SUPPLEMENTAL MATERIAL FOR ARTICLE

SEX RATIO OF CALVES BORN AFTER AI WITH "SEXED SPERM"

George E Seidel, Jr. and Rupert P Amann
Animal Reproduction and Biotechnology Laboratory
Colorado State University, Ft Collins, CO 80523-1683

Literature of Separation of X- and Y-chromosome Bearing Sperm

The potential to separate X- and Y-sperm has intrigued scientists for decades. Background material through 1982 was summarized in the proceedings of a symposium held at Colorado State University (Amann and Seidel, 1982). At this meeting, it was first reported that a flow cytometer could be used to separate X- and Y-chromosome bearing sperm (Gledhill et al., 1982). This is the only method to prepare sexed sperm that has been proven to be effective. After 10 years of intensive R&D, commercial use of flow-sorted, sexed sperm is a reality. By approximately 2000, it was evident that flow-sorting of sperm could lead to a commercial product, and it was only a matter of time until sales to producers was initiated. Although commercial sales of sexed semen was initiated in the United Kingdom in 2000, for several reasons commercial sales of flow-sorted semen was not initiated in the USA until 2005. Selected references are listed at the end of this article.

Binominal Variation

When there are only 2 possible outcomes for an observation, it is called a binomial response and well established mathematical rules of binominal variation apply. Flipping a coin (heads or tails), sex of newborn calf (female or male), and pregnancy status (pregnant or non-pregnant) are examples familiar to operators of an animal enterprise. When a data set contains 6 or more observations for each of the 2 possible outcomes, the distribution of outcomes becomes like a "normal distribution."

A logical question is "based on observations for 1 small data set, what am I likely to obtain in subsequent data sets using the same semen?" The anticipated dispersion of observed values around the "true value" can be can be calculated as $I = t\sqrt{[(p(1-p))/n]}$, where I = confidence interval above or below the mean (average) value, p = the proportion of heifer calves (expressed as 0.50 or 0.90 rather than 50% or 90%), n = the number of calves examined, and t = a value reflecting the desired width of the confidence range (i.e., 50, 70, 90, or 95% of all future data sets of this size). The values for t are 0.674, 1.062, 1.645, or 1.960 for encompassing 50, 70, 90, or 95% of data sets. The confidence range is calculated as mean ∀ I. This pair of equations was used to calculate displays in Figure 1 of the primary article and also the supplemental figure in a later section.

Impact of Number of Observations on Certainty of a Proportion

In the case of coin flipping or sex of newborn calf, there is essentially no error in making the measurement (deciding if a coin is a head or tail, or a calf female or male). Hence, one can calculate the confidence range as discussed in the previous section. In respect to pregnancy rate, however, there are a number of factors that might affect an individual observation. These include method to determine pregnancy, interval after mating or AI, technician making the decision, herd, etc. Hence, any individual report of pregnant or non-pregnant might be wrong. The combined impact of both known and unknown sources of variation have been estimated to add at least 20% to the estimated imprecision in any average measurement, above that associated with the number of observations and magnitude of the observed average response (reviewed in Amann, 2005). Hence, for pregnancy rate confidence rates should be wider than for sex ratio.

From the formula $I = t\sqrt{[(p(1-p))/n]}$ introduced in the previous section, it is obvious that the value of n affects the value for I. As *n* increases, the value of I decreases and, consequently, the confidence range decreases. This is evident in Supplemental Table 1.

Supplemental Table 1.
Confidence ranges in percent (%) for a binominal distribution with true value of 50%

Sample size	70% confidence range	95% confidence range
10	34 - 66	19 - 81
20	39 - 61	28 - 72
30	41- 59	32 - 68
50	43 - 57	36 - 64
100	45 - 55	40 - 60
250	47 - 53	44 - 56
1000	48 - 52	46 - 54

