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Sex-Selected Semen

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Sexed semen for cattle is now available commercially in a number of countries including the United States. The only proven, repeatable and reliable method of sperm sexing is using an instrument called a flow cytometer/cell sorter. Other methods claiming to separate sperm by sex without damaging them severely have proven unsuccessful. Factors that must be considered when using sexed semen, include the added cost for a straw of semen, that the product is only available from a few bulls, and that fertility is compromised because of the nature of the product. Nevertheless, there is a place for sexed semen in many breeding programs. In this article, we will discuss the current benefits and limitations of sexed sperm. Understanding this product is a first step in determining whether and under what conditions to apply sexed sperm in a breeding program.

How Sperm are Sexed

It is important to recognize that sexed semen technology is continually being improved and becoming more efficient as procedures are refined. Perhaps the most serious limitation of current technology for widespread commercial use and application is that it takes considerable time to sex millions of sperm for an insemination dose. Sperm are analyzed and sexed one sperm at a time. Furthermore, the sperm sorter is complex, relative expensive and requires highly trained personnel to operate. Procedures describing currently available sperm sorting methods have been presented in detail by Seidel and Garner (2002). The principles used are well known and scientifically sound. Personnel at the United States Department of Agriculture (USDA) (Johnson and Welch, 1999) developed many of the earlier aspects of sperm sorting, and this technology was improved over the years. The principles work for nearly all mammalian species including humans. The basic principle is that almost exactly half of mammalian sperm in any ejaculate have an X chromosome and produce females, while the other half have a Y chromosome and produce males. The X chromosome in cattle contains about 4% more genetic material (DNA) than the Y chromosome. Except for this chromosomal difference, no discernible differences between X and Y sperm have been found to date.

To determine the amount of DNA in sperm, they are incubated with a DNA-binding dye, Hoechst 33342. This dye fluoresces a deep blue color if exposed to an appropriate wavelength of light, which is provided by a laser. Because X sperm have about 4% more DNA than Y sperm, they fluoresce brighter. Stained sperm can be viewed under a microscope, but our eyes and brains are not designed to be able to discriminate such a small difference in brightness reliably, so X and Y sperm appear identical. However, with proper electronic equipment and a powerful computer, brightness of DNA-stained sperm can be divided into 3 populations with about 90% reliability. The brightest 20-30% of sperm are mostly X sperm; the least bright 20-30% are mostly Y sperm; and the remaining 40-60% cannot be categorized as X or Y reliably for various reasons (Seidel and Garner, 2002).

All of the above measurements and subsequent sorting of sperm are made possible by a remarkable instrument called a flow cytometer/cell sorter. Stained sperm are pumped through this instrument in tubing past a detector that measures the brightness of individual sperm exposed to laser light. That information is processed by computer and used to sort sperm, one at a time, at a rate of about 25,000 sperm/second. On the average, about 4,000 of these can be sorted as X sperm at 90% accuracy (10% will be Y sperm); about 4,000 can be sorted as Y sperm at 90% accuracy; and the remainder are discarded. Both X and Y sperm can be sorted at the same time if desired. There is, however, considerable variability in sorting speed due to the individual bull or ejaculate. Because of various losses in processing after sorting (Seidel and Garner, 2002), only about 75% of the sorted sperm end up being packaged in straws for freezing. Thus, the number of useable X and Y sperm produced per hour at 90% accuracy is around 11 million sperm of each sex (3,600 seconds x 4,000/second x 75%), which is enough for one conventional dose of semen of each sex. Very importantly, for some ejaculates this rate is less than 8 million per hour, and for others it may be up to 20 million/hour. Recently, the Monsanto Corporation claims to have developed a multi-nozzle flow cytometer sperm sorter, but information on the performance characteristics of this equipment has not been published and remains proprietary. The information presented by Monsanto indicates 85% accuracy, but speed of sorting has not been presented nor have experimental field trial results.

Low Dose Insemination

Machines in current use cost around $340,000, and because they only produce about one conventional dose of each sex of sperm per hour per machine, this obviously is impractical. The main solutions have been to have multiple machines at each site and to decrease the number of sperm packaged per straw of semen to around 2 million. Since it only takes one sperm to fertilize an egg, 2 million sperm/dose is adequate for normal fertility of unsexed sperm for some bulls and only slightly reduced fertility for most others (Den Daas et al., 1998). However, fertility of 10-20% of bulls decreased 10-20 percentage points at 2 million unsexed sperm/dose as measured by non-return rates. Note that these data were obtained using professional inseminators with dairy cows in Europe. We have shown that, for bulls of above average fertility, 0.5 million unsexed sperm/dose results in the same fertility as 10 million sperm when inseminating heifers into the uterine horns (Seidel et al., 1996).

