Sex-sorted semen for dairy heifers: Effects on reproductive and lactational performances

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ABSTRACT

The objectives of this study were to evaluate the effects of using sex-sorted semen for first AI of heifers on health and productivity during first lactation. Holstein heifers (herd A = 227 and herd B = 1,144) received first artificial insemination (AI) with sex-sorted semen (SX; $n = 343$) or conventional semen (CS; $n = 1,028$), and all heifers that displayed estrus after first AI were reinseminated with conventional semen up to 11 times before being culled. Age at first AI was 13.1 ± 0.1 and 13.8 ± 0.1 mo for SX and CS heifers, respectively, in herd A and 12.9 ± 0.1 mo for both SX and CS heifers in herd B. Pregnancy per AI after first AI was greater for CS heifers than for SX heifers (51.8 vs. 40.2%). From heifers initially enrolled, 70.2% calved in herds A ($n = 188$) or B ($n = 774$) and first-lactation data were collected. Interval from first AI to calving was greater for SX heifers than for CS heifers (10.2 ± 0.1 vs. 9.9 ± 0.1 mo). Among heifers conceiving to first AI, SX heifers were more likely than CS heifers to deliver a female calf (85.7 vs. 47.7%), but because SX heifers were more likely to deliver a dead calf (8.8 vs. 3.4%), the difference in proportion of SX and CS heifers delivering a live female calf was smaller than expected (SX = 79.1%; CS = 47.2%). Rearing cost from first AI to calving was greater for SX heifers than for CS heifers ($\$775.3 \pm 6.7$ vs. $\$750.0 \pm 5.9$), but calf revenue tended to be greater for SX heifers ($\$142.0 \pm 7.2$ vs. $\$126.7 \pm 6.4$) and cost per female calf produced was smaller for SX heifers than for CS heifers ($\$-809.4 \pm 10.8$ vs. $\$-1,249.7 \pm 10.9$). Treatment did not affect calving difficulty, proportion of heifers needing assistance, and incidence of retained fetal membranes or metritis. Among heifers that conceived to first AI, however, SX heifers were more likely to be culled within 30 DIM (3.3 vs. 1.6%) and tended to be more likely to be culled within 60 DIM (5.5 vs. 3.4%) than CS heifers, but overall replacement cost was not different ($\$136.8 \pm 13.4$). Total milk yield

($9,245.5 \pm 84.7$ kg) and income over feed cost ($\$554.7 \pm 5.1$) were not different. Overall economic return was greater for SX heifers than CS heifers ($\$-83.7 \pm 36.7$ vs. $\$-175.3 \pm 33.4$). Use of sex-sorted semen for first insemination of virgin heifers reduced the cost per female calf produced and increased the economic return during the first lactation.

Key words: sex-sorted semen, heifer, economics

INTRODUCTION

Selection for offspring sex has always been a desired goal of food animal producers. This goal has become a reality with the advances made in sorting sperm based on the presence of X or Y chromosome. The control of offspring sex could allow dairy producers to selectively breed their animals to produce female offspring from genetically superior dams, which would serve as replacement animals, or to produce male offspring from genetically inferior dams, which would be raised as steers for slaughter.

Although considerable advances have been made in the technology of sperm sorting based on chromosome born, to date, pregnancy per AI (P/AI) after insemination with sex-sorted semen is considerably smaller than insemination with conventional semen. Seidel et al. (1999) reported P/AI approximately 10 percentage units smaller for heifers inseminated with sex-sorted semen compared with those inseminated with conventional semen. Data from subsequent studies support this initial finding and demonstrate that P/AI of sex-sorted semen is expected to be approximately 70 to 80% of P/AI of conventional semen (Bodmer et al., 2005; Borchersen and Peacock, 2009; DeJarnette et al., 2009). Although one of the possible reasons why reduced P/AI is observed after insemination with sex-sorted semen is the reduced number of sperm per dose of semen, it is also likely that damage may occur during the process of sperm staining, identification, and separation, resulting in reduced P/AI (Schenk and Seidel, 2007).

Because P/AI of lactating dairy cows is reduced and considerably smaller than that of heifers, and because extended calving interval resulting from insemination

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with sex-sorted semen is not an acceptable consequence of the use of this technology, dairy producers have been advised to use sex-sorted semen for first insemination of heifers (Schenk et al., 2009). Although heifers are the most fertile animals in any given dairy herd, to overcome the potential increase in interval from birth to first parturition when using sex-sorted semen, some have suggested that heifers be inseminated with sex-sorted semen at a slightly younger age than when they are inseminated with conventional semen. Further, it has also been recommended that heifers be inseminated with sex-sorted semen once and subsequently with conventional semen if they do not conceive. It could be argued that some heifers that are inseminated earlier would conceive and would be more likely to have periparturient diseases for calving at a younger age, but because sex-sorted semen should yield 85 to 90% of female calves and incidence of postparturient diseases is related to calf sex, the risk of postparturient diseases is not likely to increase when heifers are inseminated with sex-sorted semen at a younger age.

Therefore, we hypothesized that heifers inseminated with sex-sorted semen at first AI would have smaller P/AI and longer interval to parturition than those inseminated with conventional semen. We also hypothesized that heifers inseminated with sex-sorted semen would be less likely to have periparturient diseases and would have improved reproductive and productive performance during first lactation compared with heifers inseminated with conventional semen. The objectives of this study were to evaluate the effects of inseminating heifers with sex-sorted semen at first AI on reproductive performance of dairy heifers and health, productive, and reproductive performance during first lactation.

MATERIALS AND METHODS

Heifers

Animals and Diets. Holstein heifers from 2 commercial herds located in the San Joaquin Valley of California (herd A = 227 cows; herd B = 1,144 cows) were used in this study. Heifers were fed once a day a TMR that met or exceeded the requirements set forth by the NRC (2001) for Holstein heifers weighing approximately 360 kg and gaining 0.6 kg/d of live BW. Heifers were inseminated for the first time between April and November of 2006 and calved from January of 2007 to March of 2008.

Treatments and Reproductive Management. In herd A, heifers assigned to be inseminated with conventional (CS) semen (minimum of 20×10^6 cells/inseminating dose) were moved to a breeding group at approximately 13 mo of age and heifers assigned to

receive sex-sorted (SX) semen (minimum of 2×10^6 cells/inseminating dose) were moved to the breeding group approximately 21 d earlier. In herd B, heifers assigned to be inseminated with SX and CS semen were moved to the breeding group at approximately 12 mo of age. Upon entrance in the breeding group, all heifers received an injection of PGF_{2α} and those detected in estrus were inseminated. Heifers not detected in estrus received PGF_{2α} injections every 14 d until detected in estrus and inseminated.

