



Overview of sexing sperm

G.E. Seidel Jr.*

*Animal Reproduction and Biotechnology Laboratory, Colorado State University, ARBL Building,
Foothills Campus, Fort Collins, CO 80523-1683, USA*

Abstract

Hundreds of thousands of off springs have been born as a result of AI with sexed sperm. Although this technology has been used for many species, the overwhelming majority of pregnancies have been in cattle, nearly all as a result of sperm that were sexed and subsequently frozen. The technology for sexing sperm has not changed greatly in the past 7 years, but refinements have speeded up the process and reduced damage to sperm.

The process of commercialization of sexed sperm has accelerated recently. However, this technology is characterized by high costs, complexity of implementation and lower pregnancy rates than with control sperm. Nevertheless, sexed, frozen bovine sperm are being produced commercially in many countries, although from a limited number of bulls.

The main application of sexed sperm to date has been to breed dairy heifers to produce female calves. Because of the slow speed of sexing sperm, fewer sperm are used per insemination dose of sexed than conventional sperm, and pregnancy rates with this product are often only slightly decreased. Successful use of sexed sperm requires excellent management of cattle, careful handling of sperm and use of skilled inseminators. As costs decline, sexed sperm will be used increasingly for cattle breeding, horse breeding and niche applications in other species.

© 2007 Elsevier Inc. All rights reserved.

Keywords: Sperm; Sexing; Bovine; Flow cytometer

1. Introduction

There has been great interest in sexing sperm ever since AI was practiced widely. More than a dozen approaches to sexing sperm have been attempted, but convincing results were not produced prior to 1980. The major breakthrough was development of flow cytometry/cell sorting in the early 1980s [1]; the initial methods separated X- and Y-sperm effectively, but killed the sperm, making the procedure impractical. A major refinement to this procedure was making the system work without killing or severely damaging the sperm [2].

The currently successful method of sexing sperm has been reviewed by Seidel and Garner [3]. This method has one major limitation: sperm are sexed one at a time, serially, rather than sexing multiple sperm simultaneously (in parallel). Another constraint is that sexing works best with fresh sperm, so sorters usually are located near the bulls, and sperm are cryopreserved after the sexing process. While other, more practical methods of sexing sperm have been proposed and continued to be tested, none of these has been found that meets two essential criteria: accuracy of sexing and retention of sperm fertility.

The instruments currently used for sexing sperm are remarkable feats of engineering, capable of evaluating thousands of sperm per second. However, this is still slow relative to needs for routine AU. Therefore, for sexed semen to be practical, fewer sperm are used per

* Tel.: +1 970 491 5287; fax: +1 970 491 3557.

E-mail address: gseidel@colostate.edu.

insemination dose than are used normally, which means that applications for sexed sperm currently only fit systems of excellent management that result in high fertility.

2. How sperm are sexed

The basic principles are simple; the X-sperm contains more DNA than the Y-sperm (approximately 4% more in the case of cattle). Although this difference is small, by attention to details, it is possible to measure DNA content of individual sperm with sufficient accuracy to distinguish between X- and Y-sperm with about 90% accuracy for 50% of the sperm. Therefore, about half of the sperm are discarded as unsexable, and there is a 10% error rate for those sexed with routine procedures [3].

The DNA content of sperm is determined using a fluorescing dye, Hoechst 33342 that readily penetrates the sperm cell membrane and binds to the DNA stoichiometrically. Thus, X-sperm ends up with about 4% more dye bound to their DNA than Y-sperm. This dye only fluoresces when exposed to a particular wavelength of light, and this is usually provided by an expensive laser. The fluorescence is measured by a detector and analyzed by computer. Since X-sperm have 4% more DNA, and therefore, bind more dye than Y-sperm, they give off 4% more fluorescence, which the computer can recognize. Note that we can also observe the fluorescence with a microscope, but our eyes and brains are not designed to discriminate a 4% difference in the amount of brightness of fluorescence, so X- and Y-sperm appear equal to us, even though they appear different to the instruments used for sexing.

