Comparison between low-dose, high-sort and high-dose, low-sort semen on conception and calf sex ratio in Jersey heifers and cows

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ABSTRACT

The objective of this clinical trial was to compare conception and newborn calf sex ratios among Jersey heifers and lactating cows inseminated with either standard sex-sorted semen (low-dose, high-sort; LDHS) containing 2.1×10^6 sorted sperm at 90% purity or high-dose, low-sort (HDLS) semen containing 10×10^6 sorted sperm at 75% purity. After a specified voluntary waiting period (VWP), female subjects, consisting of nulliparous heifers (VWP 10 mo of age) and lactating cows (VWP 50 d in milk), received their first service and were systematically allocated to each treatment group in the order in which they presented for artificial insemination (AI). Females were bred to the same sire and type of sex-sorted semen for up to 2 additional services. Animals that were not pregnant after 3 breeding attempts were excluded. A total of 1,846 services were performed on 1,011 eligible females (LDHS; $n = 494$, HDLS; $n = 517$), which consisted of 516 nulliparous heifers and 495 lactating cows. Study groups were comparable with respect to the mean age at first AI for nulliparous heifers and the mean days in milk at first AI for parous cows. Insemination with HDLS semen did not result in a higher proportion of pregnancies per AI (P/AI) compared with LDHS semen for either nulliparous heifers (P/AI = 43 vs. 38%) or parous cows (P/AI = 47 vs. 43%). Insemination of nulliparous heifers using HDLS resulted in a lower proportion of newborn female calves compared with those bred to LDHS (76% vs. 87%). Similarly, lactating cows bred to HDLS gave birth to a lower proportion of newborn female calves compared with those bred to LDHS (79

vs. 90%). The odds ratio for a female calf to be born to an animal inseminated with HDLS compared with LDHS was 0.32 for nulliparous heifers and 0.19 for parous cows. Overall, the use of HDLS resulted in fewer females compared with LDHS, which may be explained by the lower concentration of X-bearing spermatozoa in HDLS compared with LDHS.

Key words: sex-sorted semen, sperm dose, pregnancy, sex ratio

INTRODUCTION

The commercial production of sex-sorted semen has been one of the major advances in dairy cattle reproduction in recent years. Based on the amount of DNA in each sperm, researchers in the 1980s were able to sort sperm bearing X and Y chromosomes by flow cytometry (Johnson et al., 1989; Seidel, 2007). Initially, sex sorting of semen was inefficient, with several difficulties including damaged sperm viability due to UV irradiation (Libbus et al., 1987; Johnson et al., 1989). The process was subsequently improved at various critical steps to allow for its commercialization. The characteristic flattened oval shape of the bovine spermatozoon required the development of a nozzle that orients the sperm heads (Rens et al., 1996), which is critical for increased accuracy of the sorting process (Johnson, 2000). Sorting speeds were increased, allowing higher sorting rates while maintaining high X-chromosome purity and reduced operating pressures of the flow cytometer, thus decreasing damage to the spermatozoa (Johnson and Welch, 1999; Suh et al., 2005).

Bovine X-bearing spermatozoa contain 3.8% more DNA than Y-bearing spermatozoa (Garner, 2006). The production of sex-sorted semen involves the staining of spermatozoa and their illumination by argon laser, allowing the greater fluorescence of the X chromosome to be distinguished and recognized by a detector (Seidel, 2007). Droplets exiting the nozzle contain an X-sperm, a Y-sperm, a combination of both, or neither. Only

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those droplets containing X-bearing spermatozoa are deflected by a charged plate, resulting in some 20% of the original sperm sample being sorted as purified X-bearing sperm (Seidel, 2007).

