



# DAIRY VETERINARY NEWSLETTER

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## How Viable is the Use of Frozen-thawed, Sex-sorted Bull Sperm in Terms of Fertility?

Recently there has been an interesting series of papers reporting on studies of frozen-thawed and subsequently sex-sorted bull sperm for AI of heifers, by S.L. Underwood, R. Bathgate et al. Three of their studies have been reported in the March 2010 (pp. 7-12) and April 2010 (pp. 171-175, electronically published ahead of print) issues of Animal Reproduction Science, and the October 15, 2009 (pp. 1001-1008) issue of Theriogenology.

### Practical interest in freezing or possibly cooling sperm before sex sorting

All of the papers are introduced with the point that most sex-sorted semen used in the dairy industry is produced from bulls located at or near a flow cytometry facility; bulls located very far from such facilities for semen sorting are usually excluded from sex-sorting technology. This is because nearly all commercial sex-sorting is done on fresh bull sperm. Therefore there is interest in transport of cooled (5° C) or frozen sperm to a sex-sorting facility thus allowing any bull in the AI industry to be a candidate for production of sex-sorted semen.

### In vitro sperm characteristics depending on handling

In this study, sperm was divided into 5 methods of handling (none of which involved cooled transport, only frozen): frozen, non-sorted (Control), frozen, thawed, refrozen with no sex-sorting (FF), frozen, thawed, sex-sorted and refrozen (FSF), frozen, thawed, sex-sorted and incubated at 15° C (FS15), or frozen, thawed, sex-sorted and incubated at 37° C (FS37). Sex sorting used staining and flow cytometry procedures typical in the AI industry. Except for the FS37 group already at 37° C, all groups were warmed to 37° C in a waterbath for evaluation of motility and other sperm characteristics.

Motility and progressive motility: At most time points from 0 to 30 hrs of incubation at 37° C, motility and progressive motility of Control, FS15, and FS37 semen groups were similar and were higher than the FF and FSF groups. After 4 hrs, motility of the FSF group decreased markedly to only approximately 10%, declining significantly faster than for any other group. Progressive motility decreased to approximately zero for the FF and FSF groups after 4 hrs, compared with 30% to 50% for the other groups, significantly faster decline for both of the twice-frozen groups.

Viability and acrosome integrity: Both measures of semen quality were significantly higher for FS15 and FS37 sperm from 0 to 30 hrs of incubation than other groups, but FS15 survived significantly better even than FS37, approximately 50% viable and acrosome intact after 30 hrs. FSF had the second-lowest and FF had significantly

lower viability and acrosomal integrity than all other groups, decreased to approximately 20% after 4 hrs and then not further reported. Mitochondrial activity results were similar, with significantly higher proportions of the FS15 and FS37 sperm retaining activity; the Control, FF and FSF groups declined significantly faster after 4 hrs and were not further reported.

The authors concluded that sex-sorting selects for a population of sperm with high functional capacity (see more on this below), resulting in better viability at some time points for the FSF group than the FF group. However, as they also noted, the freezing, thawing, and re-freezing decreased viability of sperm compared with the control or frozen, thawed, sex-sorted and incubated sperm; this is not surprising considering that earlier studies have shown that each freeze-thaw cycle damages plasma membranes.

### **Establishment and survival of pregnancies in heifers in association with semen handling**

The next study examined pregnancy and methods of handling sex-sorted semen. The sperm treatment groups were frozen, non-sorted (Control), frozen, thawed, sex-sorted and refrozen (FSF) and cooled (5° C), sex-sorted and frozen (CSF). Sex sorting again used standard methods. All groups' straws were warmed to 37° C in a waterbath for 30 sec before AI breeding of 183 Holstein heifers between 15 and 20 mo old. All heifers were inseminated 12 hr after initial observation of standing estrus, but only those assigned sex-sorted semen were inseminated again 20 hr after initial observation of standing estrus.

The results table below is adapted directly from the March 2010 paper by Underwood et al.:

Bull	Semen type	No. heifers inseminated	No. heifers pregnant (%)	No. Pregnancies Lost (%)
1	Control	46	29 (63) <sup>a</sup>	3 (11)
	<b>FSF</b>	35	<b>3 (9)<sup>c</sup></b>	<b>3 (100)</b>
	<b>CSF</b>	20	<b>2 (10)<sup>c</sup></b>	<b>1 (50)</b>
2	Control	22	10 (46) <sup>b</sup>	0
	<b>FSF</b>	39	<b>0<sup>c</sup></b>	NA
	<b>CSF</b>	21	<b>1 (5)<sup>c</sup></b>	<b>1 (100)</b>

Different superscripts (a, b, c) indicate significant difference ( $p < 0.05$ ) within columns

It can be seen from the table that there was a marked decrease in conception and increase in pregnancy loss (5/6 = 83% of sex-sorted semen pregnancies were lost) associated with sex-sorted semen that was either cooled or frozen before sex sorting. Overall conception rate for Control semen was 39/68 (57%) vs. 6/115 (5%) for sex-sorted semen, significant at  $p < 0.001$ . It is noteworthy that two semen straws were used on each heifer bred with sex-sorted semen and this still resulted in only one (female) calf born from 115 heifers.

