Effects of 2.1 and 3.5×10^6 sex-sorted sperm dosages on conception rates of Holstein cows and heifers

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ABSTRACT

The objective was to compare conceptions rates of Holstein cows and heifers after artificial insemination (AI) with 2.1 or 3.5×10^6 sex-sorted sperm or 15×10^6 conventional sperm. Ejaculates collected from 7 Holstein sires were cryopreserved conventionally at 15×10^6 sperm per dose or sorted to 90% purity for X-chromosome-bearing spermatozoa using flow cytometry and cryopreserved at either 2.1 or 3.5×10^6 sperm per dose. All treatments were processed in an egg-yolk (20%), Tris, glycerol (7%) extender and packaged in color-coded 0.25-mL French straws. Straws ($n = 700$ straws/dosage per sire) were packaged and distributed in aliquots of 12 (4 straws/sperm dosage) to 69 Holstein herds with an across-herd goal of achieving ~50% use in heifers and cows. Straw color was recorded in the on-farm recordkeeping system at the time of AI and retrieved by electronic download. Data for cows and heifers were analyzed separately. Among heifers, 6,268 services were retrieved from 45 herds (298 ± 4.2 services/sperm dose per sire; range: 244 to 344). Conception rate of heifers was influenced by the sire by treatment interaction. Conception rate of the 2.1 and 3.5×10^6 sex-sorted sperm dosages were comparable in 6 of 7 sires. Conception rate of both sex-sorted dosages were less than those of conventional semen for 6 of 7 sires. Across sires, heifer conception rates for 2.1 and 3.5×10^6 sex-sorted sperm dosages and 15×10^6 conventional dosages were 44, 46, and 61%, respectively. Among cows, 5,466 services were retrieved from 52 herds (260 ± 3.3 services/sperm dose per sire; range: 236 to 289). Conception rates of cows were influenced by herd, sire, and sperm dosage. Conception rates of the 2.1 and 3.5×10^6 sex-sorted sperm dosage were comparable for all 7 sires. Conception rates of 2.1×10^6 sex-sorted sperm dosage were less than those of conventional semen for 4 of 7 sires and conception rates of the 3.5×10^6 sex-

sorted sperm dosage were less than those of conventional semen for 2 of 7 sires. Across sires, conception rates for 2.1 and 3.5×10^6 sex-sorted sperm dosages and 15×10^6 conventional dosages in cows were 23, 25, and 32%, respectively. In conclusion, these data could not confirm that a meaningful improvement in conception rates should be expected in cows or heifers from increasing sex-sorted sperm dosage from 2.1 to 3.5×10^6 sperm per dose.

Key words: sex-sorted semen, sperm dosage, flow cytometry, conception rate

INTRODUCTION

The ability of flow cytometry to accurately distinguish between X- and Y-chromosome-bearing sperm populations was first reported by Garner et al. (1983). Johnson et al. (1989) later validated the technology with the birth of live offspring of the predicted sex. Flow sorting procedures were refined to facilitate commercial application in cattle, while documenting the ability of the technology to consistently produce ~90% sex bias among offspring (Schenk et al., 1999; Seidel et al., 1999). Despite progress made to date, the highly invasive nature of the procedure has detrimental effects on sperm viability and quality (Seidel and Garner, 2002; Seidel, 2007). Additionally, the equipment expense, speed, and sorting efficiency dictate that commercial application is presently only economically possible with extremely low (~2 million) sperm numbers per AI dose (Amann, 1999; Seidel, 2007). For these reasons, commercial application has focused on use of sex-sorted semen in virgin heifers to capitalize on the greater opportunity for conception (compared with lactating dairy cows) and to provide a greater opportunity for a more positive return on investment for the producer adopting this novel and expensive technology.