The dairy industry has an economic imperative to produce female offspring. The ability to alter sex ratios has allowed producers who utilize sex-sorted semen on nulliparous heifers to expand replacement inventories. The use of sex-sorted semen decreases the cost per female calf produced compared with nulliparous heifers bred to nonsorted semen and additionally increases the economic return during the first lactation (Chebel et al., 2010). To date, the use of sex-sorted semen in the dairy industry has been limited because of its poor reproductive efficiency and high cost (Seidel, 2003). The rate of sorting sperm is limited to 9 to 14×10^6 spermatozoa per hour (Schenk et al., 2009). Consequently, the rate of production of sex-sorted semen doses may not justify the capital investment in the required equipment (Moore and Thatcher, 2006). As a result of such expenses and the inefficiencies of the sorting process, the commercial production of sex-sorted semen has been at low insemination doses (Amann, 1999). Typically, a dose of sex-sorted semen contains 2.1×10^6 spermatozoa at 90% purity of X-chromosome spermatozoa, henceforth referred to as low-dose, high-sort (**LDHS**). Conception rates reported in virgin heifers using LDHS sex-sorted semen have consistently yielded about 75 to 80% of that obtained using non-sex-sorted semen in well-managed systems (Bodmer et al., 2005; DeJarnette et al., 2009, 2010). Similarly, conception rates reported for lactating cows have ranged from 23 to 29%, or 75 to 80% of the conception rate achieved with conventional semen in the same herds (Bodmer et al., 2005; DeJarnette et al., 2008). As a result, commercial use of LDHS has been mostly restricted to virgin heifers (Schenk and Seidel, 2007; DeJarnette et al., 2011).

Recent studies have focused on the effect of increasing the dosage of 90% X-chromosome sex-sorted semen above the 2.1×10^6 concentration; however, these studies did not find significant differences in conception rates between different doses in heifers or cows (DeJarnette et al., 2008, 2010). Data from a subsequent study showed that a semen dose of 10×10^6 sperm significantly improved conception rates compared with 2.1×10^6 in heifers (44 vs. 38%; DeJarnette et al., 2011). Although such an increase in dose resulted in higher conception rates, approximately 5 times the number of ejaculates per bull were necessary to produce the same number of these higher concentration LDHS semen doses. Other trials have examined the effect of dose as well as the effect of the sorting process on reproductive efficiency. A field trial comparing sex-sorted and nonsorted semen at a dose of 2×10^6 spermatozoa showed significantly

improved pregnancy rates in heifers inseminated with nonsorted semen but not in cows (Bodmer et al., 2005).

In an effort to improve reproductive efficiency and reduce the cost of producing sex-sorted semen, a higher dose (10×10^6), lower sort (75% X-spermatozoa) product (termed high-dose, low-sort; **HDLS**) was formulated. During the production of HDLS, combined sperm droplets (i.e., droplets that contain both X- and Y-bearing spermatozoa) are retained in the sex-sorted semen, thus reducing the X-sperm purity to approximately 75%. The objectives of this clinical trial were to compare the pregnancy per AI (**P/AI**) and resulting calf sex ratios due to insemination with HDLS (10×10^6 spermatozoa per dose at 75% purity of X chromosome) or LDHS (2.1×10^6 spermatozoa per dose at 90% purity) semen in nulliparous heifers and lactating cows in a California Jersey herd.

MATERIALS AND METHODS

Heifer and Cow Housing and Diets

The trial was conducted in a 3,000-cow Jersey herd in the San Joaquin Valley of California. Enrolled nulliparous heifers were eligible to be bred at 10 mo of age, whereas lactating cows of 6 lactations or fewer were eligible after a 7-wk postcalving voluntary waiting period (**VWP**). Heifers and cows were housed in dry-lot pens and fed a TMR twice daily. Diets were formulated to meet the NRC (2001) nutritional requirements for growing heifers and for lactating dairy cattle. Lactating cows were milked twice daily and produced an average of 28 kg of 3.5% FCM per day.