The principles just discussed are combined to make a system to sex sperm. The basic instrument used is a flow cytometer/cell sorter (Fig. 1). It consists of a pump to move the fluid containing sperm past a detector of fluorescence. A laser provides the correct wavelength of light to cause fluorescence without damaging the DNA. A powerful computer also is needed to analyze the fluorescence.

The cell sorting part of the system works as follows: when the stream of fluid exits the flow cytometer, it is broken into little droplets by a vibrator, forming about 70,000 to 80,000 droplets per second. About one-third of the droplets contain a sperm and about two-thirds are empty; a few droplets contain two or more sperm. If a droplet contains an X-sperm as analyzed by the computer, a positive electrical charge is added to the droplet; if the droplet contains a Y-sperm, a negative charge is added; and if the droplet contains no sperm,

Beltsville Sperm Sexing Technology

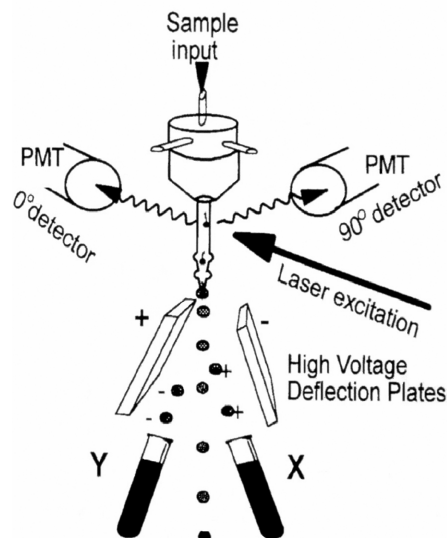


Fig. 1. Schematic diagram of a sperm sorter. The electronic signals from the photomultiplier tubes (PMT) are sent to a computer for analysis. Modified from Johnson and Welch, 1999 [13].

multiple sperm, damaged sperm, or sperm that are indistinguishable relative to DNA content, no charge is placed on the droplet. As the droplets fall when they exit the nozzle of the flow cytometer (at a speed of about 80 km/h), they pass through electric fields that are positive on one side and negative on the other. Since opposite electrical charges attract each other, the droplets with a positive charge (containing X-sperm) move toward the negative part of the field, those with a negative charge move toward the positive field, and those with no charge continue straight down. Thus, three streams of droplets are produced that can be collected into three test tubes, thereby separating the X- from the Y-sperm. In practice, about 20% of sperm end up in the X-fraction, 20% in the Y-fraction and 60% are damaged or not sexable for one reason or another.

The method for detecting and discarding dead and damaged sperm is to add food coloring to the sperm to be sorted. Sperm with a damaged cell membrane become colored, whereas those with healthy cell membranes exclude the food coloring. The food coloring in dead and damaged sperm quenches the fluorescence of the Hoechst 33342, and those poorly fluorescing sperm are discarded in the process. This is a “fringe” benefit of sexing sperm.

The equipment for sexing sperm functions quite well, but is fairly complicated and expensive, over \$350,000 per sperm sorter. It also is expensive to install and maintain. Skilled operators are required, which results in training costs. Because of these large fixed

costs, most sorters are operated in shifts for 14–16 h or more per day.