Heifers were observed for estrus daily in the morning and those determined to be in estrus, based on removal of tail paint, were inseminated on the same morning. After first insemination, heifers detected in estrus were reinseminated with conventional semen. There were 2 and 3 AI technicians in herds A and B, respectively, and they were well distributed between treatments. A total of 3 sires were used for first AI of SX heifers (81, 110, and 152 heifers inseminated with semen from each sire) and a total of 29 sires were used for first AI of CS heifers. Heifers observed in estrus after first AI or diagnosed not pregnant were reinseminated with semen from sires used for first AI of CS heifers.

Pregnancy Diagnosis and Calculation of Reproductive Outcomes. Heifers were diagnosed for pregnancy at 39 ± 3 d after AI by palpation per rectum of uterine contents and those diagnosed pregnant were reexamined at 67 ± 3 d after AI. Pregnancy per AI was calculated as the number of heifers diagnosed pregnant at 39 ± 3 or 67 ± 3 d after AI divided by the number of heifers inseminated. Pregnancy loss was calculated as the number of heifers diagnosed not pregnant at 67 ± 3 d after AI divided by the number of heifers examined. Interval between first and second insemination was calculated by subtracting the date of second insemination from the date of first insemination. Heifers that aborted after being diagnosed pregnant at 39 ± 3 d after AI were not included in this calculation because the objective was to determine whether the use of SX semen would alter the pattern of return to estrus. The interval from first AI until conception was calculated only for heifers that started the first lactation in herds A and B.

Culling Rate and Proportion of Heifers Initiating a First Lactation in Herds A or B. In both herds, heifers were selected according to phenotype to be sold as springer heifers. Therefore, the number of heifers that were sold as springer heifers, the number of heifers that were culled, and the number of heifers that died were calculated. Information regarding reason for culling was extracted from Dairy Comp 305 (Valley Ag Software, Tulare, CA). Proportions of heifers enrolled in the study that calved in herds A and B were calculated by dividing the number of heifers that calved in

herds A or B by the number of heifers enrolled in the study.

First-Lactation Cows

Health Parameters During First Lactation.

Heifers that calved in herds A or B were followed during the first lactation until they were dried off, until they left the herd, or until 305 DIM. At parturition, calving difficulty score (**CDS**; 1 = no assistance needed to 5 = cesarean section needed), sex of the calf born, and calves born dead (**CBD**) were recorded. For purposes of statistical analysis, CDS was divided into CDS 1, CDS 2 to 3, and CDS 4 to 5. Cows were monitored daily during the first 14 d postpartum by herdsmen and occurrence of retained fetal membranes (**RFM**) and metritis was recorded. Retained fetal membrane was defined as retention of fetal membranes for more than 24 h after calving.

Cows were diagnosed as having metritis when presence of fetid, pink-brown, watery uterine discharge was observed within the first 14 d postpartum. Cows were examined 3 times daily before each milking for diagnosis of mastitis, which was characterized by alteration in milk appearance or inflammation of the mammary gland. Because it was expected that the incidence of periparturient diseases would be affected by exposure to heat stress around the time of calving, heifers that calved from May through September were considered to have calved during the hot season and those that calved from October through April were considered to have calved during the cold season.

Reproductive Management, Pregnancy Diagnosis, and Calculation of Reproductive Outcomes. All cows were presynchronized with 2 injections of PGF_{2α} at 35 ± 3 and 49 ± 3 DIM, and those determined to be in estrus by removal of tail paint after the second injection of PGF_{2α} were inseminated. Those cows not inseminated by 60 ± 3 DIM were enrolled in an ovulation synchronization protocol and were timed inseminated at 70 ± 3 DIM. After first AI, cows observed in estrus were reinseminated and considered to be not pregnant and those not reinseminated were examined for pregnancy at 39 ± 3 and 67 ± 3 d after AI. Cows diagnosed not pregnant were enrolled in an ovulation synchronization protocol for reinsemination. There were 2 and 3 AI technicians in herds A and B, respectively, and AI was conducted once a day in the morning. Pregnancy per AI was calculated as the number of cows diagnosed pregnant at 39 ± 3 or 67 ± 3 d after AI divided by the number of cows inseminated. Pregnancy loss was calculated as the number of cows diagnosed not pregnant at 67 ± 3 d after AI divided by the number of cows examined.

Productive Parameters. In both herds, cows were milked 3 times daily and were treated with recombinant bST every 14 d starting at approximately 9 wk postpartum. Cows were fed a TMR designed for lactating dairy cows producing 45 kg of milk at 3.5% fat and DMI of 28 kg/d. Cows had electronic identification devices and milk yields were recorded daily. Once monthly, Dairy Comp 305 retrieved summary milk production data from the milking system software; this information was used to calculate milk yield during the study period.

Economic Outcomes

Economic outcomes were calculated for all heifers enrolled in the study. For calculation of economic outcomes, data regarding total cost of semen, rearing cost (number of days from first AI until parturition multiplied by the average daily cost of rearing a replacement heifer from 12 mo of age to parturition), value of a day-old live female and male calf, cost/price of a pregnant 24-mo-old replacement heifer, salvage value of culled heifers and cows, and income over feed cost (**IOFC**) were used. The values and costs used for each of the variables described above were based on historical averages paid and received by the dairies used in the study. Therefore, cost of a dose of conventional semen = \$15, cost of a dose of sexed semen = \$40, average rearing cost of heifers = \$2.5/heifer/d, replacement heifer cost/value = \$1,400, value of a day-old female calf = \$250, value of a day-old male calf = \$50, salvage value of a culled heifer = \$300, salvage value of a culled cow = \$400, and IOFC = \$0.06/kg of milk.

A certain number of CS and SX heifers were sold as replacements, were culled, or died before starting the first lactation. Each of these heifers was given a value according to their destination; for those that were culled and died, the cost of a replacement heifer was assessed (balance of heifers sold). Within herd and treatment, the value of these heifers was added and then divided by the number of heifers that started the first lactation in either herd and included in the calculation of the overall economic outcome.

Cost per female calf produced was calculated as [calf revenue – (rearing cost + total semen cost)] of heifers that did not deliver a female calf divided by the number of heifers that delivered female calves, within each treatment and herd.

Study Design and Statistical Analysis

Heifers were assigned to one of 2 treatments at random by farm personnel when heifers reached approximately 11 mo of age. Heifers assigned to the SX treatment were inseminated with sex-sorted semen from one of

Table 1. Reproductive performance of heifers according to herd and treatment (TRT)¹

Item	Herd A		Herd B		<i>P</i> -value		
	SX (n = 123)	CS (n = 104)	SX (n = 220)	CS (n = 924)	TRT	Herd	TRT × herd
Age at first AI, mo (±SEM)	13.1 ± 0.1 ^a	13.8 ± 0.1 ^b	12.8 ± 0.1 ^a	13.0 ± 0.1 ^a	<0.01	<0.01	<0.01
Heifers pregnant to first AI, %	38.2	51.9	41.4	51.7	<0.01	0.64	0.57
Pregnancy loss after first AI, %	10.6	7.4	0.0	1.9	0.72	<0.01	NA ²
Interval between first and second AI, d (±SEM)	27.7 ± 2.7	33.1 ± 3.3	29.4 ± 2.0	31.0 ± 1.1	0.13	0.92	0.41
Heifers starting first lactation, %	83.7	81.7	63.6	68.6	0.95	0.09	0.62

^{a,b}Values within a herd and row with different superscripts differ ($P < 0.05$).