Reproductive Management

Inseminations were performed by 3 farm technicians twice a day after visual estrus detection and evaluation of tail head chalk (All-Weather Paintstick, La-Co Industries, Chicago, IL) as a secondary sign of estrus expression. Eligible cattle detected in estrus in the morning were bred in the afternoon and vice versa. Lactating animals were presynchronized using 2 i.m. injections of PGF_{2α} (25 mg of dinoprost tromethamine; Lutalyse, Pfizer Animal Health, New York, NY) 14 d apart at 36 ± 3 and 50 ± 3 DIM. Cows that were not detected in estrus by 61 ± 3 DIM were enrolled into an ovulation-synchronization protocol (**OVS**). This consisted of an intramuscular injection of GnRH (100 μg of gonadorelin diacetate; Cystorelin, Merial Ltd., Duluth, GA) on d 0, 25 mg of PGF_{2α} by i.m. injection on d 7, a second i.m. injection of 100 μg of GnRH on d 9 and a timed AI 24 h later. Pregnancy status was determined weekly by rectal palpation of females who

had not returned to estrus 39 ± 3 d after AI. Cows found not to be pregnant were re-enrolled in OVS on the day of their negative pregnancy diagnosis.

Sire Information

Semen straws from 12 Jersey sires were included in the study and were available at both sperm doses and concentrations: 2.1×10^6 sperm/mL with a 90% X-chromosome purity (LDHS) and 10×10^6 sperm/mL with 75% X-chromosome purity (HDLS). All sires had a history of producing semen with >80% normal sperm morphology. Semen was collected from bulls on a routine collection schedule using an artificial vagina (Schenk, 1998). Ejaculates were screened for morphology and those with $\geq 80\%$ normal morphology were sex-sorted to approximately 90% purity for X-chromosome for LDHS semen (Seidel et al., 1999; Seidel and Garner, 2002; Garner and Seidel, 2003) or to 75% X-chromosome purity for HDLS semen. The X-chromosome purity was quantified using an STS Sexed Semen Purity Analyzer (Sexing Technologies, Navasota, TX). All samples were processed in a Tris-egg yolk buffer and 7% glycerol extender and packaged in color-coded 0.25-mL French semen straws. The LDHS semen was packaged into red straws and the HDLS semen was packaged into yellow straws. Straws were frozen in liquid nitrogen vapor before submersion in liquid nitrogen (Robbins et al., 1976). Straws were packed into canes and shipped to the dairy.

Study Design

Between November 2009 and June 2010, individual females were systematically assigned to either HDLS or LDHS based on alternating semen type at their first insemination. Animals that returned to estrus or were found to be nonpregnant at pregnancy diagnosis were bred to the same sire and type of sex-sorted semen for up to 2 additional services. Cattle that returned to estrus after a positive pregnancy diagnosis were assigned an abortion date at the time of their re-insemination event and were censored. Subjects that were bred in error to a different sire or type of sex-sorted semen compared with their initial allocation were excluded from the analysis. A 72-h interestrus interval was used to distinguish services. All data from subjects re-inseminated within this interval were excluded. Subjects that failed to stay in the breeding pool before a pregnancy diagnosis were excluded from all analyses. These included cattle with reproductive tract abnormalities, cows culled due to mastitis or low production, or cows that died or were sold as replacements. Individual cow data were extracted from dairy computer records

(Dairy Comp 305, Valley Ag Software, Tulare, CA) and hand-written farm breeding records.

Calving Data

A gestation length of 280 ± 10 d was used to match calving data with conception data. Cattle without an abortion date and a >10-d difference from the expected freshening date were further examined for the possibility of stillbirths (in the case of a gestation shorter than 280 d) or missing breeding information (when gestation exceeded 290 d). Because nulliparous heifers were moved to natural service groups after positive pregnancy diagnosis, freshening dates greater than 10 d from the expected freshening date were assumed to be bull conceptions and thus excluded from the calving analysis.

Final Proportion Pregnant per Group

The final proportion pregnant in each treatment group was calculated as the total number of cows diagnosed pregnant after the first 3 services post-VWP divided by the total number of animals enrolled in each group. Pregnancy per AI (**P/AI**) was calculated as the number of animals diagnosed pregnant divided by number of animals inseminated at each service number.