The potential fertilizing ability of sexed sperm is somewhat compromised as sperm are subjected to the sorting processes. Even when 10 million sexed sperm/dose are inseminated, pregnancy rates are slightly lower than unsexed sperm. Because 10 million sexed sperm/dose is impractical with today's sperm sorters, we have conducted more than a dozen studies inseminating cows and (mostly) heifers with 1 to 3 million sexed, frozen sperm/dose. We found that 1 million sexed sperm/dose often is too low, but that there was little or no improvement in fertility when increasing numbers of sexed sperm per dose above 2 million.

It is not surprising that fertility of sexed sperm is lower than unsexed sperm because the sexing process has many steps that can be detrimental to sperm, including:

1. Sperm are held for up to half a day from ejaculation to accumulate a reasonable number of sexed sperm for processing and packaging.

2. Incubating sperm with a high concentration of the DNA-binding dye takes time and may negatively affect sperm.

3. The sperm are exposed to a powerful laser beam during sorting and exit the nozzle of the machine at around 50 miles per hour (they must go fast to evaluate 25,000 each second, in series, one at a time).

4. The sperm must be centrifuged to concentrate them after sorting so that 2,000,000 fit in a 0.25-ml straw.

Success Rates

The majority of studies on sexed sperm have been done with breeding heifers, although some have been done with cows, and a few, with superovulation and in vitro fertilization. Success rates that will be described are for 2 million frozen sperm per insemination using 0.25-ml straws and inseminating into the uterine body as is done normally; few studies are available using the more common 0.5-ml straws. Importantly and not surprisingly, success varies markedly with management, female age and parity. Because sperm are slightly compromised in the process of sexing and the low number of sperm per dose, everything must be done optimally for good success rates. The following are especially important:

1. Well managed animals, including good nutrition.
2. Extremely careful handling of semen including rapid transfer of straws from container to container, thawing in a 95°F water bath, and inseminating within 10 min of thawing.
3. Excellent estrus detection. Success using sexed sperm with fixed-time AI programs where animals are inseminated whether seen in estrus or not have been poor. With conscientious detection of estrus and inseminating <u>only</u> those actually standing to be mounted, success rates have been acceptable. We have found little difference when heifers were inseminated 0.5 or 1.0 days after first observing <u>standing</u> estrus. Also, most commonly used estrus synchronization methods are appropriate as long as they are combined with estrus detection.
4. Well trained inseminators. Inseminators who get slightly lower pregnancy rates than average with unsexed semen likely will get much lower pregnancy rates with sexed sperm.

In nearly all of our trials with sexed sperm, we have simultaneously had a control group using normal numbers of unsexed sperm per inseminate from the same bulls. This provides the background, normal fertility for that herd under the conditions of the field trial. Our success rates with sexed sperm have ranged from about 35 to 100% of controls, depending on the particular herd management, the bulls used, whether animals were bred only after observing standing estrus, etc. The results fall into two categories: 1) Excellent management in which all four of the above items were done correctly, and 2) Average to below average conditions where one or more of these items were compromised. With excellent management, pregnancy rates with sexed sperm are almost always 70-90% of controls, so if the control pregnancy rate is 70%, the sexed rate is 49 to 63% pregnant. With average conditions, the pregnancy rates usually are 50 to 70% of controls, but can be lower.

No matter what the pregnancy rates are, the accuracy is almost always 85 to 95% of the sex chosen. Note, however, that one needs at least 20 calves to get a fair estimate of the sex ratio. There will be an average of around 10% of the "wrong" sex, and having 1 or 2 calves of the "wrong" sex out of 4 or 5 calves is meaningless in determining the true accuracy.

One other extremely important observation is that despite lowered fertility, the calves actually born seem to be completely normal except for the sex ratio (Tubman et al., 2004). In that study, 1,169 calves resulting from sexed sperm were compared with 793 calves from control sperm. There were no differences in neonatal death rates, birth weights, weaning weights, gestation length, incidence of abnormalities, nor in any other trait studied. There also was no increase in abortion rates with sexed sperm compared to controls.

Costs of Sexed Sperm

The current prices for commercially available sexed sperm in the United States are about $30 more per straw than normal doses of semen from the same bulls. Currently, sexed semen is being produced and marketed by Sexing Technologies (Inguran) located in Navasota, TX and marketed by Select Sires, Inc. and ABS Global, Inc. A big problem is that sexed sperm are not available for most bulls, and especially not from the more popular bulls, because genetic companies can sell all the semen such bulls produce without sexing it. This situation obviously will change with time.