Commercial application of sex-sorted semen in the United States has been consistent with developmental research in that conception rates in virgin heifers are approximately 75 to 80% of that obtained using conventional semen (DeJarnette et al., 2009). DeJarnette

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et al. (2008) determined that a portion of the compromised conception rate with sex-sorted semen could be overcome in virgin heifers by a sire-dependent response to increased sperm dosage (3.5 or 5.0 vs. 2.1×10^6). Yet, lactating cows in that study had similar conception rates across all sires ($n = 3$) and sperm dosages. To ensure appropriate interpretation and before application (or lack thereof) of these results in a commercial sex-sorting semen laboratory, it is imperative that the results of DeJarnette et al. (2008) are demonstrated to be repeatable across a larger sample of the Holstein sire population. The objectives were 1) to determine if a 3.5×10^6 sex-sorted sperm dosage will provide a meaningful increase in conception rates of Holstein cows or heifers as compared with the standard 2.1×10^6 sex-sorted sperm dosage, and 2) to achieve a large-scale unbiased estimate of the relative compromise in sex-sorted semen conception rates (2.1 and 3.5×10^6 sperm dosages) compared with a 15×10^6 conventional sperm dosage used as a blind control.

MATERIALS AND METHODS

Seven Holstein sires with January 2009 USDA genetic evaluations were selected for this study. To be eligible, each sire was required to 1) possess breed average or below PTA for calving ease, 2) have a history of producing semen with $>80\%$ normal sperm morphology, and 3) have a documented normal fertility potential (≥ -3) as assessed by USDA-Animal Improvement Programs Laboratory (Beltsville, MD) sire conception rate (Norman et al., 2008). Ejaculates were collected via artificial vagina and screened for normal sperm morphology at $600\times$ magnification using differential interference contrast optics after fixing a $10\text{-}\mu\text{L}$ sample of neat semen in 0.5 mL of Tyrode's albumin lactate pyruvate buffer containing 0.2% formalin. Abnormalities related to sperm head defects were classified as primary defects and those involving the sperm tail were classified as secondary defects (Barth and Oko, 1989). Samples with $<80\%$ normal sperm morphology were rejected. Samples with $\geq 80\%$ normal sperm morphology were sex-sorted for X-chromosome-bearing sperm according to procedures described previously (Seidel and Garner, 2002; Seidel, 2007). A split ejaculate technique was not practical because of the inefficient nature of the sorting process, which would have yielded too few doses per treatment within ejaculate (~ 25 straws) to provide meaningful statistical power. Ejaculates ($n = 60$) were sorted to approximately 90% purity for X-chromosome-bearing spermatozoa and extended to 8.4 or 14.0×10^6 sperm/mL in a Tris-egg-yolk, 7% glycerol extender. Extended semen was packaged in 0.25-mL French straws to provide AI dosages of 2.1 or 3.5×10^6

sperm, respectively. A nonsorted conventional semen treatment was processed similarly at 15×10^6 sperm per dose. For simplicity of discussion, semen treatments will be generically referred to as sperm dosages with recognition that actual dosages (2.1 , 3.5 , and 15×10^6) and semen type (sex-sorted vs. conventional) are confounded. Semen treatments were identified by color-coded straws that were alternated across sires to avoid the potential for perception bias during field use. A total of >700 straws per dosage per sire were processed for field use. Straws were frozen in liquid nitrogen vapor at rates within documented tolerance limits (Robbins et al., 1976) before plunging in liquid nitrogen. Sample straws were thawed in water at 37°C for 1 min before evaluation for progressive sperm motility. Sperm motility and acrosome integrity were evaluated in sample straws after 3 h of incubation at 37°C . Motility evaluations were subjective visual estimates of 5 or more random fields of view (avoiding slide perimeter) at $200\times$ magnification using phase-contrast optics and rounded to the nearest 5% . Acrosomal integrity evaluations were conducted at $400\times$ magnification using differential interference contrast optics (Saacke and Marshall, 1968). The results of postthaw semen quality evaluations by treatment across sires are presented in Table 1. Postsort purity of X-chromosome-bearing spermatozoa (Welch and Johnson, 1999) was $90 \pm 0.3\%$ (range: 87 to 95%).

Straws were packaged 12 per cane (4 straws of each dosage) to facilitate balanced and random field use. Within sire, $\sim 50\%$ of the semen (350 straws/sperm dosage per sire) was designated for use in virgin heifers and the remainder for use in lactating cows. Commercial herds were selected based on evidence from on-farm records that the herd had the potential to achieve conception rates $\geq 60\%$ in virgin heifers and $\geq 30\%$ in lactating cows. Each herd used a minimum of 36 total doses ($12/\text{treatment}$ for any combination of sires) for each parity group selected. Experimental semen was used in 45 herds of heifers and 52 herds of lactating cows. Herd personnel were required to use semen in a random fashion within 2 to 3 mo of delivery, which occurred during mid to late March 2009. At the time of AI, straw color was recorded in the on-farm computer records as an appendage to the sire identification. Pregnancy was diagnosed at 30 to 50 d after AI according to each farm's standard operating procedures (rectal palpation or ultrasound). Upon completion of the study, an electronic back up of on-farm records was used to retrieve AI and pregnancy data.

