

## USING SEXED SEMEN



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### **Background**

For cattle production and many other endeavors, sex is the most important genetic trait. Furthermore, for any given mating, one sex of the offspring almost always will be worth more monetarily than the other sex. There are three points during the production process of cattle where sex can be determined before birth: sexing sperm before artificial insemination (AI), sexing embryos recovered nonsurgically about a week after AI, and sexing fetuses at about 2 to 3 months of pregnancy. I will concentrate on sexing sperm, but the other two procedures are greater than 95% accurate when done by skilled persons, and the sex information can be used in various ways, such as discarding embryos or aborting fetuses of the less desired sex, or using the information for management or marketing purposes.

### **How is Semen Sexed?**

There are hundreds of patents for sexing semen involving nearly a dozen methods. However, there is only one method that is efficacious and does not destroy the fertility of the sperm: flow cytometry/cell sorting (Seidel and Garner, 2002). Even that method is far from ideal, but it is the only reasonably accurate procedure available, despite claims for some other procedures. The principle behind this procedure is to measure the DNA (genetic material) content of individual sperm. Bovine X chromosome-bearing sperm (which result in females) have about 3.8% more DNA than Y chromosome-bearing sperm (which produce males), so accurate measurement of sperm DNA enables distinguishing X from Y sperm. There are many problems in measuring this small difference accurately, rapidly, and with minimal damage to the sperm, but most of these problems have been solved sufficiently well so that sexing sperm is now done commercially on a large scale (Seidel, 2007). Sexing sperm is a 2-stage process: 1) determining the sex, and 2) separating the 3 sexes of sperm – X-sperm, Y-sperm, and those for which sex cannot be determined accurately. Well over half of the sperm end up in the latter category with current procedures (Seidel and Garner, 2002).

### **Limitations to Sperm Sexing Procedures**

One of the most important characteristics of the current method of sexing sperm, and also the greatest limitation, is that sperm are sexed one at a time (in series), and there are limits as to how fast this can be done. The process is actually remarkably fast, with over 20,000 sperm evaluated each second. However, since over half of those evaluated are unsexable, and half of the rest are of the “wrong” sex, this results in fewer than 5,000 sperm of each sex sorted per second, or less than 18 million sperm/hour. This amounts to one or two standard AI doses of semen. A second problem is that the procedure is only

about 90% accurate, although accuracy can be increased to over 95% for most bulls if fewer sperm are collected per unit time. Under most circumstances, setting the accuracy above 90% is too expensive, which brings up another limitation, expense. A 2-nozzle sorter (like a 2-cylinder engine) costs around half a million dollars. Sorters also require well-trained technicians, as well as constant supplies and a suitable laboratory. Mostly because of these and other costs, sexed semen typically is priced around \$20/dose higher than doses of unsexed semen. To keep costs manageable, fewer sperm are packaged per dose of sexed semen than for unsexed semen, which together with the stresses of sexing, results in lowered fertility; more on that later.

Another limitation is that sexed semen is not available for all bulls for a variety of reasons, mostly related to supply and demand, but occasionally the sexing procedure simply does not work well for a particular bull. Also, bull studs rarely sex sperm of bulls with below average fertility. A final limitation is that sperm do not survive well if thawed, sexed, and re-frozen. This means that freshly collected semen usually is sexed, which practically means that the bulls must be close to the sorting laboratory for best results. Procedures are being developed to ship freshly collected semen to sorting labs by overnight courier services, but this does not work as well as using freshly collected semen. Another development is the portable sexing laboratory, placed in a huge trailer truck that can go to where bulls are located. Currently there is one laboratory that sexes sperm on a custom basis: [www.sexingtechnologies.com](http://www.sexingtechnologies.com).

### **Success Rates**

As alluded to earlier, fertility of sexed semen is lower than that of conventional semen, partly due to fewer sperm per insemination dose and partly because of the stress on sperm from the sexing process. Under ideal circumstances, pregnancy rates are only slightly depressed and can be within 10% of pregnancy rates with unsexed sperm (Schenk et al., 2009). However, with typically well-managed herds, pregnancy rates almost always average between 70 and 90% of those of unsexed semen (DeJarnette et al., 2009). Even this requires doing everything right – proper handling of semen, skilled inseminators, good heat detection, excellent nutrition, etc. Conventional AI usually results in low pregnancy rates in poorly managed herds; superimposing sexed semen in such situations usually leads to extremely low pregnancy rates.

Fixed time AI programs, for example breeding 60 hours after a prostaglandin injection, have many advantages including no need for heat detection. However, they require more semen per pregnancy than only breeding those detected in heat, so such programs are not recommended for expensive semen. High fertility semen also is needed for optimum results with fixed time AI, and sexed semen is lower in fertility than normal for the two reasons of slight damage due to sexing and low numbers of sperm per insemination dose. Thus, sexed semen is not recommended for fixed time AI programs, both due to cost and compromised fertility.