3. Low sperm dose insemination

Typically, a dose of frozen bull sperm for artificial insemination contains $\geq 20 \times 10^6$ sperm. However, for most bulls, fertility is satisfactory at 10×10^6 sperm, and for some bulls fertility remains high at 2×10^6 frozen sperm per dose [4]. Routine operation of a flow cytometer/cell sorter for sexing sperm results in sexing about 10×10^6 sperm/h of each sex [3]. Thus, use of typical insemination doses of sperm that are sexed is impractical. There are two approaches to deal with this problem, and both involve using fewer sexed sperm per dose (usually 2×10^6 sperm). The first approach is to select bulls with good fertility at low doses of sperm, and the second is to use sexed sperm under management conditions in which normal fertility occurs even if sperm numbers per dose are low. It usually is impractical to screen bulls to use the first approach, although bulls with lower fertility than average usually have unacceptably low fertility with low doses of sexed sperm. Therefore, the second approach is almost always used. With excellent management (nutrition, disease control, estrus detection, semen handling and insemination techniques), fertility of heifers usually is higher than for lactating cows, especially dairy cows. It has been demonstrated repeatedly that with good management, pregnancy rates with breeding heifers are only slightly lower than normal using low doses of sexed sperm [5,6]. In one study with substantive numbers of heifers balanced among three bulls and two inseminators [6], the pregnancy rate for 2×10^6 sexed sperm per inseminate was 56%, whereas the control pregnancy rate with 10×10^6 unsexed sperm was 61%. However, pregnancy rates with sexed sperm are very low with marginal management [7], and usually are low with estrus synchronization protocols in which heifers are bred by appointment instead of on the basis of observed standing estrus. Pregnancy rates with sexed sperm in lactating dairy cows can be similar to controls when selecting only those cows with completely normal reproductive characteristics using ultrasound examination, records, etc. [8]; such selection usually is impractical.

4. Normality of calves

One concern of using sexed sperm is that the calves produced might have abnormalities. This concern is primarily because of using the DNA binding dye

Table 1
Normality of calves from sexed sperm (least squares means)

	Sexed ^a	Control
Numbers ^b	1158	787
Abortion rate (%)	4.5	5.0
Gestation length (d)	279	279
Neonatal death (%)	3.5	4.0
Calving ease score	1.22	1.23
Birth weight (kg)	33.9	34.1
Live at weaning (%)	91.7	91.5
Weaning weight (kg)	239	241

^a There were no differences between sexed and control groups for any response ($P > 0.10$).

^b Numbers for gestation length, neonatal death and live at weaning; *N* were lower for the other responses because not all data were collected at each farm (adapted from Tubman et al., 2004 [5]).

Hoechst 33342 plus exposing sperm to laser light of low wavelength. We, therefore, studied calves resulting from our field trials with sexed sperm, including calves from control semen from the same bulls used in the same herds [5]. We found no evidence of abnormalities in the calves (Table 1), nor could we document increased abortion rates. In a few cases, calves were produced from sexed sperm whose mothers also were produced from sexed sperm. While these studies do not provide absolute proof that there is no genetic damage to sperm from the sexing process, they indicate that the calves produced are phenotypically normal. Genetic damage likely is very low, or does not occur at all.

5. Applications of sexed sperm for cattle

Because sexed sperm have been used to breed heifers successfully on many occasions, one obvious application is to breed heifers to have female calves. These should result in excellent replacements for beef and dairy herds, since the youngest cattle in any herd with a genetic improvement program are genetically superior to the older cows. A major additional benefit is that on the average, female calves weigh about 2 kg less at birth than male calves, so the incidence of dystocia in first calf heifers will decrease with this application.

Another application is to obtain male calves from the very best cows in the herd to use as breeding bulls. One example is dairy cows that artificial insemination companies contract for their bull calves. Sexed sperm could be especially useful for superovulation, in which case it often is desirable to obtain calves of one sex or another for a particular mating. One must be careful when using sexed semen for this application because more, rather than fewer, sperm typically are used for superovulated cattle, so embryo production will be

lower and more variable than with unsexed sperm [9]. Nevertheless, the calves produced will be about 90% of the desired sex, so even if embryo production is reduced by one-third, this application may be appropriate.

One concern with dairy cattle breeding is that when use of sexed sperm becomes widespread, female calves will be produced in excess numbers, and their value will decline. This will be offset somewhat if sexed sperm are used primarily in heifers; in this case, many older, genetically less valuable cows might be bred to beef sires (with sexed or unsexed sperm) to produce dairy X beef crossbred calves for meat production. For example, Charolais X Holstein cross calves are excellent for growth and fattening, especially the males, and likely could be sold for considerably more than male Holstein calves. One other obvious application is to increase the number of heifer calves when increasing the size of a herd. Herd expansion could occur more quickly, and possibly without purchasing females. This would be very valuable from a biosecurity standpoint, and possibly from a genetic standpoint, too. An analysis of the economics of sexed sperm was done by Seidel [10], and some ideas about how sexed sperm might fit the AI industry have been summarized [11].