Statistical Analysis

Statistical tests were used to assess the comparability of the groups at enrollment. Days in milk at first insemination and mean lactation number for parous cows and mean age at first breeding for heifers were compared using a 2-sample *t*-test. The proportion of animals that aborted was compared between treatment groups. The final proportion of females pregnant after all 3 breedings, and P/AI for each breeding were compared between HDLS and LDHS for nulliparous heifers and lactating cows. Similarly, the final proportion pregnant after all 3 breedings and P/AI for each breeding were compared between heifers and cows for each sex-sorted semen type. Finally, the proportion of female calves born to HDLS compared with LDHS breedings in nulliparous heifers and lactating cows were compared. Proportions were compared using the *z*-test for proportions.

Logistic Regression

A random effects logistic regression model was performed to investigate the association between type of sex-sorted semen and conception by comparing the odds of a female conceiving to HDLS semen with the

odds of a female conceiving to LDHS semen for nulliparous heifers and lactating cows, separately. A second model was specified to compare the odds of a live heifer calf being born to HDLS compared with LDHS semen used to inseminate nulliparous heifers and lactating cows, separately. For both outcomes, variables in the full model included HDLS, breeding number (**BN**), AI technician (**AITech**), and sire (**Sire**) as fixed effects, whereas the j th heifer was modeled as a random intercept u_{0j}^{heifer} and the k th breeding number ($k = 1, 2, 3$) as a random slope $u_{1j}^{heifer} BN_{jk}$. The full logistic regression model for conception in nulliparous heifers can be summarized as follows:

$$\text{logit}[P(\text{conception})] = \beta_0 + \beta X_{HDLS} + \beta X_{Sire} + \beta X_{AITech} + \beta X_{BN} + u_{0j}^{heifer} + u_{1j}^{heifer} BN_{jk},$$

and that for a female calf born to a nulliparous heifer can be summarized as follows:

$$\text{logit}[P(\text{female calf})] = \beta_0 + \beta X_{HDLS} + \beta X_{Sire} + \beta X_{AITech} + \beta X_{BN} + u_{0j}^{heifer} + u_{1j}^{heifer} BN_{jk}.$$

In addition to these predictors, each of the cow models also included lactation (**LACT**) and OVS as fixed effects with the random effects u_{0j}^{cow} and $u_{1j}^{cow} BN_{jk}$. All 2-way interactions were investigated, and a manual backward stepwise elimination procedure was implemented using 5% level of significance. The odds ratio (**OR**) for conception models was interpreted as the ratio of the odds of a female conceiving using HDLS compared with LDHS. Similarly, the OR for a newborn heifer calf was interpreted as the ratio of odds of a female giving birth to a live female calf from a HDLS breeding compared with a live female calf from a LDHS

breeding. Statistical analyses were performed using commercial statistical software packages (Minitab 15, Minitab Inc., state College, PA; Stata 11.2, Stata Corp., College Station, TX).

RESULTS

The mean age at first AI among nulliparous heifers was not significantly different between LDHS and HDLS groups (336 vs. 335 d; $P = 0.45$). The mean DIM at first AI for lactating cows was not significantly different between LDHS and HDLS groups (58 and 57 d, respectively; $P = 0.52$). Similarly, the mean lactation number was not significantly different between LDHS and HDLS (2.71 and 2.90, respectively; $P = 0.28$). The proportion of abortions was not significantly different between treatment groups for either heifers (LDHS = 7.3%, HDLS = 10%; $P = 0.34$) or cows (LDHS = 11.2%, HDLS = 6.4%; $P = 0.09$). Table 1 summarizes the frequency distribution of conception by type of sex-sorted semen and breeding number for heifers and cows. A total of 1,846 services were performed on 1,011 eligible subjects (LDHS; $n = 494$, HDLS; $n = 517$), which consisted of 516 nulliparous heifers and 495 lactating cows.

