O-3

Sexing Bovine Sperm[a]

George E. Seidel, Jr.

Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO 80523 USA

Key Points

No. Sperm can be sexed with 90% accuracy by flow cytometry/cell sorting.

No. The current speed of sexing is about 5,000 live sperm of each sex per second, remarkably fast considering that each sperm is individually sexed.

No. Although fast, sperm sexing is not fast enough to use standard numbers of sperm per AI dose.

No. With well managed heifers, pregnancy rates with low doses of sexed, frozen sperm are 70~80% of those with unsexed sperm with normal sperm numbers. Pregnancy rates are lower in lactating dairy cows.

No. Calves from sexed sperm appear to be normal.

No. Sexed, frozen semen from a few bulls currently is available commercially in the United Kingdom, and likely will be available in several other countries in 2002, probably at a premium of US $30~50 per straw.

Introduction

Many methods of sexing sperm have been attempted, and there have been sufficient failures to cause much skepticism about sperm-sexing claims. However, recent work has clearly shown that one method is successful with various species, including cattle and horses. This procedure, flow cytometry/cell sorting, is 85 to 95% accurate. It was developed for sexing live sperm by Dr. Larry Johnson in the United States Department of Agriculture laboratory at Beltsville, Maryland, and patented by the U.S. government. Exclusive worldwide rights to the patent have been assigned to XY, Inc., a company in Colorado, for applications in non-human mammals. XY, Inc. has subsequently spent millions of dollars in further research, including development of faster sperm sorters. Much of this work was done in collaboration with Colorado State University.

---

[a]Portions of this have appeared in several other articles and proceedings.

Procedures for Sexing Sperm

Bovine X-sperm, which produce heifers, have 3.8% more DNA (genetic material) than Y-sperm, which produce bulls. Freshly collected sperm are bathed in a DNA-binding stain for about 45 min. This particular stain fluoresces a deep blue when exposed to short wavelength light, provided by a laser. Thus, X-sperm give off 3.8% more blue light under these conditions than Y-sperm. Although a 3.8% difference is too small for people to discriminate using a simple microscope, with the proper electronic equipment plus a powerful computer, this difference can be detected rapidly and accurately for most, but not all sperm.

For the actual sexing procedure, fluid containing the stained sperm passes a detector that measures the brightness of the sperm-bound dye. The fluid then exits a vibrating nozzle that causes individual droplets to form. Ideally, each droplet would contain a single sperm; in practice, over half of the droplets contain no sperm, 20-30% of droplets contain one sperm, and a few droplets contain 2 or more sperm.

Information from the detector is processed by the computer and relayed to the nozzle where a positive charge is given to droplets containing X-sperm, a negative charge, to droplets with Y-sperm, and no charge to droplets with 1 X- and 1 Y-sperm, no sperm, unsexable sperm, or dead sperm. The exiting droplets pass by positively and negatively charged electrical plates; since opposite charges attract, the positive droplets veer toward the negative plate; the negatively charged droplets, toward the positive plate, and the uncharged droplets are unaffected and simply continue falling. These three streams are collected separately, resulting in physical separation of sperm by sex.

Speed of Sexing

This sexing procedure has been improved greatly over the past several years, and improvements continue to be made. About 90,000 droplets are formed per second, and they exit the nozzle at nearly 100 km per hour. Currently about 5,000 live sperm of each sex at about 90% accuracy can routinely be collected per second (nearly double this under ideal circumstances), the rate depending on characteristics of individual semen samples. As procedures improve, these rates likely will increase considerably. Possibly multi-nozzle sorters will be developed, analogous to multi-cylinder engines.

Numbers of Sexed Sperm Needed for Successful Artificial Insemination

Performance of current sperm sorters is impressive; routinely, more than 15,000,000 sperm of each sex can be sorted per hour. However, this is just in the range of the number of sperm in a typical dose of semen for artificial insemination. Thus, even a 2- or 3-fold increase in sorting throughput would be too slow for standard AI practices. It would, however, be quite practical for in vitro fertilization.

For several years, we have been breeding heifers successfully with sexed, frozen sperm.

Insemination of well managed heifers with fewer than 1 million unsexed frozen-thawed sperm per dose results in near normal pregnancy rates with semen of some bulls if skilled inseminators are employed. Similarly, workers in the Netherlands recently found that non-return rates were normal when breeding dairy cows with fewer than 2,000,000 sperm with semen from the most fertile 20% of bulls (Den Daas et al., 1998).

Several years ago, we used the concept of low-dose insemination to inseminate 1,000 heifers with sexed sperm along with 370 heifers with unsexed sperm from the same bulls (Seidel et al., 1999). Sperm were from 22 bulls selected for good semen quality; actual fertility information was not available for most of these bulls before the trials. The 2-month pregnancy rates determined by ultrasound averaged 47% for sexed sperm, mostly at 1 to 3 million frozen sperm/dose, and 60% for unsexed, control sperm, mostly at 20,000,000 frozen-thawed sperm/dose. We and others have since confirmed these results with thousands of additional heifers. However, pregnancy rates are lower when heifers are not managed well in terms of nutrition, estrus detection, etc. Pregnancy rates with low doses of sexed sperm usually are 70~80% of controls with normal numbers of sperm when breeding well-managed heifers.