Sex Ratio at Birth after Natural Mating or AI with Regular Semen

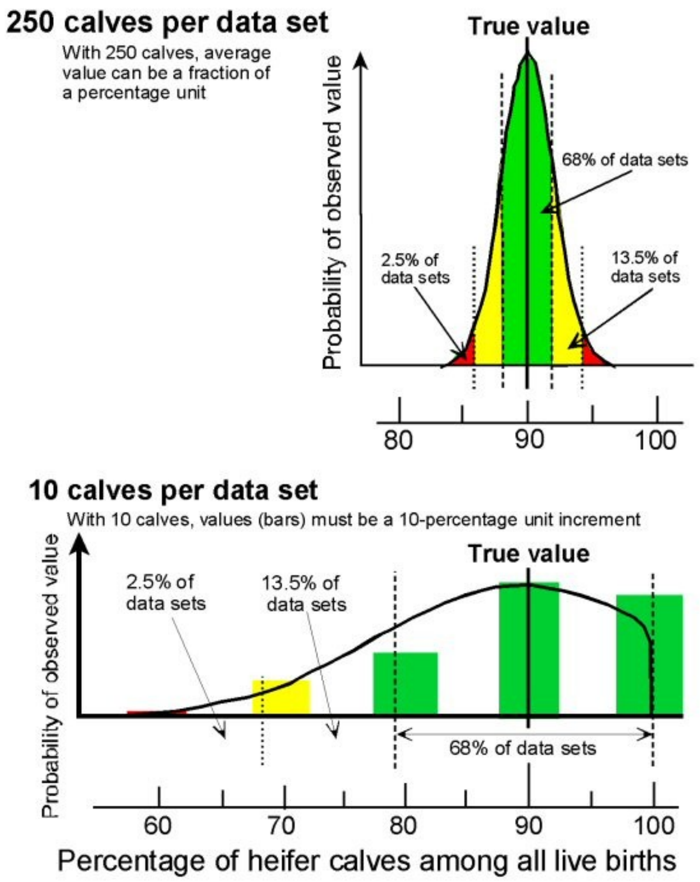
Sex ratio of calves at birth is the number males divided by the number of females in a population. Sex ratio at birth is slightly in favor of bull calves, and might average 1.06 males per female. There is extensive literature on the sex ratio of calves at birth. A good summary of this was provided by Foote (1977).

Sex ratio after AI with sexed sperm

The flow-sorter used to prepare sexed sperm can be operated to provide a nominal 80, 85, 90 or even 95% X- or Y-sperm, or collection of both types concurrently in separate tubes. Principles are discussed in Seidel and Garner (2002). The higher the desired purity, the fewer the number of cells that can be recovered. Hence, high purity is expensive, and flow sorters frequently are operated to provide 90% of the desired sperm type (i.e., 90% X-sperm). There are slight differences between individual instruments possibly contributing cells to a pool of sorted sperm for a given freeze code, and variation over time for a given instrument. The actual percentage of X-sperm in a pool of semen processed for distribution usually is measured by “analytical re-analysis,” which has a small measurement error. The actual percentage of X-sperm for individual freeze codes or batches usually will fall within a range of 0-3 percentage units below or above the target (i.e., 87-93% X-sperm).

The impact of number of newborn calves in a data set on the possible deviation of percentage of heifer calves from a theoretical 90%, based on proportion of X-sperm in the semen, is shown in Supplemental Figure 1. The upper plot shows the distribution that is expected for data sets based on 250 newborn calves. This is a symmetrical and rather narrow plot. Virtually all data sets based on 250 observations should give an observed average value of >85% heifers. It is highly unlikely that an average value of 80% or less, based on 250 observations, would be observed with this semen.

The lower plot shows the distribution expected for data sets based on 10 newborn calves, using the same semen. The plot is rather broad (because of the far smaller number of observations). A substantial number of data sets would have an average value of less than 80% heifers, when based on 10 newborn calves.



As noted in respect to Figure 1 in the primary article, it is an inescapable fact that for about half of all data sets, of any size, the percentage of heifers will be less than that anticipated based on percentage of X-sperm in the semen. Further, with small data sets of 10-20 newborn calves, there is a measurable probability that there will be fewer than 80-85% heifers. Nevertheless, there is a high probability that the vast majority of newborn calves in a given data set indeed will be heifers.

Pregnancy Rate with Sexed Semen

It is imperative that producers recognize that conception/pregnancy rates with sexed semen will be lower than obtained with regular semen, especially with AI of cows rather than heifers. This difference might be 10-15 percentage units lower with sexed sperm than control sperm used in the same herds (Seidel et al., 1999). Importantly, in one study Tubman et al. (2004) there was no evidence for an excess of abnormalities among 1,169 calves produced from sexed semen.