Nulliparous Heifers

Final proportion of females pregnant after all 3 breedings in heifers bred to HDLS and LDHS was 77.6 and 73.2%, respectively ($P = 0.24$). Similarly, the P/AI proportion after each breeding to HDLS was not significantly different from those to LDHS ($P = 0.23$, 0.15, and 0.82 for first, second, and third breeding, respectively). The median time to conception was 49 d (95% CI: 46, 55 d) and 56 d (95% CI: 50, 62 d) for HDLS and LDHS, respectively. Sire, AI technician, and

Table 1. Frequency distribution of conceptions in Jersey females bred using low-dose, high-sort (LDHS) or high-dose, low-sort (HDLS) semen stratified by breeding number and parity in a field trial conducted on a California dairy¹

Animals	Breeding Number	LDHS			HDLS		
		No. Conceived	No. Bred	%	No. Conceived	No. Bred	%
Heifers	1	111	261	42.5	122	255	47.8
	2	49	148	33.1	54	130	41.5
	3	31	99	31.3	22	74	29.7
	Total	191	508	37.6	198	459	43.1
Cows	1	110	233	47.2	128	262	48.9
	2	53	124	42.7	64	132	48.5
	3	18	63	28.6	23	65	35.4
	Total	181	420	43.1	215	459	46.8

¹LDHS semen contained 2.1×10^6 spermatozoa per dose at 90% purity of X-chromosome; HDLS semen contained 10×10^6 spermatozoa per dose at 75% purity of X-chromosome.

breeding number had no significant effect on conception in heifers and hence were not included in the final logistic regression model. We observed no significant difference in the odds of conception in heifers bred to HDLS versus LDHS (P/AI = 43.0 vs. 37.6%; adjusted OR = 1.33; $P = 0.10$).

A total of 145 heifers (LDHS: $n = 76$; HDLS: $n = 69$) were excluded from data analyses, with 137 of those heifers having conception dates that did not match calving records, 5 heifers with late abortions (>170 d carried calf), and 3 excluded because of twin calving. The majority of the calvings excluded from this analysis were because of inconsistent conception and calving dates. The proportion of female calves born to heifers bred using HDLS and LDHS were 76.3 and 86.5%, respectively ($P = 0.01$). Table 2 summarizes the final logistic regression model for odds of a female offspring in heifers. A heifer inseminated with HDLS semen had 68% lower odds of giving birth to a female calf compared with a heifer inseminated with LDHS semen ($P = 0.02$). We observed no significant effect for sire or AI technician; however, heifers presented for a third service had significantly lower odds of conceiving compared with those bred at first service ($P = 0.03$). Out of 371 nulliparous heifer calvings, LDHS yielded 160 live heifer calves out of 185 heifer calvings (86.5%; 95% CI: 81.5, 91.4), whereas HDLS yielded 142 live heifer calves out of 186 heifer calvings (76.3%; 95% CI: 70.2, 82.5).

Lactating Cows

Final proportion pregnant after all 3 breedings in cows bred to HDLS and LDHS was 82.1 and 77.7%, respectively ($P = 0.22$). Similarly, the P/AI proportion after each breeding to HDLS was not significantly different from those to LDHS ($P = 0.71, 0.35$, and 0.41 for first, second, and third breeding, respectively). The

median time to conception was 26 d (95% CI, 22–29 d) and 28 d (95% CI, 25–31 d) post-VWP for HDLS and LDHS, respectively. We found no significant difference between P/AI in cows compared with heifers after each of the first, second, or third services and for each of the sexed semen types ($P > 0.05$).

A total of 144 cows (LDHS: $n = 69$; HDLS: $n = 75$) were excluded from data analyses: 137 had conception dates that did not match calving date records, 2 cows had late abortions (>245 d carried calf), and 5 were excluded because of twin calving. Sire, technician, BN, LACT, and OVS had no significant effect on conception in cows and hence these variables were removed from the final logistic regression model. In addition, the odds of conception did not differ in cows bred to HDLS compared with LDHS (P/AI 46.8 vs. 43.1%; adjusted OR = 1.15; $P = 0.38$).