Recommendations for semen use were as follows: synchronization of estrus was acceptable, but semen should only be used in females displaying standing estrus. The use of experimental semen at fixed-time AI or in the

Table 1. Descriptive statistics of semen quality characteristics¹ of sex-sorted and conventional semen across 7 Holstein sires

Sperm dose ($\times 10^6$)	Ejaculates (n)	Motility: 0 h	Motility: 3 h	Acrosome integrity: 3 h	Abnormalities	
					Primary	Secondary
2.1 sex-sorted	24	59 $\pm$ 1.0	48 $\pm$ 1.6	68 $\pm$ 1.5	4.9 $\pm$ 0.7	3.1 $\pm$ 0.4
3.5 sex-sorted	36	62 $\pm$ 0.8	55 $\pm$ 1.3	72 $\pm$ 1.2	4.3 $\pm$ 0.6	2.9 $\pm$ 0.4
15 conventional	10	62 $\pm$ 1.3	53 $\pm$ 1.7	70 $\pm$ 2.7	NA ²	NA

¹Sperm motility evaluations were performed subjectively at 200 $\times$ magnification using phase contrast optics after thawing in a 37°C water bath and incubating for 0 or 3 h. Acrosomal integrity evaluations were performed at 400 $\times$ magnification using differential interference contrast optics after thawing and incubating sample straws in a 37°C water bath for 3 h. Sperm morphology of each ejaculate was screened before sorting and was performed at 600 $\times$ magnification using differential interference contrast optics after fixing in a Tyrode's albumin lactate pyruvate buffer containing 0.2% formalin. Abnormalities of the sperm head and tail were classified as primary and secondary abnormalities, respectively. Ejaculates with >20% total sperm abnormalities were discarded before sorting.

²NA = morphology evaluations were not performed on conventional sperm dosages.

absence of observed standing estrus was discouraged. Inseminations should occur 8 to 12 h after initial detection of estrus. Cows should be <150 DIM at the time of AI with no evidence of calving problems, metritis, or early postpartum metabolic disorders.

Heifer and lactating cow data were analyzed separately using least squares means backward stepwise regression ANOVA models. All models included the effects of herd, sire, sperm dosage (2.1 vs. 3.5 $\times 10^6$ sex-sorted sperm dosages vs. 15 $\times 10^6$ conventional), service number (1, 2, or ≥ 3) and all 2-way interactions with sperm dosage. Models for analysis of lactating cow data included the effects of parity (1, 2, or ≥ 3) and DIM at AI (<60 vs. ≥ 60 d) and all 2-way interactions with sperm dosage. In each model, interactions and main effects lacking significance ($P > 0.10$) were removed in a backward stepwise manner and data were reanalyzed with the exception of the sire by treatment interaction, which remained in all models irrespective of level of significance. Means comparisons were performed using the Student's *t*-test. All analyses were performed using SAS JMP Statistical Discovery Software (version 8.0; SAS Institute Inc., Cary, NC).

RESULTS

Virgin Heifers

A total of 6,268 services were recovered from use in virgin heifers in 45 herds, which provided a mean of 298 $\pm$ 4.2 services per sperm dosage within sire (range: 244 to 344), 895 $\pm$ 18 services for each sire, 2,089 $\pm$ 12 services for each sperm dosage. Eighty-nine percent of all reported AI occurred at first service, and insemination number had no influence ($P > 0.05$) on conception rates. The age at AI for heifers bred to experimental semen (424 $\pm$ 0.6 d for 3,993 heifers with available birth dates) was not different by sperm dosage ($P > 0.10$) within or across sires. Conception rates in virgin heifers were influenced ($P < 0.01$) by the sire $\times$ sperm

dosage interaction (Table 2). Within 6 of the 7 sires evaluated, conception rates of both sex-sorted sperm dosages were less than ($P < 0.05$) those of conventional semen (Figure 1). In only 1 sire (sire G) did the 3.5 $\times 10^6$ sex-sorted sperm dosage achieve conception rates greater than ($P < 0.05$) those of the 2.1 $\times 10^6$ sexed sperm dosage. Across sires, conception rates of the 2.1 and 3.5 $\times 10^6$ sex-sorted sperm dosages and the 15.0 $\times 10^6$ conventional sperm dosages were 43.9, 45.7, and 60.7%, respectively.

