fertility of cyclic cows. Conception rates on d 45 were increased by bST treatment, and part of this effect was due to the increased pregnancy maintenance in bST-treated cows. Because bST increases fertilization rates (Moreira et al., 2002b), accelerates early embryonic development (Moreira et al., 2002a; Moreira et al., 2002b), might increase peripheral concentrations of progesterone (Lucy et al., 1994; Morales-Roura et al., 2001), and can potentially modulate $PGF_{2\alpha}$ synthesis (Badinga et al., 2002), it is expected that GH and IGF-I will have direct positive effects on fertility of lactating dairy cows.

In lactating dairy cows, each antral follicle larger than 3 mm has a life span of 7 to 10 d as it progresses through emergence, deviation, dominance, and atresia or ovulation. When the period of dominance is extended, either by exogenous progestins (Austin et al., 1999) or when cows have estrous cycles with 2, compared with 3, waves of follicle growth (Townson et al., 2002), fertility is compromised. Compared to untreated controls, cows treated with bST have a similar number of follicular waves and similar estrous cycle lengths, but the period of dominance of the first-wave dominant follicle is reduced, and emergence of the second follicular wave occurs 24 to 48 h earlier than in controls (Lucy et al., 1994; Kirby et al., 1997a). Such effects of bST result in a second-wave dominant follicle with extended period of dominance, which is associated with reduced oocyte viability and subsequent fertility (Austin et al., 1999). Therefore, when bST-treated cows are inseminated at the second postpartum service following spontaneous estrus, the dominant follicle of the second follicular wave might result in lowered fertility. This might partially explain the lack of a positive effect of bST on fertility at the second postpartum AI (Moreira et al., 2000; Moreira et al., 2001) or the lack of a bST effect on overall fertility in studies in which the control of follicle emergence and CL regression was not implemented (Cole et al., 1992; Collier et al., 2001).

The lack of a bST effect on the second postpartum AI might also be related to BCS. When cows were inseminated for the second time, they averaged 105 DIM, which coincided with the period when BCS of bST-treated cows was lower than that of control cows. However, when BCS at 103 DIM was included in the statistical analysis of conception rate in the second postpartum AI, no significant effect was observed for BCS or interaction between bST and BCS on second service conception rate ($P > 0.15$).

Studies have indicated either similar or higher conception rates in cows inseminated at detected estrus compared with TAI following the Ovysnch protocol (Burke et al., 1996; Pursley et al., 1997a; Pursley et al., 1997b; Chebel et al., 2002). Chebel et al. (2002)

evaluated over 7000 AI from over 3600 lactating cows in 3 large commercial farms and observed similar conception rates among cows inseminated at fixed-time following the Ovysnch protocol and cows inseminated at detected estrus following spontaneous estrus or exogenous $PGF_{2\alpha}$-induced estrus. Similarly, Pursley et al. (1997a, 1997b) and Burke et al. (1996) observed similar conception rates in lactating cows when inseminated following the Ovsynch protocol or after estrus induced by exogenous $PGF_{2\alpha}$. Interestingly, our results suggest that estrus induced by a combination of GnRH and $PGF_{2\alpha}$ 7.5 d later, when cows are presynchronized with $PGF_{2\alpha}$, results in higher conception rates than when cows are inseminated following the Ovysnch protocol.

Several factors may explain the reduced conception rates for cows inseminated following TAI, compared with cows inseminated in the ED treatments. Cows inseminated at detected estrus following injections of GnRH and $PGF_{2\alpha}$ are expected to display signs of estrus when a newly recruited follicle achieves maturity and is highly steroidogenic. These follicles are usually at their optimal stage of development to result in a successful fertilization. Also, detection of estrus eliminates the problem of anovulatory/anestrous animals affecting fertility as in Ovsynch groups, because only cows displaying estrus are inseminated, and most cows are expected to ovulate 27 to 30 h after the onset of estrus. Also, cows that display premature estrus, between the injections of GnRH and $PGF_{2\alpha}$, are unlikely to respond to the luteolytic effect of $PGF_{2\alpha}$ and, therefore, are not expected to be observed in estrus and inseminated. On the other hand, cows subjected to the TAI treatments were inseminated at fixed times, regardless of the aforementioned factors that can potentially alter synchrony between timing of CL regression, induced ovulation of a competent follicle, and AI. Another explanation may be related to the 7.5-d interval between the first GnRH and the $PGF_{2\alpha}$ injections in the Ovsynch treatments. This lengthens the interval between the first and second GnRH injections, which might have reduced the percentage of cows that ovulated to the second GnRH injection. Unfortunately, ovulation rate was not determined in the current study.

An important finding of the current study was the similar pregnancy loss between cows inseminated at detected estrus or following the Ovsynch protocol. In a comprehensive review of the literature on reproductive loss in high-producing lactating dairy cows, Lucy (2001) suggested that one of the many factors involved in the high, late embryonic mortality in lactating dairy cows might be the increased adoption of reproductive programs involving insemination at fixed-time. Cows inseminated at detected estrus were suggested to be less likely to experience late embryonic loss compared with

cows inseminated following TAI. Present results provide no evidence that cows inseminated at fixed-time following the Presynch/Ovsynch protocol experience more pregnancy losses than those inseminated following detected estrus. Chebel et al. (2002) studied factors involved in late embryonic loss in over 1500 pregnant, lactating Holstein cows in 3 dairy farms. They observed similar pregnancy loss between 31 and 45 d after AI for cows inseminated when observed in estrus or at fixed-time following the Ovsynch protocol.

Few studies have observed an association between bST treatment and reduction in detected estrus in dairy cows (Morbeck et al., 1991; Cole et al., 1992; Kirby et al., 1997b). In some studies (Morbeck et al., 1991; Cole et al., 1992; Kirby et al., 1997b), the number of animals was limited, and others (Morbeck et al., 1991; Cole et al., 1992) did not use a standardized reproductive protocol to monitor estrous behavior.