Measurement of pregnancy rate with sexed or regular semen is profoundly affected by the cows or heifers on which the average is based; the number of animals, their status (parity, lactation), and nutritional and management factors associated with herd (Amann and Hammerstedt, 2002). In general, estimates of pregnancy rate for a bull, or for sexed vs regular semen from a given bull, based on fewer than 100 cows or heifers are too imprecise to be meaningful (Amann, 2005). Further, pregnancy rate measured on the basis of a relatively small sample has minimal utility in predicting future pregnancy rates with semen from the same bull.

References

Amann RP. 2005. Weaknesses in reports of "fertility" for horses and other species. Theriogenology 63:698-715.

Amann RP, Hammerstedt RH. 2002. Detection of differences in fertility. J Androl 23:317-325.

Amann RP, Seidel GE Jr. 1982. Prospects for Sexing Mammalian Semen. Colorado Associated University Press, Boulder, 288 pp.

Foote RH. 1977. Sex ratios in dairy cattle under various conditions. Theriogenology 8:349-356.

Garner DL. 2001. Sex-sorting mammalian sperm: Concept to application in animals. J Androl 22:519-526.

Garner DL, Seidel GE Jr. 2003. Past, present and future perspectives on sexing sperm. Can J Anim Sci 83:375-384.

Gledhill BL, Pinkel D, Garner DL, Van Dilla MA. 1982. Identifying X- and Y-chromosome-bearing sperm by DNA content: retrospective perspectives and prospective opinions. In: Prospects for Sexing Mammalian Semen (RP Amann and GE Seidel Jr, eds). Colorado Associated University Press, Boulder, pp. 177-191.

Johnson LA, Flook JR, Hawk HW. 1989. Sex preselection in rabbits: Live births from X and Y sperm separated by DNA and cell sorting. Biol Reprod 41:199-203.

Johnson LA, Seidel GE Jr (eds). 1999. Current status of sexing mammalian sperm. Theriogenology 52:1267-1484.

Schenk JL, Suh TK, Seidel GE Jr. 2006. Embryo production from superovulated cattle following insemination of sexed sperm. Theriogenology 65:299-307.

Seidel GE Jr. 1999. Sexing mammalian spermatozoa and embryos – state of the art. J Reprod Fertil (Suppl) 54:475-483.

Seidel GE Jr. 2003. Economics of selecting for sex: The most important genetic trait. Theriogenology 59:585-598.

Seidel GE Jr. 2003. Sexing mammalian sperm – Intertwining of commerce, technology, and biology. Anim Reprod Sci 79:145-156.

Seidel GE Jr. 2005. Sexado de semen. Available online at: http://www.cvmbs.colostate.edu/bms/sexcalves_supp.pdf

Seidel GE Jr, Garner DL. 2002. Current status of sexing mammalian spermatozoa. Reproduction 124:733-743.

Seidel GE Jr, Schenk JL, Herickhoff LA, Doyle SP, Brink Z, Green RD, Cran DG. 1999. Insemination of heifers with sexed spermatozoa. Theriogenology 52:1407-1420.

Tubman LM, Brink Z, Suh TK, Seidel GE Jr. 2004. Characteristics of calves produced with sperm sexed by flow cytometry/cell sorting. J Anim Sci 82:1029-1036.

SEXING SPERM

George E. Seidel, Jr.
ARBL, Colorado State University.

Summary

Many thousands of offspring have been born as a result of artificial insemination with sexed sperm. While this technology has been used for many species, the overwhelming majority of pregnancies have been in cattle, nearly all as a result of sexed, then frozen semen. 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 continues steadily but slowly. There are 3 main reasons for slow commercialization: high costs, complex technology to implement, and lower pregnancy rates than with control semen. Nevertheless, it is possible to purchase sexed, frozen bovine semen produced in several countries.

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

Introduction

There has been great interest in sexing sperm ever since artificial insemination started to be 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 (Garner and Seidel, 2003); procedures used 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 (Johnson et al., 1989).

The currently successful method of sexing sperm has been reviewed by Seidel and Garner (2002). 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 frozen after the sexing process. While other, more practical methods of sexing sperm have been proposed and continue to be tested, none 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 artificial insemination. Therefore, for sexed semen to be practical, fewer sperm are available per 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.