¹SX = heifers inseminated with sex-sorted semen at first AI; CS = heifers inseminated with conventional semen at first AI.

²NA = not applicable.

3 sires and heifers assigned to the CS treatment were inseminated with semen from one of 29 sires. After first insemination, all heifers observed in estrus and those diagnosed not pregnant were reinseminated with semen from sires used for first AI of CS heifers.

Dichotomous data were analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS Institute Inc., Cary, NC). The final logistic regression model removed variables by a backward elimination based on the Wald's statistics criterion if $P > 0.10$. When independent variables had more than 1 degree of freedom, the contrast statement of the LOGISTIC procedure was used to compare the levels of the independent variables.

Continuous data were analyzed by ANOVA using the GLM procedure of SAS and continuous repeated data were analyzed by ANOVA for repeated measures using the MIXED procedure of SAS. Ordinal data were analyzed by the GENMOD procedure of SAS using a Poisson distribution.

Hazard ratios for conception after parturition and for culling after parturition were analyzed by the Cox proportional hazards regression using the PHREG procedure of SAS. The final regression model removed variables by a backward elimination based on the Wald's statistics criterion if $P > 0.10$. Explanatory variables identified as affecting the hazard ratios were further analyzed by survival analysis using the product limit method of the Kaplan-Meier model by the LIFETEST procedure of SAS.

All models included treatment (SX vs. CS), herd (A vs. B), and the interaction between treatment and dairy. Other independent variables were included in the model as covariates when they were observed to affect the outcome according to a univariate analysis. Initial models did not include variables affected by treatment as covariates to avoid collinearity. However, subsequently, to further clarify how treatment affected the outcomes evaluated, variables affected by treatment

were included in the model as covariates only when univariate models demonstrated significance.

RESULTS

Heifer Data

Average age at first AI was different ($P < 0.01$) between treatments and herds (Table 1). The interaction between treatment and herd affected ($P < 0.01$) age at first AI because, in herd A, SX heifers were younger at first AI than CS heifers, but age at first AI was not different in herd B (Table 1). Pregnancy per AI after first AI was affected ($P < 0.01$) by treatment, and P/AI of SX heifers was 77.7% that of CS heifers (Table 1). Among SX heifers, P/AI to first AI was not affected ($P = 0.22$) by sire (range: 35.5–46.9%). Although treatment did not affect ($P = 0.72$) proportion of heifers that had pregnancy loss after first AI, a greater ($P < 0.01$) proportion of heifers in herd A had early fetal loss compared with heifers in herd B (Table 1). Interval between first and second AI was not affected ($P = 0.13$) by treatment (Table 1), and the pattern of return to estrus of heifers reinseminated before pregnancy diagnosis is depicted in Figure 1. Proportions of heifers that did not become pregnant within 12 mo after entering the breeding group or before leaving the herd were 3.2 and 4.8% ($P = 0.22$) for SX and CS heifers, respectively.

Proportion of heifers sold as replacements was not affected by treatment ($P = 0.30$) or herd ($P = 0.30$). Heifers that conceived to first AI were more likely ($P < 0.01$) to be sold as replacements than heifers that did not conceive to first AI (19.4 vs. 16.0%). The interaction between treatment and pregnancy to first AI affected ($P = 0.01$) the proportion of heifers sold as replacement because a greater proportion of SX heifers that conceived to first AI were sold as replacement compared with CS heifers (26.8 vs. 17.5%), whereas a similar proportion of heifers that did not conceive to first AI were sold as replacement heifers (SX = 14.1%;

Figure 1. Distribution of return to estrus of heifers reinseminated after first AI and before pregnancy diagnosis at 39 ± 3 d after first AI ($n = 472$). SX = heifers inseminated for the first time with sex-sorted semen; CS = heifers inseminated for the first time with conventional semen.

CS = 16.7%). There were no effects of treatment ($P = 0.21$) or herd ($P = 0.38$) on the likelihood of heifers being culled from the herd, but heifers that conceived to first AI were less likely ($P < 0.01$) to be culled from the herd than heifers that did not conceive to first AI (9.1 vs. 16%). Finally, there were no differences in the proportion of heifers that died before starting the first lactation (0.4%) according to treatment ($P = 0.34$) or herd ($P = 0.60$).

First-Lactation Data

Proportions of heifers that calved in herds A and B were not different ($P = 0.95$) between treatments (Table 1), but there was a tendency ($P = 0.09$) for a greater proportion of heifers from herd A to remain in the herd until the start of the first lactation compared with heifers from herd B (Table 1).

Heifers that Conceived to First AI. There were 91 SX heifers and 388 CS heifers that conceived to first AI and started the first lactation in herds A and B. When all heifers were included in the statistical analysis, gestation length was affected ($P < 0.01$) by treatment and calf sex, and the interaction between treatment and calf sex also affected ($P < 0.01$) gestation length. This interaction was mainly because, among SX heifers, 1 sire was associated with reduced gestation length of heifers

delivering male calves (Figure 2). When data from SX heifers that conceived to this outlier sire were removed, treatment ($P = 0.14$), calf sex ($P = 0.31$), and the interaction between treatment and calf sex ($P = 0.98$) did not affect gestation length (Table 2). Proportion of SX heifers delivering twins was 0%, whereas 1.7% of CV heifers delivered twins. Heifers delivering twins had shorter ($P < 0.01$) gestation length than those delivering singletons (270.4 ± 2.3 vs. 276.7 ± 0.5 d).