Although conception rates tended to be influenced by the herd $\times$ sperm dosage interaction ($P = 0.06$), the mean sample size per sperm dosage within herd (46 $\pm$ 4.1) typically lacked meaningful statistical power. Nonetheless, conception rate differences due to sperm dosage were detected ($P < 0.05$) in 16 of the 45 herds evaluated. In 12 of these 16 herds, the conception rates of the 2 sex-sorted sperm dosages were comparable ($P > 0.05$) but less than ($P < 0.05$) those of conventional semen. In only 4 herds were the conception rates of the 3.5 $\times 10^6$ sexed sperm dosage greater ($P < 0.05$) than those of the 2.1 $\times 10^6$ sex-sorted sperm dosage. Interestingly, in 2 herds, conception rates of the 3.5 $\times 10^6$ sex-sorted sperm dosage were less than ($P < 0.05$) those of conventional semen, whereas the 2.1 $\times 10^6$ sex-

Table 2. Analysis of variance of model effects included in analysis of conception rate in heifers

Source	df	Mean squares	F-ratio	P-value
Herd	44	0.977	4.1	<0.001
Sire	6	1.800	7.6	<0.001
Dose ¹	2	8.450	35.9	<0.001
Herd $\times$ Dose	88	0.293	1.2	0.06
Sire $\times$ Dose	12	0.567	2.4	<0.01
Error	6,115	0.237		

¹Dose generically refers to treatment effects of 2.1 and 3.5 $\times 10^6$ sex-sorted sperm per dose and 15 $\times 10^6$ conventionally processed sperm per dose.

sorted sperm dosage achieved intermediate conception rates.

Lactating Cows

A total of 5,466 services were recovered from use of experimental semen in lactating cows in 52 herds, which provided 260 ± 3.3 services/sperm dosage within sire (range: 236 to 289), 781 ± 14 services per sire, and $1,822 \pm 20$ services per sperm dosage. Eighty-six percent of all reported AI occurred at first service, and insemination number had no influence ($P > 0.05$) on conception rates. The percentage of services recovered by parity (1 = 46%, 2 = 27%, and ≥ 3 = 27%) and by postpartum interval at AI (100 ± 0.72 d, range 35 to 568 d) did not differ ($P > 0.05$) by sperm dosage, indicating adequately randomized usage across these potentially confounding factors.

Conception rates in cows were not influenced ($P > 0.05$) by any interactions with sperm dosage, but were influenced ($P < 0.05$) by the main effects of herd, sire, sperm dosage, and DIM to AI (Table 3). Across sires, conception rates of the 2.1 and 3.5×10^6 sex-sorted sperm dosages were comparable ($P > 0.05$), but less than ($P < 0.05$) those achieved by the 15×10^6 conventional sperm dosage (23.0 and 25.4% vs. 31.5%, respectively). Conception rates of the 3.5 and 2.1×10^6 sex-sorted sperm dosage were comparable ($P > 0.05$) within all sires evaluated (Figure 2). Conception rate

Table 3. Analysis of variance of model effects included in analysis of conception rate in lactating cows

Source	df	Mean squares	F-ratio	P-value
Herd	51	0.410	2.1	<0.0001
Sire	6	1.433	7.3	<0.0001
Dose ¹	2	3.450	17.6	<0.0001
DIM to AI	1	1.600	7.9	<0.01
Sire $\times$ Dose	12	0.158	0.8	0.64
Error	5,393	0.196		

¹Dose generically refers to treatment effects of 2.1 and 3.5×10^6 sex-sorted sperm per dose and 15×10^6 conventionally processed sperm per dose.

of cows ≥ 60 DIM at AI (29.2%, $n = 4,665$) was greater ($P < 0.05$) than that of cows < 60 d DIM at AI (24.1%, $n = 801$).