How Sperm Are Sexed

The basic principles are simple. X-sperm contain more DNA than Y-sperm, about 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 correctly about 90% of the time. Therefore, there is a 10% error rate with routine procedures.

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. Thus, X-sperm end 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 a 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, which is illustrated in Figure 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.

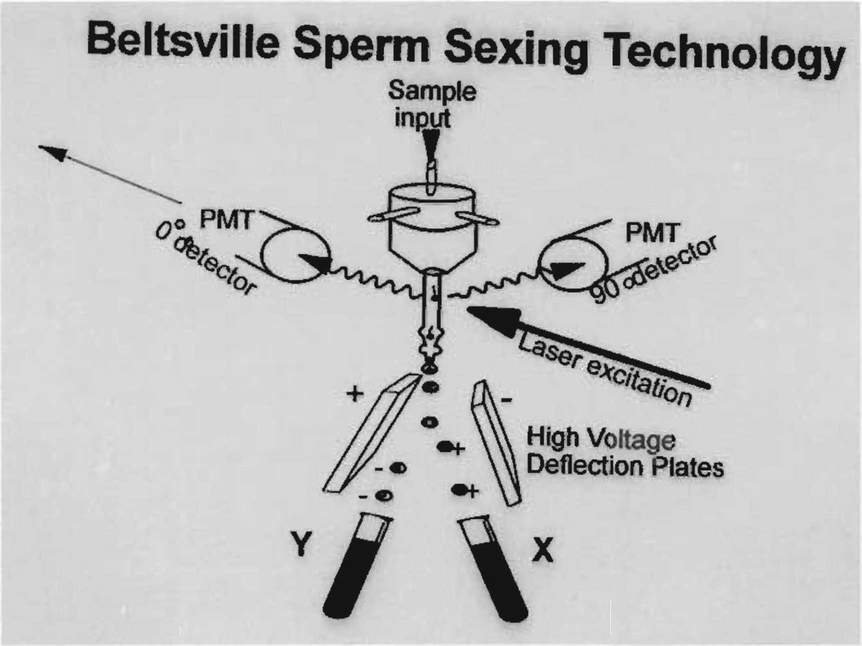


Figure 1. Diagram of sperm sorter.

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 2 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, 2 sperm, damaged sperm, or a sperm that is unsexable, 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 90 km/hour), they pass through an electric field that is 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 3 test tubes, which separates the X- and 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.

This equipment functions quite well, but is fairly complicated and expensive, over $300,000 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 to 16 hours per day.

Low Sperm Dose Insemination

Typically, a dose of frozen bull semen for artificial insemination contains 20 million or more sperm. However, for most bulls fertility is satisfactory at 10 million sperm, and for some bulls fertility remains high at 2 million sperm per dose (Den Daas et al., 1996). Routine operation of a flow cytometer/cell sorter for sexing sperm results in sexing about 10 million sperm per hour of each sex (Seidel and Garner, 2002). Thus, use of typical doses of sperm that are sexed is impractical. There are two approaches to dealing with this problem, and both involve using fewer sexed sperm per dose, usually 2 million 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 recommended. 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 pregnancy rates with breeding heifers are only slightly lower than normal using low doses of sexed sperm. However, pregnancy rates with sexed sperm are very low with marginal management, and 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.; such selection usually is impractical.

Normality of Calves

One concern of using sexed sperm is that the calves produced might have abnormalities. This concern is primarily because of the DNA binding dye 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 (Tubman et al., 2004). 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 zero 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.

Table 1. Normality of calves from sexed sperm

	Sexed	Control
No.[a]	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] 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.

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 difficult births in 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 less, sperm typically are used for superovulated cattle, so embryo production will be lower and more variable than with unsexed sperm. (Schenk et al., 2005). 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 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 recently (Seidel, 2003a), and some ideas about how sexed sperm might fit the artificial insemination industry have been summarized (Seidel, 2003b).

One special application of sexed sperm is for in vitro fertilization. One dose of semen can be used to produce many embryos. Of course, this application only is appropriate if an in vitro fertilization program makes sense for other reasons. One application may be to produce embryos to increase pregnancy rates under hot, humid conditions. Hansen and Block (2004) have shown that embryo transfer results in higher pregnancy rates than artificial insemination with Holstein cows under these conditions in the southeastern United States.