A greater ($P < 0.01$) proportion of SX heifers delivered a female calf compared with CS heifers (Table 2). Among SX heifers, sire did not affect ($P = 0.22$) proportion of female calves delivered (range: 79.4–95.7%). Treatment did not affect ($P = 0.98$) calving difficulty, but heifers delivering a male calf tended ($P = 0.08$) to have greater calving difficulty than those delivering a female calf (Table 2). The proportion of heifers with CDS = 1 was not affected ($P = 0.19$) by treatment, but the proportion of heifers delivering female calves with CDS = 1 was greater ($P < 0.01$) than that of those delivering a male calf (Table 2). The proportion of SX heifers with CBD was greater ($P = 0.01$) than that of CS heifers, and there was a tendency ($P = 0.09$) for calf sex to affect proportion of CBD (Table 2). Increased CDS was associated ($P < 0.01$) with increased proportions of CBD (CDS 1 = 2.2, CDS 2–3 = 8.5, and CDS 4–5 = 9.7%). Among the 91 SX heifers that conceived

Table 2. Gestation length, sex distribution, calving difficulty, incidence of calves born dead, retained fetal membranes, and metritis, and proportion of cows culled and becoming pregnant according to treatment (TRT) and calf sex (CS) among heifers that conceived to first AI¹

Item	SX		CS		P-value		
	Female (n = 78)	Male (n = 13)	Female (n = 185)	Male (n = 203)	TRT	CS	TRT × CS
Gestation length, ² d	273.9 ± 1.4	275.1 ± 2.6	271.9 ± 1.2	273.2 ± 1.2	0.14	0.31	0.98
Sex distribution, %	85.7	14.3	47.7	52.3	<0.01	NA ³	NA
Calving difficulty ⁴ (±SEM)	1.37 ± 0.10	1.68 ± 0.22	1.32 ± 0.08	1.72 ± 0.07	0.98	0.08	0.81
Heifers with calving difficulty score = 1, %	75.6	61.5	81.1	53.2	0.19	<0.01	0.53
Calves born dead, %	7.7	15.4	1.1	5.4	0.01	0.09	0.70
Heifers delivering a live female or male calf, %	79.1	12.1	47.2	49.5	<0.01	NA	NA
Incidence of retained fetal membranes, %	7.7	0.0	3.2	4.4	0.29	0.70	NA ⁵
Incidence of metritis, %	46.2	61.5	50.8	52.7	0.61	0.51	0.65
Heifers culled by 30 DIM, %	3.9	0.0	1.6	1.5	0.02	0.59	NA ⁵
Heifers culled by 60 DIM, %	5.1	7.7	3.2	3.5	0.06	0.90	0.98
Heifers pregnant to first postpartum AI, %	26.0	46.2	31.0	34.5	0.80	0.19	0.31
Heifers pregnant at 305 DIM, %	85.7	91.7	87.7	87.6	0.86	0.88	0.73

¹SX = heifers inseminated with sex-sorted semen at first AI; CS = heifers inseminated with conventional semen at first AI.

²Data from SX heifers that were inseminated with a sire that resulted in outlier gestation length were not included.

³NA = not applicable.

⁴Calving difficulty: 1 = no assistance needed, 5 = cesarean section needed.

⁵Interaction not included in the statistical analysis because of quasi-complete separation of data points.

to first AI and calved in herds A and B, the sire distribution was 23, 34, and 34 and the incidences of CBD among heifers inseminated to these sires were 13.0, 2.9, and 11.8%, respectively ($P = 0.32$). Even though SX heifers were more likely to have CBD, the proportion of SX heifers delivering a live female calf was still greater ($P < 0.01$) compared with CS heifers (Table 2).

Incidence of RFM was not different ($P = 0.29$) between SX and CS treatments (Table 2), but a greater ($P = 0.05$) proportion of heifers that calved in herd A had RFM compared with those that calved in herd B (8.0 vs. 3.7%). Incidence of RFM was increased ($P < 0.01$) by the occurrence of twins (twins = 50.0%; singleton = 3.8%). Similarly, incidence of metritis was

not affected ($P = 0.61$) by treatment (Table 2), but heifers that calved in herd B were more likely ($P < 0.01$) to be diagnosed with metritis than those that calved in herd A (55.2 vs. 29.3%). Interestingly, there was no association ($P = 0.17$) between occurrence of RFM and occurrence of metritis, but increasing CDS was associated ($P < 0.01$) with increased incidence of metritis (CDS 1 = 45.9; CDS 2 to 3 = 69.5; CDS 4 to 5 = 54.2%).

Proportion of cows culled within 30 DIM was greater ($P = 0.02$) for the SX than the CS treatment (3.3 vs. 1.6%). Cows that calved during hot seasons were more likely ($P = 0.02$) to be culled within 30 DIM than those that calved during cull seasons (3.1 vs. 0.5%). There was a tendency ($P = 0.06$) for more SX heifers than CS heifers to be culled within 60 DIM (5.5 vs. 3.4%). Occurrence of metritis (metritis = 5.3%; no metritis = 2.1%; $P = 0.05$) and calving during the hot season (hot season = 5.0%; cull season = 2.3%; $P = 0.03$) increased the likelihood of cows being culled within 60 DIM. Risk of culling up to 305 DIM, however, was not affected by treatment ($P = 0.13$), occurrence of metritis ($P = 0.56$), or calving during the hot season ($P = 0.23$). Cows from herd A tended ($P = 0.08$) to have smaller culling risk than cows in herd B [adjusted hazard ratio (AHR) = 0.35; 95% CI = 0.11, 1.11]. Cows that did not have mastitis had a slower ($P = 0.02$) culling rate than those that had mastitis (AHR = 0.47; 95% CI = 0.25–0.87). Incidence of mastitis was not affected by treatment (SX = 18.7, CS = 16.5%; $P = 0.65$) and herd (A = 20.0, B = 16.3%; $P = 0.75$).

Figure 2. Gestation length of heifers inseminated with sex-sorted semen and conceived to first AI according to sire. Effect of calf sex ($P = 0.02$), sire ($P < 0.01$), and calf sex × sire ($P < 0.01$).

Table 3. Effect of treatment (TRT) and herd on interval from first AI to calving, age at calving, calving difficulty, proportion of female calves born, stillbirths, incidence of retained fetal membranes and metritis, culling at 30 and 60 DIM, and reproductive performance during first lactation for all heifers¹

Item	Herd A		Herd B		P-value		
	SX (n = 123)	CS (n = 104)	SX (n = 220)	CS (n = 924)	TRT	Herd	TRT × herd
Interval between first AI and calving, mo ($\pm$ SEM)	10.2 $\pm$ 0.1	10.0 $\pm$ 0.1	10.3 $\pm$ 0.1	9.8 $\pm$ 0.1	<0.01	0.52	0.15
Age at calving, mo ($\pm$ SEM)	23.1 $\pm$ 0.2	23.5 $\pm$ 0.2	22.8 $\pm$ 0.2	22.7 $\pm$ 0.1	0.41	<0.01	0.16
Gestation length, ² d ($\pm$ SEM)	276.8 $\pm$ 1.2	275.8 $\pm$ 0.9	277.4 $\pm$ 1.0	275.9 $\pm$ 0.3	0.22	0.62	0.79
Female calves, %	63.1	47.1	65.7	51.6	<0.01	0.60	0.50
Calving difficulty ³ ($\pm$ SEM)	1.46 $\pm$ 0.08	1.61 $\pm$ 0.09	1.42 $\pm$ 0.07	1.44 $\pm$ 0.03	0.42	0.34	0.53
Heifers with calving difficulty = 1, %	70.9	65.9	70.7	69.2	0.38	0.37	0.43
Calves born dead, %	10.7	4.7	7.1	3.8	<0.01	0.32	0.78
Live female calves, %	56.3	47.1	62.9	50.5	<0.01	0.41	1.00
Incidence of retained fetal membranes, %	4.9	3.5	5.0	3.2	0.21	0.93	0.87
Incidence of metritis, %	27.2	30.6	50.0	45.9	0.39	<0.01	0.23
Incidence of mastitis, %	23.3	22.4	17.9	15.6	0.51	0.07	0.75
Heifers culled by 30 DIM, %	2.9	1.2	4.3	2.2	0.32	0.40	0.87
Heifers culled by 60 DIM, %	2.9	1.2	5.7	3.9	0.27	0.26	0.66
Heifers pregnant to first postpartum AI, %	30.4	31.0	26.4	30.1	0.50	0.63	0.82
Heifers pregnant at 305 DIM, %	87.6	83.3	88.2	90.6	0.60	0.36	0.45

¹SX = heifers inseminated with sex-sorted semen at first AI; CS = heifers inseminated with conventional semen at first AI.