The proportion of female calves was lower in cows bred using HDLS compared with LDHS (78.64 vs. 90.2%, $P = 0.003$). A cow inseminated with HDLS had 81% lower odds of giving birth to a female calf compared with LDHS ($P = 0.03$; Table 3). We observed no significant effects due to LACT, BN, AI technician, or OVS; hence, these were dropped from the final model. Out of 351 calvings in parous cows, 148 heifer calves were born using LDHS semen in 164 cows (90.2%; 95% CI: 85.7, 94.8) compared with 147 heifer calves born using HDLS semen in 187 cows (78.6%; 95% CI: 72.7, 84.5).

DISCUSSION

The use of HDLS semen in this study had no effect on the proportion of animals becoming pregnant among either nulliparous or parous animals compared with insemination with LDHS semen. Previous studies that have examined the reduction of fertility associated with sex-sorted semen have found that sperm

Table 2. Final random effects logistic regression model for the odds of a female calf being born to a heifer bred to 1 of 2 types of sex-sorted semen in a Jersey herd ($n = 371$)

Variable	Odds ratio	SE	P-value	95% CI
Semen type ¹				
LDHS	Reference			
HDLS	0.32	0.15	0.02	0.12; 0.82
Breeding number				
1	Reference	—	—	—
2	0.21	0.22	0.13	0.29; 1.60
3	0.07	0.09	0.03	0.01; 0.78
Random effects	Variance			
Intercept (heifer)	3.02	1.16	—	1.42; 6.40
Breeding number	1.38	1.01	—	0.33; 5.80

¹LDHS (low-dose, high-sort) semen contained 2.1×10^6 spermatozoa per dose at 90% purity of X-chromosome; HDLS (high-dose, low-sort) semen contained 10×10^6 spermatozoa per dose at 75% purity of X-chromosome.

Table 3. Final random effects logistic regression model for the odds of a female calf being born to a cow bred to 1 of 2 types of sex-sorted semen in a Jersey herd ($n = 351$)

Variable	Odds ratio	SE	<i>P</i> -value	95% CI
Semen type ¹				
LDHS	Reference			
HDLS	0.19	0.14	0.027	0.04; 0.82
Random effects	Variance			
Heifer	3.00	1.14	—	1.42; 6.32
Breeding number	0.93	0.43	—	0.37; 2.30

¹LDHS (low-dose, high-sort) semen contained 2.1×10^6 spermatozoa per dose at 90% purity of X-chromosome; HDLS (high-dose, low-sort) semen contained 10×10^6 spermatozoa per dose at 75% purity of X-chromosome.

dose plays an important, if somewhat complex, role. Previous research on the effect of insemination dose showed that the reproductive efficiency of most bulls remained high at 10×10^6 , whereas only highly fertile bulls maintained high reproductive efficiency at 2×10^6 (Den Daas et al., 1998). Bulls that respond to increases in sperm dosage are thought to possess a greater proportion of compensable sperm defects (Saacke, 1998). One study attributed approximately two-thirds of the loss of fertility of sex-sorted semen to low dose (Frijters et al., 2009), with the remainder attributed to effect of the sorting process. However, both effects were found to differ among bulls. Sperm dosage and sire interactions were also observed in another study, where conception rates of heifers were significantly improved in one sire that had a sperm dose increase from 2.1×10^6 to 5×10^6 (DeJarnette et al., 2008), but not in 2 other sires. Interestingly, the same study found no effect of dose or sire among parous cows. DeJarnette et al. (2008) posit that fertility in the adult female population constrains maximal sire fertility, whereas increased sperm dose allows the deleterious effects of compensable sperm defects to be mitigated among more fertile heifers. This conclusion was reinforced by a further study (DeJarnette et al., 2011), which found that conception rates in heifers improved by increasing dose of sex-sorted semen from 2×10^6 to 10×10^6 sperm. However, the higher dose of sex-sorted semen did not result in P/AI comparable with that of nonsorted semen at either dose, implying that the sorting process does induce damage that cannot be entirely overcome by increasing the number of spermatozoa per insemination. In the present study, one might speculate that the increase in sperm dose associated with HDLS semen was insufficient to overcome compensable sperm defects due to either the sires used in the study or as a result of the sorting process itself.