DISCUSSION

Despite the use of a very liberal method of means comparison in the present trial, the 3.5×10^6 sex-sorted sperm dosage did not result in an improvement in conception rates over the 2.1×10^6 sex-sorted sperm dosage in either cows or heifers. These observations are consistent with those of similarly designed dosage trials in both cows and heifers (DeJarnette et al., 2008; Seidel and Schenk, 2008). Conception rates of both sex-sorted sperm dosages were less than those of conventional se-

Figure 1. Effects of sire and sperm dosage on conception rates of virgin Holstein heifers. Sperm dosage generically refers to treatment effects of 2.1 and 3.5×10^6 sex-sorted sperm per dose and 15×10^6 conventionally processed sperm per dose. ^{a-c}Values within sire or total lacking common letters differ, $P < 0.05$. Sire effect, $P < 0.001$; sperm dosage effect, $P < 0.001$; sire by sperm dosage interaction, $P < 0.01$.

Figure 2. Effects of sire and sperm dosage on conception rates of lactating Holstein cows. Sperm dosage generically refers to treatment effects of 2.1×10^6 sex-sorted sperm per dose and 3.5×10^6 sex-sorted sperm per dose and 15×10^6 conventionally processed sperm per dose. ^{a,b}Values within sire or total lacking common letters differ, $P < 0.05$. Sire effect, $P < 0.001$; sperm dosage effect, $P < 0.001$; sire by sperm dosage interaction, $P = 0.64$.

men in almost all comparisons with the exception of a very few individual bull interactions (sire E in cows and heifers; sire F in cows). An important observation is that the conception rate of sex-sorted semen was only 74% for the 2.1×10^6 dosage and 78% for the 3.5×10^6 dosage when expressed as a percentage of a blind conventional control. These results support others (Seidel et al., 1999; Schenk et al., 2009).

DeJarnette et al. (2009) used on-farm records to compare the conception rates of commercially available sex-sorted and conventional semen. Although they reported that sex-sorted semen achieved conception rates $>80\%$ of conventional semen in both cows and heifers, the authors warned of biases that likely existed in these on-farm comparisons. Because of the expense of semen, low sperm numbers per dose, and likely compromised sperm functional viability, the recommendations for use of sex-sorted semen intentionally place a positive bias on the probability of a successful outcome (i.e., first service, standing estrus, proficient technicians). By default, animals not meeting these criteria are destined for AI with conventional semen, which would be accompanied by negative biases influencing conception success. This distinction is an important consideration to avoid over-interpretation of the economic impact of sex-sorted semen on an individual herd basis (Olynk and Wolf, 2006; Fetrow et al., 2007; Cabrera, 2009).

The observation that the 3.5×10^6 sex-sorted sperm dose resulted in little to no advantage in conception

over the 2.1×10^6 sex-sorted sperm dose and failed to achieve conception rates comparable to conventional semen seems to imply that sex-sorted sperm are less responsive to increasing sperm dosages than are conventionally processed sperm. This may be a reflection of compromised sperm viability (compensable trait; Saacke et al., 2000) as a result of the invasive sorting procedure. Schenk et al. (2009) presented the results of experiments wherein 10×10^6 dosages of sex-sorted and unsorted sperm were compared. In the first experiment, pregnancy rates in beef heifers were virtually identical between the 10×10^6 dosages of sex-sorted versus unsorted sperm (62%, $n = 180$; 61%, $n = 88$, respectively), and thus, appeared fully compensable for the 3 bulls. In the second experiment, 2.1×10^6 and 10×10^6 sex-sorted sperm dosages and 10×10^6 conventionally processed sperm were used in lactating Holstein cows ultrasonically prescreened for normal ovarian and uterine status. Although conception rates were comparable across treatments (41%, $n = 57$ vs. 44%, $n = 57$ vs. 56%, $n = 58$, respectively), the small sample size lacked statistical power to confirm significance of the 12 to 15 percentage point numeric difference favoring conventionally processed semen.

Alternatively, the sorting procedure may impart a degree of uncompensable sperm damage (Saacke et al., 2000) that attenuates conception responses to increased sperm dosage. The latter interpretation may be supported by observations of an increased incidence of

degenerate embryos following superovulation and AI with sex-sorted semen (Sartori et al., 2004; Schenk et al., 2006; Potter et al., 2009). Similarly, Frijters et al. (2009) compared the nonreturn rates of 7 sires achieved by 2×10^6 sperm dosages of sex-sorted and conventional semen with those obtained by 15×10^6 conventional sperm dosages and concluded that only a portion of the reduction in conception rates was due to sperm dosage and the remainder was due to the sorting process itself. Although some studies support a reasonable suspicion that the reduction in conception rates with sex-sorted semen may be uncompensable, none are conclusive. Additional large-scale field trials across a large sampling of the sire population are necessary to clarify the effect of the sex-sorting procedure on compensable and uncompensable semen quality factors.