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 making a sperm sorter with multiple nozzles, instead of one. This is analogous to having an 8-cylinder engine instead of one-cylinder. If this develops commercially, it would certainly speed up the sperm sexing process, and likely would 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 semen from the same bull.

References

Den Daas, J.H., DeJong, G., Lansbergen, L.A.M. and Van Wagtendonk-DeLeeuw, A.M. 1998. The relationship between the number of sperm inseminated and the reproductive efficiency of individual dairy bulls. J. Dairy Sci. 81:1714-1723.

Garner, D.L. and Seidel, G.E. Jr. 2003. Past, present and future perspectives on sexing sperm. Can. J. Anim. Sci. 83:375-384.

Hansen, P.J. and Block, Jr. 2004. Towards an embryocentric world: The current and potential uses of embryo technologies in dairy production. Reprod. Fertil. Develop. 16:1-14.

Johnson, L.A., Flook, J.P. and Hawk, H.W. 1989. Sex preselection in rabbits: Live births from X and Y sperm separated by DNA and cell sorting. Biol. Reprod. 41:199-203.

Schenk, J.L., Suh, T.K. and Seidel, G.E. Jr. 2005. Embryo production from superovulated cattle following insemination with sexed sperm. Theriogenology 64:in press.

Seidel, G.E. Jr. 2003a. Economics of selecting for sex: The most important genetic trait. Theriogenology 59:585-598.

Seidel, G.E., Jr. 2003b. Sexing mammalian sperm – Intertwining of commerce, technology and biology. Anim. Reprod. Sci. 79:145-156.

Seidel, G.E. Jr. and Garner, D.L. 2002. Current status of sexing mammalian spermatozoa. Reproduction 124:733-743.

Tubman, L.M., Brink, Z., Suh T.K. and Seidel, G.E. Jr. 2004. Characteristics of calves produced with sperm sexed by flow cytometry/cell sorting. J. Anim. Sci. 52:1029-1036.

Sexado de semen

George E. Seidel, Jr.
ARBL, Colorado State University
Fort Collins, CO 80523-1683 USA
Correo-e: gseidel@colostate.edu

Resumen

Muchos miles de crías han nacido como resultado de la inseminación artificial con semen sexado. Aunque esta tecnología ha sido usada en muchas especies, la abrumadora mayoría de preñeces se han logrado en bovinos, casi todas como resultado del uso de semen sexado y congelado. Esta tecnología no ha cambiado sustancialmente en los últimos 7 años, aunque se ha refinado en vías de acelerar el proceso y reducir el daño a los espermatozoides.

El proceso de comercialización del semen sexado avanza en forma constante pero lenta. Existen 3 razones principales para esta lenta comercialización: altos costos, la complejidad de la tecnología a implementar y menores tasas de preñez en comparación con semen convencional. No obstante lo anterior, es posible comprar semen sexado bovino congelado en varios países.

La aplicación principal del semen sexado a la fecha es inseminar vaquillas lecheras para producir crías hembras. En virtud de la lentitud del proceso del sexado de semen, se usa una menor cantidad de espermatozoides por dosis en comparación con una dosis de semen convencional y las tasas de preñez que se obtienen son por lo general solo ligeramente inferiores cuando se sirven vaquillas. El uso exitoso del semen sexado requiere un manejo excelente del ganado, manejo cuidadoso del semen y el uso de inseminadores experimentados. El semen sexado será usado cada vez mas conforme los costos se vayan reduciendo, en la reproducción bovina y equina y en aplicaciones especificas en otras especies.

Introducción

Desde que la inseminación artificial se empezó a practicar de forma generalizada, ha habido un gran interés en el sexado del semen. Mas de una docena de aproximaciones se habían intentado sin haberse obtenido resultados convincentes antes de 1980. El avance mas significativo fue el desarrollo de la citometría de flujo / separación de células a principio de los años 1980s (Garner and Seidel, 2003); los procedimientos usados separaban los espermatozoides X y Y de manera efectiva, pero mataban las células espermáticas, haciendo el procedimiento impráctico. Un mejoramiento importante a este procedimiento, fue lograr que el sistema funcionara sin matar o causar daño severo a los espermatozoides (Johnson et al., 1989).