The study sires' semen did not result in significantly different odds of conception in heifers and cows. Interestingly, we observed no significant difference between P/AI percentages for the first, second, or third services

in heifers compared with cows for each of the sexed semen types. These results were in contrast to other studies in which nulliparous heifers attained higher conception rates than lactating cows (Bodmer et al., 2005; DeJarnette et al., 2010). One possible explanation may be the lack of statistical power to detect a difference in proportion conceived between semen type in each of heifers and cows. However, unlike the previous studies, our study restricted the use of either LDHS or HDLS semen to the same animals during the first 3 services. Breed differences, as well as management practices, should be taken into consideration when making comparisons across studies. Average first-service conception achieved by LDHS heifers (42.5%) was comparable to that previously reported by Borchersen and Peacock (2009) in Jersey heifers (46%); however, a decline in conception was observed in the remaining 2 breedings (33.1 and 31.1% in second and third services). Such a marked reduction in conception was not observed in heifers bred to HDLS in our study until the third breeding (47.8, 41.2, and 29.3% for first, second, and third services, respectively).

The current study is the first to report on the reproductive performance and expected calf sex ratios with the use of HDLS. The odds of a female offspring in heifers and cows bred with HDLS semen were significantly lower than those bred with LDHS semen. The percentage of female calves conceived with LDHS semen in this trial was consistent with other reported sex ratios in the literature, which were consistently above 85% (Bodmer et al., 2005; Borchersen and Peacock, 2009; Chebel et al., 2010). In comparison, HDLS produced approximately twice as many male calves as LDHS.

It is unclear whether the sorting process to produce HDLS decreased mechanical damage to the sorted semen. A study comparing baseline levels of DNA damage between nonsorted and LDHS semen showed that the sorting process significantly reduces the subpopulations of fragmented DNA (Gosálvez et al., 2011). However, the post-sorting rate at which sex-sorted semen undergoes DNA fragmentation was higher than that for

nonsorted semen (Gosálvez et al., 2011). No congenital abnormalities were observed in our study, and field data from another study failed to show an association between sex-sorted semen and gross anatomical abnormalities in calves born to sex-sorted semen (Tubman et al., 2004).

One limitation of our study was the lack of randomization of heifers and cows to LDHS or HDLS; however, systematic allocation resulted in baseline comparability among treatment groups with respect to age at first service for nulliparous heifers and DIM at first service for lactating cows. The use of 12 sires in the study could have been another source of variation; however, each sire was available for LDHS and HDLS. Furthermore, the same numbers of straws was available from each sire. During the study, AI technicians were aware of the color scheme used to differentiate between treatments, which could have introduced a source of bias on the use of the semen in the trial. However, we observed no significant differences in conception between AI technicians that bred heifers and cows; hence, a bias due to unmasking of semen straw color is unlikely.

Future areas of research might compare the use of HDLS to sex-sorted semen with standard X-bearing spermatozoa concentrations (90%) but at a higher dose (10×10^6 spermatozoa). Alternatively, lower concentration and lower dose sex-sorted semen may be promising in terms of cost effectiveness. Hence, optimum combinations of dose and sorting purities need to be investigated. Furthermore, the cost effectiveness of one dose-sort combination compared with another should be investigated within the context of today's dynamic market for female and male calves. Breed differences should also be explored by comparing LDHS and HDLS semen in Holstein heifers. Within European dairy breeds, DNA content differences have been found, in which Jersey bulls were found to have a greater difference in the DNA content of the X and Y chromosomes (4.22%) compared with Holstein bulls (4.01%; Garner et al., 1983). Similarly, sire differences should be considered because some sires exhibit higher fertility. The economics of this new sorting technique should be explored further and compared with LDHS semen and nonsorted semen.

CONCLUSIONS

The use of HDLS sex-sorted semen did not significantly improve conception in heifers or cows. The use of HDLS resulted in fewer females compared with LDHS, which may be explained by the lower proportion of X-bearing sperm in HDLS compared with LDHS. Nevertheless, the use of HDLS may be cost effective given the

simpler processing technique and lower cost required to sort semen into HDLS compared with LDHS.

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