One special application of sexed sperm is for in vitro fertilization. One dose of sexed sperm can be used to produce many embryos. Of course, this application only is appropriate if an in vitro fertilization program, including the costs and logistics of embryo transfer, makes sense for other reasons. One application may be to produce embryos to increase pregnancy rates under hot, humid conditions. Hansen and Block [12] have shown that embryo transfer results in higher pregnancy rates than artificial insemination with Holstein cows under these conditions in the southeastern United States.

6. Future possibilities

Although no promising alternative methods of sexing sperm are currently available, it is likely that someone eventually will find a method of mass sexing of sperm in parallel, so that low dose insemination is not a constraint. This also would make sexing sperm less expensive.

One recent development is configuring sperm sorters with two or more nozzles, instead of one. This is analogous to having an eight-cylinder engine instead of one-cylinder. Even two nozzles speed up the sperm sexing process, and decrease costs. The current cost of a low dose of sexed sperm in most countries is usually

more than twice the cost of a normal dose of frozen sperm from the same bull.

Acknowledgements

Numerous students and colleagues contributed to the research summarized here. I especially acknowledge the many farmers and ranchers who allowed us to use their cattle and facilities. Most of the research was funded by XY, Inc., Fort Collins, CO, and the Colorado State University Experiment Station. This is an updated version of “Sexado de Semen” from Proc. XLI Reunion Nacional de Investigacion Pecuaria, pp. 154–160 (2005), which first appeared in Spanish.

References

- [1] Garner DL, Seidel Jr GE. Past, present and future perspectives on sexing sperm. *Can J Anim Sci* 2003;83:375–84.
- [2] Johnson LA, Flook JP, Hawk HW. Sex preselection in rabbits: live births from X- and Y-sperm separated by DNA and cell sorting. *Biol Reprod* 1989;41:199–203.
- [3] Seidel Jr GE, Garner DL. Current status of sexing mammalian spermatozoa. *Reproduction* 2002;124:733–43.
- [4] Den Daas JH, DeJong G, Lansbergen LAM, Van Wagendonk-DeLeeuw AM. The relationship between the number of sperm inseminated and the reproductive efficiency of individual dairy bulls. *J Dairy Sci* 1998;81:1714–23.
- [5] Tubman LM, Brink Z, Suh TK, Seidel Jr GE. Characteristics of calves produced with sperm sexed by flow cytometry/cell sorting. *J Anim Sci* 2004;52:1029–36.
- [6] Schenk JL, Brink Z, Suh TK. Use of competitive fertilization to evaluate a simple laser for flow cytometric sexing of bovine sperm. *Reprod Fertil Dev* 2005;17:306 (abstract).
- [7] Schenk JL, Seidel Jr GE. Pregnancy rates in cattle with cryopreserved sexed spermatozoa: effects of laser intensity, staining conditions and catalase. In: Juengel JL, Murray JF, Smith MF, editors. *Reproduction in Domestic Ruminants VI*. Nottingham, UK: Nottingham University Press; 2007. p. 165–77.
- [8] Crichton E, Huffman S, McSweeney K, Schenk J. Artificial insemination of lactating Holstein cows with sexed sperm. *Reprod Fertil Dev* 2006;18:281 (abstract).
- [9] Schenk JL, Suh TK, Seidel Jr GE. Embryo production from superovulated cattle following insemination of sexed sperm. *Theriogenology* 2006;65:299–307.
- [10] Seidel Jr GE. Economics of selecting for sex: the most important genetic trait. *Theriogenology* 2003;59:585–98.
- [11] Seidel Jr GE. Sexing mammalian sperm—intertwining of commerce, technology and biology. *Anim Reprod Sci* 2003;79:145–56.
- [12] Hansen PJ, Block J. Towards an embryocentric world: the current and potential uses of embryo technologies in dairy production. *Reprod Fertil Dev* 2004;16:1–14.
- [13] Johnson LA, Welch GR. Sex preselection: high-speed flow cytometric sorting of X- and Y-sperm for maximum efficiency. *Theriogenology* 1999;52:1323–413.