²Data from SX heifers that were inseminated with a sire that resulted in outlier gestation length were not included.

³Calving difficulty: 1 = no assistance needed, 5 = cesarean section needed.

The proportion of cows that conceived to first postpartum AI (32.1%) was not affected ($P = 0.80$) by treatment (Table 2). Occurrence of RFM tended ($P = 0.09$) to reduce P/AI after first postpartum AI (RFM = 32.9%; no RFM = 14.3%). Proportion of cows pregnant at 305 DIM (87.4%) was not affected ($P = 0.86$) by treatment (Table 2). Cows that had RFM were less likely to be pregnant at 305 DIM than cows that did not have RFM (88.3 vs. 71.4%, respectively; $P = 0.03$). Risk of pregnancy was not affected by treatment ($P = 0.33$), but cows that did not have RFM had greater risk of pregnancy (AHR = 1.73; 95% CI = 1.03, 2.91; $P = 0.04$).

There was no effect of treatment (SX = 32.8 $\pm$ 0.5 kg/d, CS = 32.2 $\pm$ 0.3 kg/d; $P = 0.26$) or herd (A = 32.4 $\pm$ 0.5 kg/d, B = 32.6 $\pm$ 0.3 kg/d; $P = 0.78$) on average daily milk yield. Cows that had metritis, however, had smaller ($P = 0.02$) average daily milk yield than those that did not have metritis (32.1 $\pm$ 0.4 vs. 32.9 $\pm$ 0.4 kg/d).

All Heifers. In total, 243 SX heifers and 719 CS heifers started their first lactation in herds A and B. Interval from first AI to calving was longer ($P < 0.01$) for SX heifers (Table 3). Such a difference was a consequence of reduced P/AI after first AI for SX heifers because when pregnancy to first AI was included in the model as a covariate, treatment did not affect ($P = 0.52$) interval between first AI and parturition, and heifers that conceived to the first AI had shorter ($P < 0.01$) interval from first insemination to parturition

than heifers that did not conceive to first AI (9.1 $\pm$ 0.1 vs. 10.9 $\pm$ 0.1 mo). There was no difference ($P = 0.41$) in age at calving between SX and CS heifers, but heifers from herd A were older ($P < 0.01$) at first calving than those from herd B (Table 3). Average age at calving of heifers that conceived to first AI was 22.2 $\pm$ 0.1 mo and age at calving of heifers that did not conceive to first AI was 23.8 $\pm$ 0.1 mo ($P < 0.01$).

Initially, treatment ($P = 0.18$) and calf sex ($P = 0.42$) did not affect gestation length. When data from SX heifers that conceived to first AI with semen from the sire that resulted in outlier gestation length (Figure 2) were removed, treatment had no effect ($P = 0.22$) on gestation length (Table 3), but heifers delivering male calves tended ($P = 0.06$) to have longer gestation length than those delivering female calves (277.4 $\pm$ 0.9 vs. 275.6 $\pm$ 0.5 d).

Overall, proportion of heifers delivering female calves was affected ($P < 0.01$) by treatment (Table 3). As expected, the interaction between treatment and pregnancy to first AI also affected ($P < 0.01$) proportion of heifers delivering female calves, because whereas a greater proportion of SX heifers that conceived to first AI delivered female calves compared with those that did not conceive to first AI (85.7 vs. 52.0%), the proportion of CS heifers that did or did not conceive to first AI that delivered female calves was not different (47.7 and 55.0%, respectively).

Treatment ($P = 0.42$) and herd ($P = 0.34$) had no effect on CDS, but cows delivering female calves had

Table 4. Effect calving difficulty, twin birth, retained fetal membranes, metritis, and mastitis on culling, reproductive performance, total milk yield, and overall economic return after first lactation

Item	Culled by 60 DIM, %	Risk of culling, AHR ¹ (95% CI)	Pregnant at 305 DIM, %	Risk of conception, AHR (95% CI)	Total milk yield (±SEM), kg	Overall return (±SEM), \$
Calving difficulty						
1	3.7	0.91 (0.50, 1.64)	91.7 ^a	1.30 (1.06, 1.61)	9,173.2 ± 160.0 ^a	-52.5 ± 26.9 ^a
2-3	3.9	1.23 (0.61, 2.49)	85.3 ^b	1.10 (0.84, 1.43)	8,715.9 ± 249.2 ^b	-172.4 ± 44.9 ^b
4-5	4.4	Referent	81.4 ^b	Referent	8,827.9 ± 243.5 ^{a,b}	-163.7 ± 44.2 ^b
Twin birth						
No	3.7 ^a	0.25 (0.09, 0.70)	89.3	1.93 (0.86, 4.32)	9,279.9 ± 83.8 ^a	-128.0 ± 27.5
Yes	16.7 ^b	Referent	75.0	Referent	6,521.0 ± 1,259.0 ^b	-350.3 ± 144.5
Retained fetal membranes						
No	3.8	1.54 (0.45, 5.26)	89.6 ^A	1.24 (0.84, 1.85)	9,257.6 ± 86.4	-41.4 ± 16.3
Yes	5.7	Referent	78.8 ^B	Referent	8,926.0 ± 441.0	-43.0 ± 84.0
Metritis						
No	3.1	0.93 (0.61, 1.42)	90.1	1.15 (0.99, 1.32)	9,348.0 ± 111.0	-47.6 ± 21.3
Yes	4.8	Referent	88.0	Referent	9,111.0 ± 131.0	-33.4 ± 24.4
Mastitis						
No	3.8	0.44 (0.28, 0.68)	90.5 ^a	1.33 (1.10, 1.62)	9,160.7 ± 152.8 ^a	-76.3 ± 25.8 ^a
Yes	4.2	Referent	83.0 ^b	Referent	8,650.6 ± 230.7 ^b	-182.7 ± 41.4 ^b

^{a,b}Values within columns and items with different superscripts differ ($P < 0.05$).

^{A,B}Values within columns and items with different superscripts differ ($P < 0.10$).