El método de sexado de semen que actualmente se usa con éxito ha sido revisado por Seidel y Garner (2002). Esta metodología tiene una limitante importante: los espermatozoides son sexados de uno en uno, serialmente, en lugar de que pudieran sexarse múltiples espermatozoides simultáneamente (en paralelo). Otra limitante es que el proceso de sexado funciona mejor con semen fresco, de modo que las maquinas separadoras deben ser ubicadas cerca de donde están los toros y el semen ser congelado inmediatamente después del proceso de sexado. Aún cuando otros métodos de sexado de semen mas prácticos han sido propuestos y continúan probándose, ninguno ha demostrado reunir los dos criterios esenciales: precisión en el sexado y retención de la fertilidad de los espermatozoides.

Los instrumentos que actualmente se usan para el sexado de semen son hazañas sobresalientes de ingeniería capaces de evaluar miles de espermatozoides por segundo. Sin embargo, esto resulta aún muy lento, en relación a lo que es requerido para la inseminación artificial rutinaria. Por lo tanto, para que la tecnología de semen sexado sea práctica, es necesario que se use un menor numero de espermatozoides por dosis de semen, lo cual significa que la aplicación actual del semen sexado se circunscribe a sistemas con excelente manejo que garantizan alta fertilidad.

Como el semen es sexado

Los principios básicos son simples. El espermatozoide X contiene mas ADN que el espermatozoide Y; alrededor de 4% mas en el caso del ganado. Aunque esta diferencia es pequeña, atendiendo a los detalles, es posible medir el contenido de ADN de un solo espermatozoide con suficiente precisión para distinguir correctamente si es X o Y con un 90% de efectividad. Existe por lo tanto un 10% de probabilidad de error usando procedimientos rutinarios de separación de esperma.

El contenido de ADN del espermatozoide es determinado usando una tinción fluorescente, Hoechst 33342, que penetra inmediatamente la membrana de la célula espermática y se une al ADN. Así, los espermatozoides X captan alrededor de un 4% mas tinción unida a su ADN que los espermatozoides Y. Esta tinción emite fluorescencia únicamente cuando es expuesta a un haz de luz con una longitud de onda particular, la cual es proveída usualmente por un costoso láser. La fluorescencia es medida por un detector y analizada por una computadora. En virtud de que los espermatozoides X tienen 4% mas ADN y por lo tanto captan mas tinción que los espermatozoides Y, estos emiten 4% mas fluorescencia, la cual puede reconocer la computadora. Es preciso aclarar que también es posible observar la fluorescencia con un microscopio, sin embargo, nuestros ojos y nuestro cerebro no son capaces de discriminar ese 4% de diferencia en la intensidad de la fluorescencia, de modo que los espermatozoides X y Y aparecen iguales ante nuestra vista, no obstante ellos se muestran diferentes para los instrumentos usados para el sexado.

Los principios anteriormente discutidos se combinan para generar un sistema para sexar semen. El instrumento básico usado es un citómetro de flujo / separador de células, el cual es ilustrado en la Figura 1. Consiste en una bomba que mueve el fluido conteniendo los espermatozoides a través de un detector de fluorescencia. Un láser provee luz con la apropiada longitud de onda para causar fluorescencia sin dañar el ADN. Una poderosa computadora es también requerida para analizar la fluorescencia.