It is possible to take your own bull to Sexing Technologies for sexing and freezing semen. The cost of collecting, sexing and freezing semen depends heavily on the number of doses desired. Large contracts for sexed sperm keep the production costs per dose affordable. Small contracts can result in quite high costs per dose sexed. There will be the occasional bull whose sperm will not tolerate the stresses of sexing, so no sexed semen will be produced. Also there are bulls whose sperm result in much slower sort rates, that will limit production and increase associated costs. Of course, some bulls cannot produce acceptable quality semen, even without sexing.

For many beef production situations, the biggest cost of using sexed sperm will be the lower fertility. Even getting 60 instead of 70% pregnancy is extremely costly for a herd, and the additional value of distorting the sex ratio to get more steers to sell at weaning will almost never compensate for the costs of sexed semen plus the lower fertility. In most cases, the same holds true for getting more replacement heifers from the best cows in the herd. If, however, one sex is worth at least $300 more than the other sex at birth, sexing semen likely can be profitable (Seidel, 2003a). This clearly will be true in some cases when selling breeding stock or expanding a herd.

Recommendations

Despite all of the concerns with a relatively new product like sexed sperm, it already has a place in some breeding programs, and the opportunities will grow as the product improves and becomes more widely available. Early adopters are likely to benefit most. Fringe benefits also need to be considered, such as less dystocia in heifers having heifer calves and special cases such as wanting to expand a herd without introducing new animals because of bio-security issues. Another option being researched is producing embryos by in vitro fertilization with sexed sperm; it takes many fewer sperm per embryo produced in vitro than when breeding females.

Specific recommendations are:

1. Don't even think of using sexed sperm unless you already have a very successful AI program.

2. Don't go "whole hog" at first, but do inseminate enough females for a fair test. Breeding 10 heifers resulting in 4 or 5 calves will not give you enough information about how well sexed sperm will work under your conditions. You need to breed at least 20-25 head and preferably 40-50 to get an honest evaluation.

3. Try sexed sperm where it is easiest to use and will not create a disaster if fertility is low. In our opinion, the best place to start is to breed replacement heifers to have heifer calves. You can do this with the first inseminations of the breeding season, so if there are problems, getting them pregnant on the second service will not be too costly. However, be certain that your heifers are of adequate size, on a positive nutritional program, and most importantly, are cycling.

4. Do not use sexed semen for superovulated cows, as this is the most difficult system for obtaining acceptable success using compromised semen. Even with multiple inseminations and good management, the number of good embryos recovered when breeding superovulated donors with sexed sperm is about half that with unsexed semen (Schenk et al., 2006). However, pregnancy rates with the embryos produced appear to be normal. The one place that sexed semen already may fit for superovulation is if one sex of calves is very valuable, and the value of the other sex is very low. This is true for some dairy cows, for which a heifer calf may be worth a few thousand dollars, while a bull calf may be worth only $100. Sexed sperm in this case, while producing only about half as many embryos, will produce just as many female embryos as unsexed sperm, and you will not waste recipients and associated costs to produce the "wrong" sex.

5. Try sexed semen if it seems to fit. With good management, it will add "spice" to your breeding program.

Future Considerations

As with any new technology, costs will decline and success rates will improve with time. Sexed sperm could eventually change the whole nature of the beef cattle industry, making it more like current swine breeding programs in which there are maternal lines and terminal cross lines for almost every breeding program. While this already is true in some cases for beef cattle, when breeding for maternal traits, the males are really a by-product, as are the females with terminal cross programs. Certainly terminal crossing would be more efficient with 90% males if costs of sexed semen were low and fertility was normal.

One other characteristic of new technologies is that most of the benefit usually accrues to the early (not too early!) adopters. After the new technology becomes widespread, nearly everyone is forced to use it, so everyone has to incorporate it to remain profitable. A clear example is lean carcass hogs. There isn't even a market for the fat hogs of decades ago at any price.

One final characteristic of sexed sperm, and indeed many other technologies, is that most of the investment in the technology was made by private industry, not by government grants or university resources. Private industry has patented key aspects of the technology, or licensed key patents held by the US government or universities. There simply were no government grants available for the size of investment required to make sexed sperm a commercial reality. This has resulted in greater input from private industry in how sexed semen is developed and marketed and priced than we are used to for most cattle breeding goods and services. Artificial insemination cooperatives or USDA would have been logical sources of funding for this research, and while there was some investment by these entities, private industry was the main driver for the steps leading to commercialization (Seidel, 2003b).

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