The authors mention that producing one calf shows that freezing (they do not appear to advocate cooling), thawing, sex-sorting and re-freezing bull semen has potential as a future technology, which is true. Possibly future refinement can make this a method to allow all AI bulls to be candidates to produce sex-sorted semen.

However, the results reported above suggest that at present there is no practicality in using twice-frozen sex-sorted semen to breed dairy heifers.

### **Birth of calves following AI of heifers in association with semen handling and semen dose per straw**

This study was similar to the one above, but focused on birth of calves from inseminated heifers and evaluated dose of semen per AI straw used in addition to semen handling methods. The sperm treatment groups were frozen, non-sorted (Control), frozen, thawed, sex-sorted and refrozen (FSF) and frozen, thawed, sex-sorted and

inseminated within 12 hr after sorting (FS). In one trial, 12 heifers were inseminated with  $4 \times 10^6$  FSF sperm and none conceived; all returned to estrus < 26 d after AI. However, 6 of 8 heifers inseminated with  $4 \times 10^6$  Control sperm were pregnant 7 wk after AI (75% conception). In another trial, 1 of 7 heifers (14%) inseminated with  $10 \times 10^6$  FSF sperm became pregnant and eventually delivered a female calf; none of 7 heifers inseminated with  $4 \times 10^6$  FS sperm conceived, and 4 of 7 (57%) inseminated with  $10 \times 10^6$  of Control sperm conceived and eventually calved.

The authors again concluded that freezing, thawing, and sex-sorting bull semen has potential as a future technology. Nevertheless, as in the trial described earlier above, use of such semen to breed cattle does not appear to be practical yet.

It is also of interest that in the first paper, the authors suggested that sex-sorting selects for a population of sperm with higher functional capacity than non-sorted sperm. That was because the frozen, thawed, sex-sorted and refrozen sperm had better in vitro survival than the frozen, thawed, refrozen sperm that was not sex-sorted. If this is the case, then it suggests that an important reason for the widely observed reduction in fertility of sex-sorted semen compared with non-sorted semen may be the reduced dose of sorted semen per AI straw instead of the sorting process itself. The reduced dose of sex-sorted semen has been described previously as accounting for approximately half of the decreased fertility associated with its use; the other half has been speculated to have something to do with the sorting process that is not yet clearly identified. The above findings and interpretations contradict that. Of course, in vitro characteristics may not be the same as actual fertility in vivo. Identification of all reasons for reduced fertility of sex-sorted semen, including the optimal dose per straw, as well as possible use of frozen, transported, thawed, and then sex-sorted semen for widespread use in the dairy industry warrant further investigation.

### **The New Option of 75% Female Sex-sorted Semen**

A new option in sex-sorted semen was described in the January 1, 2010 issue of Progressive Dairyman. The article was titled, “Genetic innovations lead into 2010” by Ray Nebel and Chuck Sattler. A new type of sexed semen with 75% “X-chromosome enriched” semen resulting in an average of three-quarters of calves being born female is now available. The article states that the reduced conception rates associated with sexed semen are similar for this product to those with the 90% female sex-sorted semen products. However, the allowance of more male sperm into each straw allows for more straws produced per bull collection and therefore reduces the price per straw. The price difference is not discussed in the article. (The price difference of 90% female sex-sorted semen vs. conventional un-sorted semen is usually reported by others as \$40 – \$50 per straw vs. \$10 - \$20 per straw, often reported in financial analysis studies as a \$30 difference).

In order to find out more about this new product, pricing, and acceptance in the industry, I talked with Amy Teplate of Genex Cooperative, Inc. She said that for bulls of highest genetic merit, a typical price difference for 90% female sex-sorted semen vs. conventional semen would be \$25, and the price for 75% female sex-sorted semen would be \$15 more than conventional, \$10 less than 90% female semen per straw.

Amy also said that since its introduction in July 2009, the 75% female sex-sorted semen has been well accepted and now makes up a substantial proportion of all sexed semen sales. This includes use by some dairy producers that never used sexed semen before but have been induced by the reduced price to try it.

The article by Nebel and Sattler in Progressive Dairyman also mentions that while “economics associated with reduced calving problems are very difficult to calculate or estimate”, another benefit widely perceived from use of sexed semen is that first lactation heifers having heifer calves have less dystocia than those having bull calves. I mentioned this to Amy Teplate at Genex and she said that the USDA reports that heifers having their first calf have twice the proportion of dystocia if they have bulls vs. heifer calves. It seems that both 75% and

90% female sex-sorted semen are tools that the dairy industry is adopting, and further research into making more bulls candidates for this sorting, and to increase fertility associated with it is needed.

Feedback from our readers is always welcome, including suggestions for future topics of interest. I can be reached at (435) 797-1899 M-W, (435) 797-7120 Th-F or [David.Wilson@usu.edu](mailto:David.Wilson@usu.edu).



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