The range in conception rates among individual sires ($\sim 15\%$) was similar within sex-sorted and conventional dosages in both cows and heifers. This result is not consistent with other studies wherein the range in sire fertility was greater for sex-sorted than for conventional semen dosages (DeJarnette et al., 2009; Frijters et al., 2009; Abdel-Azim, 2010). Because of the small sample of sires, no attempt was made to correlate sire conception rates among sperm dosages in the present trial, although comparison of Figures 1 and 2 by visual inspection shows a similarity of the pattern of results for heifers and cows. Consistent with the observations of others (DeJarnette et al., 2009; Abdel-Azim, 2010), Figures 1 and 2 provide examples of sire interactions that preclude the use of fertility estimates based on conventional semen to accurately predict success with sex-sorted semen.

Consistent with the results of DeJarnette et al. (2008), the current data provide no evidence to support the concept that a separate sex-sorted sperm dosage for lactating cows would be useful. In that study, 1 of 3 sires evaluated responded to sperm dose in heifers, but conception rates of cows were not influenced by sperm dose within or across any of the 3 sires. In the present study, the response to sex-sorted sperm dose in heifers (or lack thereof) was virtually mirrored in lactating cows. This does not imply that the economic returns from use of sex-sorted semen in lactating cows are similar to those of virgin heifers. On the contrary, the net present value of sex-sorted semen is highly dependent on the actual conception rate achieved in addition to other factors including differential value of calf sex, semen price, and genetic merit of the cow being inseminated (Olynk and Wolf, 2006; Fetrow et al., 2007; Cabrera, 2009). Excluding genetic value, the probability of achieving the goal (conception with heifer fetus) from a given AI with sex-sorted sperm in cows ($\sim 27\%$ conception $\times$ 90% heifers = 0.243 heifers per dose of semen) is approximately

60% of that from use in heifers (45% conception $\times$ 90% heifers = 0.405 heifers per dose of semen).

Because all sperm are subjected to identical processing procedures irrespective of sort purity, the preponderance of sex-sorted sperm dosage studies in the range of 3.5 to 6×10^6 sperm per straw implies that this approach is highly unlikely to result in meaningful improvement in conception rates. Therefore, advantages to the producer from use of reduced purity products ($<90\%$ X-chromosome bearing sperm) must come from more economically priced products, which is doubtful in consideration of current technology efficiency (Sharpe and Evans, 2009) and price competition in the AI market with standard 90% purity sex-sorted products.

The variation in conception rates achieved among individual herds of cows (13 to 48%) and heifers (32 to 74%), despite the use of common semen samples, indicates that tremendous opportunities exist to enhance the efficiency of both sex-sorted and conventional semen through identification of on-farm management techniques and procedures that are conducive to more optimum fertility potential in the female population. Postpartum interval was identified as a significant contributor to conception potential with indications that optimum conception is not achieved until ≥ 60 d after calving.

CONCLUSIONS

These data could not confirm that a 3.5×10^6 sexed sperm dose should be expected to achieve an improvement in conception rates of Holstein cows or heifers compared with a 2.1×10^6 dose. Although evidence of individual sire interactions suggests some sires may be more responsive to sexed sperm dose than others, in most sires the 3.5×10^6 sex-sorted sperm dosage had lower conception rates than conventional semen. Consistent with similar sex-sorted sperm dosage trials, both dosages of sex-sorted semen achieved conception rates that were approximately 75% of that of conventional unsorted semen. These observations imply that 1) conception rates of sex-sorted sperm are less responsive to sperm dosage than are conventionally processed sperm or 2) the sex-sorting procedure imparts a negative effect on uncompensable semen quality attributes and thereby attenuates maximum fertility potential irrespective of sperm dose. Additional research is needed to distinguish the effects of sperm dose and intrinsic sperm quality on conception rates; however, the answers may be largely academic at the present state of sorting speed, expense, and efficiency.

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