Treatment with 500 mg of bST every 14 d reduced visual observation of estrus in lactating dairy cows (Kirby et al., 1997b). Morbeck et al. (1991) demonstrated that treatment with 16.5 mg of bST/d starting at 5 wk postpartum extended the interval from calving to first postpartum-observed estrus and AI. Using estradiol primed ovariectomized heifers, Lefebvre and Block (1992) observed a tendency ($P = 0.15$) for bST-treated heifers to have a delayed onset of estrus, which was of shorter duration and with fewer mounts. The authors hypothesized that bST might have affected behavioral centers within the brain that control expression of estrus.

We observed that treatment with bST at the moment of GnRH, when a new follicular wave is expected to be recruited, tended to reduce the rate of detected estrus in multiparous cows in the 7-d observation period after a luteolytic dose of $PGF_{2\alpha}$. The reduction in observed estrous behavior was observed across all cows and particularly among cyclic cows. These data indicate a possible direct effect of bST on expression of estrus in dairy cows because increases in milk yield would have been small in the first 14 d of treatment. In fact, milk production, either during the first 3 mo of lactation or in the test day subsequent to the first bST treatment, had no effect on rate of detected estrus, and no interaction between bST and milk yield was observed for detected estrus ($P > 0.20$). It is possible that bST might, in fact, influence behavioral centers within the brain that control expression of estrus, thereby reducing estrous behavior, as suggested by Lefebvre and Block (1992).

Nonetheless, bST also reduced returns to estrus in nonpregnant cows within the first 21 d after the initial AI, as well as before pregnancy diagnosis on d 31 after AI. Surprisingly, the proportion of all nonpregnant cows that were reinseminated within the first 21-d estrous

cycle after the initial AI was small (21.3%). Reduction in returns to estrus in the first 31 d after the initial AI in nonpregnant cows might be associated with the beneficial effects of bST on embryo preservation. It is plausible that some of the nonpregnant cows on d 31, after the initial AI, might have actually been pregnant at the time of maternal recognition of pregnancy, which would have delayed return to estrus. Because bST accelerates embryo development and improves pregnancy maintenance, it is possible that timing of embryonic loss between control and bST-treated cows differed. Such an effect could alter the cellular cross-talk between the embryonic trophectoderm and the maternal endometrium, resulting in delayed luteolysis. Therefore, delayed embryonic mortality occurring immediately after maternal recognition of pregnancy may be associated with reduced returns to estrus in bST-treated cows.

When milk response to bST was evaluated by DIM, Bauman et al. (1999) observed a response of 1.0 to 3.5 kg/d between 60 and 170 DIM. These responses are similar to those observed in the current study. Collier et al. (2001), also using test-day information, observed a milk response to bST of approximately 3.9 kg/d for the approximately 242 d of bST treatment. When milk is measured daily, response to bST treatment given every 14 d relative to a control group is immediate, but it increases as cows are injected during the first 2 or 4 injection cycles (Hartnell et al., 1991). Using monthly test-day information, milk response to bST treatment, starting in the ninth week after calving, seemed to continue, increasing past 170 DIM (Bauman et al., 1999). Milk response to bST treatment was influenced by BCS at the first bST injection, and cows with a BCS ≤ 2.75 did not respond to bST as well as cows with a BCS ≥ 3.0.

During the first 4 to 8 wk of bST treatment, energy balance of dairy cows decreases and production of milk is supported to the detriment of replenishing body energy stores. As a consequence, an immediate reduction in BCS gain or a temporary loss in BCS is expected. Changes in BCS after 70 DIM were of small magnitude, but cows receiving bST continued to lose BCS, whereas control cows gained some BCS from 70 to 103 DIM. Cows with low BCS at 70 DIM produced more milk than cows with a BCS ≥ 3.0, but this effect was not dependent on changes in BCS from 37 to 70 d postpartum.

Treatment with bST, according to label recommendation, has usually minimal effects on the health of lactating dairy cows (Collier et al., 2001). We observed that bST treatment did not affect incidence of cows with at least one clinical case of mastitis, which is similar to findings by Collier et al. (2001). We observed no overall effect of bST on SCS, but after 81 DIM, bST increased SCS in primiparous cows, compared with primiparous control cows. Although milk production after the initial bST treatment affected SCS, the effects of bST on SCS of primiparous cows after 81 DIM were independent of milk yield because no interaction between bST and milk production were observed for SCS.

CONCLUSIONS

Both bST treatment and AI protocol affected fertility of dairy cows, as measured by conception to first postpartum AI. Cows inseminated at detected estrus had higher conception rates than those inseminated at fixed-time following the Ovsynch protocol, and bST improved conception rates in cyclic cows. Insemination protocol had no impact on pregnancy loss, but treatment with bST tended to reduce pregnancy loss in all cows and improved pregnancy maintenance in cyclic dairy cows. However, bST tended to reduce the rate of detected estrus in multiparous cows and reduced returns to estrus in all nonpregnant cows, which extended the interval between the first and the second postpartum AI in nonpregnant cows. Treatments had no effect on pregnancy rates because bST tended to reduce detection of estrus in multiparous cows, and TAI resulted in lower conception compared with insemination following detected estrus. Incidence of cyclic cows was greatly influenced by BCS, and cyclic cows had higher fertility than anovulatory cows. Therefore, bST increases fertility of cyclic lactating cows at the first postpartum AI, when insemination is performed either following TAI or detected estrus. However, further research is warranted to determine the effects of incorporating resynchronization protocols that optimize returns to estrus to maximize reinsemination rates of nonpregnant bST-treated cows.

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