¹AHR = adjusted hazards ratio.

smaller ($P < 0.01$) CDS than those delivering male calves (1.58 ± 0.11 vs. 1.99 ± 0.12). Similarly, treatment ($P = 0.38$) and herd ($P = 0.37$) did not affect proportion of cows with CDS = 1 (Table 3). Heifers delivering female calves were more likely ($P < 0.01$) to have CDS = 1 than heifers delivering male calves (79.2 vs. 57.5%). Those delivering singletons were more likely ($P < 0.02$) to have CDS = 1 than those delivering twins (69.6 vs. 50.0%).

Although incidence of CBD was affected by treatment ($P < 0.01$; Table 3), when only heifers that did not conceive to first AI were used in the analysis, treatment did not affect ($P = 0.25$) incidence of CBD. Herd ($P = 0.32$) had no effect on the incidence of CBD (Table 3). Cows delivering male calves (3.4 vs. 7.1%; $P < 0.01$) and cows delivering twins (5.0 vs. 16.7%; $P = 0.03$) were more likely to have CBD than those delivering females and singletons, respectively.

Proportion of heifers delivering live female calves was larger ($P < 0.01$) for SX heifers than for CS heifers (Table 3). As expected, the interaction between treatment and pregnancy to first AI also affected ($P < 0.01$) the proportion of heifers delivering a live female calf because SX heifers that conceived to first AI were more likely to deliver live female calves than those not conceiving to first AI (79.1 and 48.7%, respectively), whereas CS heifers that did or did not conceive to first AI delivered a similar proportion of live female calves (47.2 and 53.5%, respectively).

Incidence of RFM was not affected by treatment ($P = 0.21$) or herd ($P = 0.93$; Table 3), but twin births affected ($P < 0.01$) the proportion of cows that had RFM (twins = 25.0%; singleton = 3.3%). Treatment did not

affect ($P = 0.39$) incidence of metritis, but incidence of metritis was affected ($P < 0.01$) by herd (Table 3). Cows that had CBD were more likely ($P < 0.01$) to be diagnosed with metritis than cows that delivered a live calf (61.2 vs. 42.2%). Incidence of mastitis was not affected by treatment ($P = 0.51$), but cows in herd A tended ($P = 0.07$) to be more likely to have mastitis than those in herd B (Table 3). There was no effect of treatment ($P = 0.32$) and herd ($P = 0.40$) on proportion of cows culled within 30 DIM. Proportion of cows culled within 60 DIM was not affected by treatment ($P = 0.27$) or herd ($P = 0.26$), but cows that delivered twins were more likely ($P = 0.04$) to be culled within 60 DIM than cows that delivered singletons (Table 4). Rate of culling up to 305 DIM was not affected ($P = 0.65$) by treatment, but cows in herd A were culled at a slower ($P = 0.04$) rate than cows in herd B (AOR = 0.46; 95% CI = 0.22, 0.97). Cows delivering singletons had slower ($P < 0.01$) rate of culling than those delivering twins (Table 4). Occurrence of mastitis increased ($P < 0.01$) the rate of culling (Table 4), and calving during the hot season increased ($P = 0.04$) the culling rate compared with calving during the cool season (AOR = 1.65; 95% CI = 1.03, 2.64).

Treatment did not affect the proportion of cows diagnosed pregnant after first AI ($P = 0.50$) and at 305 DIM ($P = 0.60$; Table 3). Similarly, herd did not affect the proportion of cows pregnant after first AI ($P = 0.63$) and at 305 DIM ($P = 0.36$; Table 3). A greater ($P < 0.01$) proportion of cows with CDS = 1 were pregnant at 305 DIM compared with those with CDS = 2 to 5 (Table 4). Cows that had mastitis were less likely ($P = 0.01$) to be pregnant at 305 DIM than those that

Table 5. Effect of treatment (TRT) and herd on economic performance and total milk yield during first lactation for all heifers¹

Item ($\pm$ SEM), \$ unless noted	Herd A		Herd B		P-value		
	SX	CS	SX	CS	TRT	Herd	TRT $\times$ herd
Rearing cost	771.9 $\pm$ 10.0	759.1 $\pm$ 11.0	778.8 $\pm$ 8.7	740.8 $\pm$ 4.3	<0.01	0.52	0.16
Balance of heifers sold/dead ²	169.1 $\pm$ 66.1	20.2 $\pm$ 71.4	163.0 $\pm$ 47.1	137.6 $\pm$ 23.6	0.10	0.32	0.24
Calf revenue	134.8 $\pm$ 10.5	123.3 $\pm$ 11.4	149.2 $\pm$ 9.1	130.1 $\pm$ 4.8	0.09	0.24	0.67
Cost per female calf produced	-894.9 $\pm$ 26.8	-1,491.6 $\pm$ 28.7	-545.6 $\pm$ 24.4	-832.5 $\pm$ 21.4	<0.01	<0.01	<0.01
Total milk yield, kg	9,287.4 $\pm$ 258.9	9,619.8 $\pm$ 284.5	9,433.5 $\pm$ 225.1	9,208.8 $\pm$ 112.4	0.81	0.56	0.23
Income over feed cost	528.3 $\pm$ 17.4	549.1 $\pm$ 18.7	545.4 $\pm$ 17.7	529.6 $\pm$ 9.2	0.86	0.94	0.19
Replacement cost	186.1 $\pm$ 37.1	202.4 $\pm$ 22.2	146.9 $\pm$ 44.6	91.8 $\pm$ 48.2	0.60	0.05	0.32
Overall return	-139.2 $\pm$ 51.1	-254.5 $\pm$ 55.5	-28.1 $\pm$ 45.8	-96.2 $\pm$ 28.1	0.04	<0.01	0.58

¹SX = heifers inseminated with sex-sorted semen at first AI; CS = heifers inseminated with conventional semen at first AI.

²Balance of heifers sold/dead = difference between income from heifers sold as replacement and heifers culled and the cost to replace culled and dead heifers.

did not have mastitis, and cows that had RFM tended ($P = 0.06$) to be less likely to be pregnant at 305 DIM compared with those that did not have RFM (Table 4). Treatment ($P = 0.98$) and herd ($P = 0.41$) did not affect rate at which cows became pregnant. Rate at which cows became pregnant was affected by CDS ($P = 0.02$) and mastitis ($P < 0.01$) and tended ($P = 0.06$) to be affected by occurrence of metritis (Table 4).

Treatment ($P = 0.64$) and herd ($P = 0.44$) had no effect on average daily milk yield (SX = 31.2 ± 0.7 kg/d, CS = 31.0 ± 0.7 kg/d). Cows delivering twins (twins = 29.1 ± 1.3 kg/d; singleton = 33.1 ± 0.2 kg/d; $P < 0.01$) and cows that had metritis (metritis = 30.8 ± 0.7 kg/d; no metritis = 31.5 ± 0.7 kg/d; $P < 0.01$) and mastitis (mastitis = 30.8 ± 0.7 kg/d; no mastitis = 31.6 ± 0.7 kg/d; $P = 0.02$) had smaller average daily milk yield. Interestingly, heifers that conceived to first AI had smaller ($P < 0.01$) average daily milk yield during first lactation than heifers that did not conceive to first AI (30.6 ± 0.7 vs. 31.6 ± 0.7 kg/d); this was independent of treatment and herd.