It is likely that fertility of sexed, frozen sperm at around 2,000,000 sperm/insemination dose would be nearly normal for well managed heifers if the most fertile 30% of bulls were used. We have not yet done experiments with such selected bulls; our work with lactating dairy cows so far (with bulls unselected for fertility) has involved few animals and has produced mixed results. We have, however, obtained 50% pregnancy (and calving) rates using sexed, frozen sperm to inseminate nursing beef cows (Doyle et al., 2000).

Normality of Calves

Over 2,000 calves have now been born from sperm sorted by sex, and many more are gestating. No gross abnormalities have been observed. To confirm that health and survival rates of calves from sexed sperm are normal, controlled studies involving hundreds of sexed and control calves in similar environments are needed. We have examined pregnancy losses between 1 and 2 months of gestation in some studies; losses were 23 of 261 (8.8%) for sexed pregnancies and 9 of 145 (6.2%) for controls. This small difference is not statistically significant. The actual sexes of calves or fetuses at 2~3 months of gestation, averaged over six studies was 86% of the desired sex (Seidel et al., 1999). In our most recent studies, accuracy exceeds 90%. Accuracy is similar for males or females.

While routine freezing of semen kills nearly half the sperm, the process of sexing sperm only damages them slightly. An important fringe benefit of sexing sperm is that the approximately 10% of dead sperm in a typical raw ejaculate (before freezing) are discarded in the sorting process; also, some sperm with abnormal chromosome content are automatically discarded during sexing.

Cost of Sexed Sperm

XY, Inc. intends to license bull studs and other genetics companies to use the technology developed. The sorters themselves currently cost nearly US $300,000 each, although this cost should decline greatly when these machines are simplified and produced in quantity. Since freshly collected semen is required for the sexing process to work well, sorters likely will be placed at bull studs for actual production and distribution of sexed, frozen sperm. Only a small portion of each ejaculate is needed to keep equipment for sexing sperm functioning for many hours, so the remainder of the ejaculate can be frozen for routine AI.

Costs for sexing a low dose of sexed sperm in the United States probably will be in the range of US $30~50 initially, when thousands of doses are sexed per year. Probably less than US $20 will be charged when the process is streamlined to produce millions of doses each year. This charge would be in addition to the normal cost of the semen itself.

When Can Sexed Sperm be Purchased?

Large field trials to determine how well sexed sperm works under farm conditions are being done. As in the early days of embryo transfer, fertility of low dose sexed sperm varies considerably, depending on the exact conditions of use. As happened with embryo transfer, procedures are improving rapidly, and success rates likely will stabilize as experience accumulates. Initially, sexed sperm likely will be recommended for use only in heifers; more research is needed for optimal application in lactating dairy cows and nursing beef cows.

Sexed sperm from a few very fertile, genetically elite bulls already are available for purchase in the United Kingdom, and likely will be available soon in several other countries, probably in North American in 2002. The process of negotiating license fees, purchasing and installing complex equipment, training personnel, and producing and marketing a new product line requires considerable investment in time and money. Cattlemen have looked forward to having this technology for many years, and it will be interesting to see it applied.

Other Methods of Sexing Sperm

Sexing sperm one at a time with a flow cytometer probably will always result in fewer sexed sperm than ideal. Several other methods are being studied by others, including immunological and electrophoretic approaches. To date, these methods have not resulted in producing offspring of the desired sex. If such procedures would work, many sperm could be sexed simultaneously, thus speeding up the process.

Sexed Sperm in Other Species

Sperm from 6 species of mammals have been sexed by flow cytometry, resulting in offspring: humans, cattle, horses, pigs, sheep, and rabbits. It appears that the process will be successful for nearly all mammals. A major constraint with horses and pigs is that many more sperm are

required to obtain reasonable pregnancy rates than with cattle, and since sperm currently are sorted one at a time, procedures using AI of sexed sperm are not as practical as for cattle. We have, however, produced more than 50 pregnancies in horses with sexed sperm to date, and undoubtedly sexing will be commercialized in this species.

There of course is great interest in applying this technology in humans. Fugger and colleagues (1999) have produced hundreds of babies so far with sexed sperm via artificial insemination and in vitro fertilization. An important application is to produce baby girls to avoid X-chromosome-linked genetic diseases.

Implications

Sexed, frozen sperm from a few bulls are likely to become available for purchase within the next year or two. Fertility will be slightly lower than normal with well-managed heifers. Fertility likely will be very variable with poor to average management of heifers, and with well-managed cows. Even with these imperfections and a premium of US $30-50 per dose, there will be opportunities for producers to profit from this technology. As with any new technology, improvements will continue to be made, and costs will decline with time.

Literature Cited

1. Den Daas, J.H.G., G. De Jong, L.M.T.E. Lansbergen and A.M. Van Wagtendonk-de Leeuw. 1998. The relationship between the number of spermatozoa inseminated and the reproductive efficiency of individual dairy bulls. J. Dairy Sci. 81:1714-1723.
2. Doyle, S.P., K.D. McSweeney, J.L. Schenk, R.D. Green and G.E. Seidel, Jr. 2000. Inseminating lactating Angus cows with sexed sperm. J. Anim. Sci. 78 (Suppl. 1):198.
3. Fugger, E.G. 1999. Clinical experience with flow cytometric separation of human X- and Y-chromosome bearing sperm. Theriogenology 52:1435-1440.
4. Seidel, G.E., Jr., J.L. Schenk, L.A. Herickhoff, S.P. Doyle, Z. Brink, R.D. Green and D.G. Cran. 1999. Insemination of heifers with sexed sperm. Theriogenology 52:1407-1420.