A situation in which sexed semen at first seems inappropriate is with superovulation. There is huge variation in fertilization rates, although certain bulls work better than others. Numerous studies show that the number of good quality embryos recovered when using

sexed semen is about half that with unsexed semen (Schenk et al., 2006; Larson et al., 2010). However, if one sex of embryos does not justify the expenses of embryo transfer, use of sexed semen is a more elegant approach than sexing the embryos and discarding those of the undesired sex. You end up with about the same number of good embryos of the desired sex, even though only half as many are recovered if sexed semen is used. Also, this is usually considerably less expensive than sexing embryos. However, due to the huge variability, this approach is not recommended if only one or two donors are involved. An important point is that pregnancy rates appear to be normal after embryo transfer when sexed semen is used for donors.

One other application of sexed semen is with in vitro fertilization. This has a big advantage of fertilizing many oocytes with one dose of semen. As with superovulation, there is great bull-to-bull variability in fertilization rates. However, if in vitro fertilization is being done anyway, sexed semen is often an attractive option.

Most semen is sexed to provide 90% accuracy, and that accuracy is almost always attained in offspring, averaged over large numbers of pregnancies. However, due to mathematical laws of chance, accuracy can vary considerably when few animals are involved, especially fewer than 10. Although fairly rare, accuracy of sex of offspring can even be 50% or less with small numbers, even though the true accuracy of sexing in the semen sample is 90%

#### **Normality of Calves**

Hundreds of thousands of calves have now been produced with sexed sperm, and the calves appear to be normal. We did a huge systematic study several years ago involving over 1900 calves, and found that the calves from sexed semen were indistinguishable from controls for every aspect measured, including birth weight, neonatal death rates, weaning weight, etc. (Tubman et al., 2004). There is one odd situation where there appears to be a small increase in the rate of neonatal death in the small percentage of bull calves born when semen is sexed to produce females. With unsexed semen, about 1% of fetuses produced have an extra chromosome, and most of these abort. Down's syndrome in human pregnancies is one examples in which some such fetuses do not abort. In most cases, calves born with an extra chromosome would not be expected to survive, although this has not been studied to any extent. When sexing semen, sperm with a Y chromosome plus an extra chromosome theoretically will sort as an X chromosome-bearing sperm, so in the rare cases that those sperm fertilize an oocyte and the pregnancy goes to term, the male calves will have problems. This phenomenon does not occur when sexing for male sperm, nor does it occur for females under any circumstances, including the 10% females born with 90% Y sperm. This whole issue is primarily a statistical artifact affecting fewer than 1% of calves from sexed semen. Sexing semen theoretically actually decreases the incidence of chromosomal abnormalities in some situations, but this advantage would be small and hardly measurable.

#### **Applying Sexed Semen for Beef Cattle**

Due to the need for AI, the extra cost of purchasing sexed semen, and the even greater cost of reduced fertility (Seidel, 2003), most sexed semen likely will be used by

seedstock producers. They likely will want to produce bulls from most matings, but also need bull mothers from other matings, or females to sell in some cases. However, commercial producers also may find sexed semen to be profitable under some circumstances. In my own herd, over the years steer calves have been worth an average of about \$50 more than heifer calves at weaning. This probably is not sufficient to justify the use of sexed semen at current costs and success rates, but as costs decline and success rates improve, this concept might become profitable.

In my opinion, the place where sexed semen fits the commercial producer best is to breed yearling heifers to have heifer calves. Heifers often are AI'd anyway so that proven easy calving service sires can be used. This use of sexed semen has 3 advantages. First, heifer calves average about 5 lbs lighter birth weight than bull calves, so there is less dystocia, even using calving ease bulls; usually it is the larger bull calves that cause the severe dystocia problems, and there will be many fewer of those when using X sperm for females. The second advantage is that the genetic value of the yearling heifers will be higher on average than for older cows, so they are ideal for producing replacements. (If the youngest animals in the herd are not the best genetically on average, the breeding program is going backwards!) The third advantage is that fewer older cows will be needed for replacement breedings, and thus can be bred to optimal terminal cross sires (in most cases naturally) to produce more valuable calves to sell at weaning.

To minimize the consequences of lower fertility with sexed semen, a successful approach is to breed the heifers about 3 weeks earlier than normal, and then use the more fertile unsexed semen on the next cycle for those not pregnant. Of course, sexed semen can be used for the second try as well, but is not recommended for third and later services.

There are other situations for which sexed semen is appropriate, such as for producing club calves; each herd manager will have to decide if and where sexed semen fits their program.

### **Future Considerations**

As with any new technology, costs of sexed semen will continue to decline with time. In addition, success rates will improve, particularly regarding fertility. Also, new products will become available, such as 75% accurately sexed semen, which costs less to produce. Sexed semen also will be available for more bulls, both for producing male and female calves. In the future, sexed semen could end up being even more fertile than unsexed semen due to being able to discard the less fertile sperm during the sexing process. However, at present, sexed semen is less fertile than unsexed semen; this difference can be minimized by doing every step optimally – semen handling, AI, heat detection, and nutritional and other management considerations.

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