Total Milk Production and Economic Outcomes

Rearing cost was affected ($P < 0.01$) by treatment, but herd ($P = 0.52$) had no effect on rearing cost (Table 5). Balance of heifers sold/dead before first lactation tended ($P = 0.10$) to be affected by treatment, but herd had no effect ($P = 0.32$; Table 5).

Calf revenue was greater ($P = 0.04$) for SX heifers compared with CS heifers, but herd ($P = 0.19$) had no effect (Table 5). Reduced calf revenue was associated with occurrence of CBD (CBD = $\$95.0 \pm 5.0$, calves born alive = $\$220.7 \pm 3.8$; $P < 0.01$) and delivery of singletons (twins = $\$228.8 \pm 7.4$, singletons = $\$86.9 \pm 2.0$). Cost per female produced was smaller ($P < 0.01$) for SX heifers than for CS heifers and for herd B than for herd A (Table 5).

Total milk production was not affected by treatment ($P = 0.81$) or dairy ($P = 0.56$; Table 5). Interestingly, heifers that conceived to first AI had smaller ($P < 0.01$) milk production than those that did not conceive to first AI ($8,624.3 \pm 202.3$ vs. $9,187.1 \pm 177.7$ kg). Total milk yield was greater for heifers delivering singletons than for those delivering twins ($P < 0.01$) and for those that did not have mastitis compared with those that had mastitis ($P = 0.02$; Table 4). Calving difficulty score tended ($P = 0.08$) to affect total milk production (Table 4). Similarly, IOFC was not different between treatments ($P = 0.82$) and herds ($P = 0.94$; Table 5). Heifers that conceived to first AI had smaller IOFC than those that did not conceive to first AI ($\$524.0 \pm 11.1$ vs. $\$549.3 \pm 10.6$; $P < 0.02$), and cows delivering twins ($\$380.8 \pm 46.4$ vs. $\$538.8 \pm 9.5$; $P < 0.01$) and those that had mastitis ($\$522.9 \pm 13.8$ vs. $\$553.2 \pm 9.0$; $P = 0.02$) had smaller IOFC than those delivering singletons and those that did not have mastitis, respectively. There was a tendency ($P = 0.08$) for CDS to affect IOFC because cows with CDS = 1 had greater IOFC than those with CDS = 2 to 3 (CDS 1 = $\$554.2 \pm 9.5$; CDS 2-3 = $\$526.7 \pm 14.9$; CDS 4-5 = $\$533.4 \pm 14.5$).

Replacement cost was not different between treatments ($P = 0.60$), but herd A had smaller ($P = 0.05$) replacement cost than herd B (Table 5). Delivery of twins resulted in increased replacement cost (twins = $\$473.2 \pm 121.7$; singleton = $\$156.6 \pm 23.0$; $P < 0.01$), as did occurrence of mastitis (mastitis = $\$220.2 \pm 34.8$; no mastitis = $\$93.5 \pm 21.6$; $P < 0.01$).

Finally, overall return during first lactation was affected by treatment ($P = 0.04$) and herd ($P < 0.01$; Table 5). As expected, delivery of a female calf was associated ($P < 0.01$) with greater overall return compared with the delivery of a male calf ($\$-83.1 \pm 43.5$ vs. $\$-242.8 \pm 42.5$). Cows that had mastitis ($P = 0.03$)

had reduced overall return compared with cows that did not have mastitis (Table 4).

DISCUSSION

As expected, heifers inseminated with sex-sorted semen had smaller P/AI than those inseminated with conventional semen. The reduction in P/AI observed in SX heifers, approximately 77.7% of the P/AI of CS heifers, is similar to that described by other researchers (Bodmer et al., 2005; Borchersen and Peacock, 2009; DeJarnette et al., 2009). The reasons for the reduced P/AI after insemination with sex-sorted semen are likely to be the smaller number of sperm cells per inseminating dose compared with conventional semen (2×10^6 vs. 20×10^6 cells) and the mechanical damage on the sperm during the process of sorting (Suh et al., 2005; Seidel and Schenk, 2008; Schenk et al., 2009). Recently it has been demonstrated that sorting sperm at 2.8 bar instead of 3.5 bar improves P/AI after insemination with sex-sorted semen (Suh et al., 2005; Schenk et al., 2009). The sex-sorted semen used in the current study was sorted at 3.5 bar because this was the standard pressure used in the sorting process at the time the study was conducted. It is important to note that whereas sperm sorting has significantly decreased fertility of certain bulls, sperm sorting does not affect fertility of other bulls (Borchersen and Peacock, 2009). Therefore, in the current study, another source of variation for P/AI after first AI was the different sires used in the SX and CS treatments. The reduced P/AI at first AI resulted in longer interval from first AI to calving in SX heifers, but the differences between SX and CS heifers were approximately 6 and 15 d in herds A and B, respectively. The extended interval from first AI to calving resulted in rearing cost approximately \$25/head greater for SX heifers than for CS heifers.

Although SX heifers in herd A started being inseminated approximately 21 d earlier than CS heifers, treatment and the interaction between treatment and herd did not affect age at calving. We conclude from these observations that starting breeding programs for heifers to be inseminated with sex-sorted semen 21 d earlier is not likely to significantly alter age at calving because heifers that fail to conceive to first AI with sex-sorted semen are likely to conceive shortly thereafter because insemination with sex-sorted semen does not alter pattern of return to estrus and does not affect the likelihood of heifers to conceive from subsequent AI. Further, as observed in this study and demonstrated by others (Hoffman et al., 1996; Ettema and Santos, 2004), heifers that calved at a younger age (<23 mo) had poorer productive and economic performance than those calving at an older age (>23.8 mo), suggesting

that when sex-sorted semen is to be used, starting insemination earlier may affect productive performance of heifers that conceive to first AI.

Insemination with sex-sorted semen did not affect the pattern of return to estrus after first AI or the incidence of pregnancy loss; therefore, one could conclude from the current data that the reduced P/AI of heifers inseminated with sex-sorted semen is not resultant from increased late embryonic and fetal losses. Seidel and Schenk (2008) also demonstrated that Holstein heifers inseminated with sex-sorted semen had similar pregnancy loss from 29 ± 1 to 57 ± 1 d after AI compared with heifers inseminated with conventional semen, and Tubman et al. (2004) observed no differences in incidence of abortion from 2 mo of gestation to parturition. Studies in which sex-sorted semen was used for in vitro production of embryos have obtained conflicting results regarding cleavage and blastocyst development rates (Lu et al., 1999; Merton et al., 2003; Xu et al., 2006). These experiments, however, demonstrated that the effects of sex-sorted semen on in vitro fertilization and embryo development rate are dependent on sire. In the current study, 3 sires were used for first insemination of SX heifers and, although not statistically significant, there was a numerical difference in P/AI among them. When used for insemination of non-superstimulated heifers and cows, there is no data that helps to elucidate whether sex-sorted semen compromises P/AI by reducing fertilization rate, early embryo development, or both.

Gestation lengths of SX heifers that conceived to one sire in particular were altered, with those delivering male calves having shorter gestation length than those delivering female calves. When data from SX heifers that conceived to such sire were removed from the analysis, gestation length was not affected by treatment. It is interesting that, among heifers that conceived to the first AI, the incidence of CBD was significantly greater for SX heifers, independent of calf sex. Tubman et al. (2004) did not observe differences in gestation length or calf mortality within the first 24 h after birth between animals inseminated with conventional or sex-sorted semen. DeJarnette et al. (2009) also indicated that there were no differences in gestation length or incidence of stillbirth between heifers inseminated with conventional and sex-sorted semen. Similarly, in a well-controlled study conducted in Denmark, the incidence of stillbirth was not different when Holstein, Jersey, and Danish Red heifers were inseminated with sex-sorted or conventional semen from the same bulls (Borchersen and Peacock, 2009). The incidence of CBD in SX heifers that conceived to first AI in the current study was within that reported by other researchers, but the incidence of CBD in CS heifers was consider-

ably smaller (Cole et al., 2007). In the current study, bulls used for first insemination of SX heifers were not the same as the ones used for first insemination of CS heifers. Therefore, the possibility that the difference in incidence of CBD between SX and CS heifers was related to sire and not sperm sorting cannot be discarded. It has been suggested that increased incidence of abortions and stillbirths may occur when pregnancies result from Y-chromosome that are mistakenly identified as sperm bearing X-chromosome during the sorting process because they could present aneuploidism, which would result in greater DNA content and increased risk for malformation (DeJarnette et al., 2009). However, to the knowledge of the authors, this hypothesis has not been evaluated.

The accuracy of sorting for sperm bearing X chromosome was 85.7%, with a range among sires from 79.4 to 95.7%. Compared with literature published to date, the accuracy of sperm sorting was significantly smaller in 1 sire used in the current experiment (Seidel et al., 1999; Borchersen and Peacock, 2009). More importantly, however, as a consequence of the increased incidence of CBD, only 79.2% of SX heifers pregnant to first AI produced a live female calf, which is a significantly smaller proportion than expected. Overall, the proportion of SX heifers delivering a live female calf was only 10 percentage units greater than that of CS heifers (60.1 vs. 50.1%). Even though the magnitude of this difference was considerably smaller than expected, SX heifers had calf revenue approximately \$15 greater than CS heifers. The average cost of production of a live female calf was approximately \$400 smaller for SX heifers than for CS heifers because, although SX heifers had greater rearing cost, their calf revenue and balance of sold/dead heifers was greater than that of CS heifers.

Although more SX heifers that conceived to first AI delivered a female calf than CS heifers that conceived to first AI, calving difficulty and proportion of heifers not requiring assistance at calving were not different between treatments, when only heifers pregnant to first AI and when all heifers were included in the statistical analysis. This was somewhat surprising because we expected that SX heifers that conceived to first AI would have smaller calving difficulty and incidence of dystocia than CS heifers because they delivered more female calves. In the current study calves were not weighed at birth, but Tubman et al. (2004) demonstrated that weight of calves produced from sex-sorted semen or conventional semen were not different. Tubman et al. (2004), however, demonstrated that female calves had smaller weight at birth and dams delivering female calves had smaller calving ease, which is corroborated by other authors (Lombard et al., 2007).

It is interesting that in herd A, in which SX heifers were approximately 21 d younger at first AI compared with CS heifers, and in herd B, in which SX and CS heifers were at the same age at first AI, calving ease and incidence of dystocia were not different between treatments. Further, in herd A SX heifers calved approximately 6 d later than CS heifers, whereas in herd B SX heifers calved approximately 15 d later than CS heifers. These differences are minimal compared with the potential loss in milk production observed in heifers that calved at <23 mo of age in the current and other studies (Hoffman et al., 1996; Ettema and Santos, 2004). Therefore, when heifers are to be inseminated with sex-sorted semen, they should start being inseminated so that those that conceive to first AI calve at >23 mo of age.

One of our hypotheses was that because more heifers conceiving to sex-sorted semen would deliver female calves, these heifers would be less likely to have dystocia and consequently puerperal diseases (i.e., RFM and metritis). In the current study, however, among heifers that conceived to first AI, the proportion of SX and CS heifers that had RFM and metritis were not different. It is intriguing that, among heifers that conceived to first AI, SX heifers were and tended to be more likely to be culled within the first 30 and 60 DIM, respectively. In light of the fact that incidences of dystocia, RFM, and metritis were not different between SX and CS heifers that conceived to first AI, these differences in incidences of culling early postpartum are difficult to explain. Further, when all heifers were included in the model, treatment did not affect incidence of culling at 30 and 60 DIM, culling risk, and cost of replacement cows.

Finally, total milk yield was not affected by treatment. Considering that there were no differences between SX and CS heifers in age at calving, incidence of twins, calving difficulty, and incidence of RFM, metritis, and mastitis, it is not surprising that treatment did not affect total milk yield. Consequently, IOFC was not affected by treatment. The overall economic return during the first lactation was greater for SX heifers because, although rearing cost of SX heifers was approximately \$25/heifer greater than CS heifers, balance of sold/dead heifers of the SX treatment was approximately \$88/heifer greater than those of the CS treatment and calf revenue was approximately \$15/heifer greater for SX than CS heifers.

CONCLUSIONS

Insemination of heifers with sex-sorted semen resulted in smaller P/AI, likely because of reduced sperm cells per inseminating dose and possible damages to sperm

cells during sperm sorting. The proportion of heifers delivering a live female calf was greater when heifers were inseminated with sex-sorted semen, but, among those that conceived to first AI, the increase was hampered by increased proportion of CBD. Further, overall, the difference in proportion of heifers delivering a live female calf was only 10 percentage units. Although insemination of heifers with sex-sorted semen resulted in greater interval from first AI to calving, the differences in age at calving were negligible. Treatment did not affect health parameters during the postparturient period; consequently, treatment had no effect on total milk yield, and IOFC. Overall economic return during the first lactation was greater for SX heifers because they had greater balance of sold or dead heifers and calf revenue. Because cost per heifer produced was significantly smaller for the SX treatment compared with the CS treatment, we conclude that herds that need to expand in size and those that market female calves and heifers should use sex-sorted semen for first AI of heifers.

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