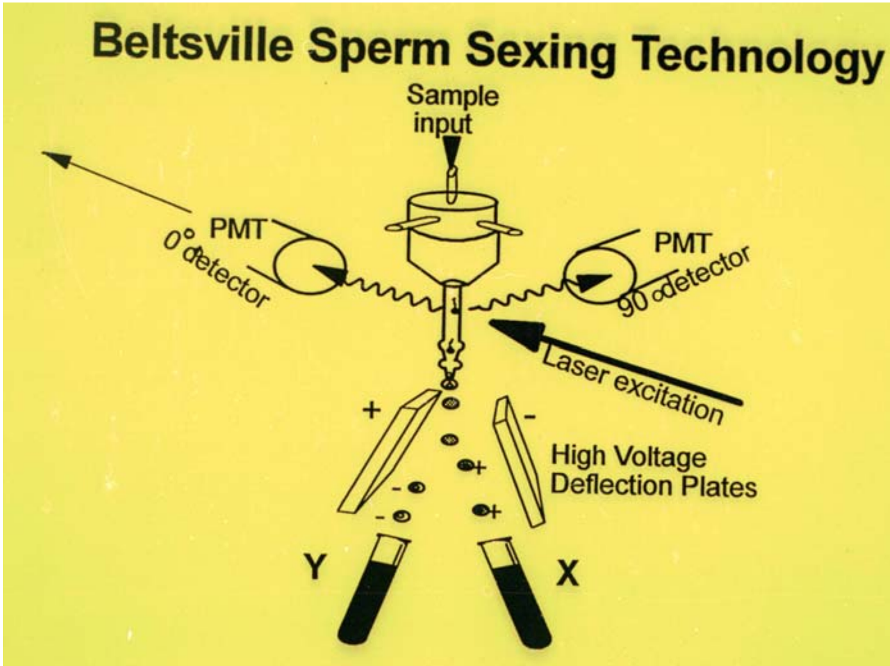


Figura 1. Diagrama del separador de semen.

La parte del sistema que separa las células funciona como sigue: Cuando la corriente de fluido sale del citómetro de flujo, ésta es fraccionada en pequeñas gotas por un vibrador, formando alrededor de 70,000 a 80,000 gotas por segundo. Aproximadamente un tercio de las gotas contienen un espermatozoide y alrededor de dos tercios están vacías; unas cuantas gotas contienen dos espermatozoides. Si la computadora detecta una gota conteniendo un espermatozoide X, una carga eléctrica positiva se agrega a la gota; si la gota contiene un espermatozoide Y se agregará una carga negativa y si la gota no contiene espermatozoides, dos espermatozoides, un espermatozoide dañado o un espermatozoide que no se puede sexar, no se agrega carga eléctrica alguna a la gota. Conforme las gotas caen al salir de la boquilla del citómetro de flujo (a una velocidad de aproximadamente 90 Km/hora), pasan a través de un campo magnético que es positivo en un lado y negativo en el otro. En virtud de que los polos opuestos se atraen, las gotas con carga positiva (conteniendo un espermatozoide X) se mueven en dirección del lado negativo del campo; aquellas con carga negativa se moverán hacia el lado positivo del campo y las que llegan sin carga, continuarán su camino sin desviarse. De esta forma, tres corrientes de gotas se producen, las cuales se pueden colectar en tres tubos de ensayo que separan los espermatozoides X y Y. En la práctica, cerca de un 20% de los espermatozoides terminan colectados en la fracción X, 20% en la fracción Y y 60% son espermatozoides dañados o que no pudieron ser sexados por alguna razón u otra.

Este equipo funciona razonablemente bien, aunque es algo complicado y caro, sobre $ 300,000 Dólares por equipo sexador, el cual es también caro de instalar y mantener. Adicionalmente, se necesita personal entrenado para su operación, lo que resulta en costos de entrenamiento. Debido a estos grandes costos fijos, la mayoría de los aparatos sexadores de semen son operados en turnos de 14 a 16 horas por día.

Inseminación con dosis reducidas de semen

Típicamente, una dosis de semen de toro congelada para uso en inseminación artificial contiene 20 millones o mas de espermatozoides. Sin embargo, para la mayoría de los toros 10 millones de espermatozoides son suficientes para lograr fertilidad satisfactoria; y para algunos toros la fertilidad permanece elevada a 2 millones de espermatozoides por dosis (Den Daas et al., 1996). La operación rutinaria de un Citómetro de flujo / separador de células produce alrededor de 10 millones de espermatozoides de cada sexo separados por hora (Seidel and Garner, 2002). Así, el uso de dosis convencionales de semen sexado es impractico. Hay dos maneras de enfrentar este problema e involucran ambas el uso de menos espermatozoides por dosis, usualmente dos millones. La primera estrategia es seleccionar toros con buena fertilidad a dosis bajas de espermatozoides y la segunda es usar el semen sexado bajo condiciones de manejo en las que se puede lograr buena fertilidad aún y cuando se use una baja dosis de semen. Es normalmente impractico buscar toros para la primera estrategia, aunque toros con fertilidad por debajo del promedio usualmente exhiben fertilidad que es inaceptablemente baja cuando se usan dosis reducidas de semen sexado. Es por lo tanto, la segunda estrategia la que se recomienda. Con manejo excelente (nutrición adecuada, control sanitario, detección de celos, manejo de semen y técnicas de inseminación), la fertilidad en vaquillas es usualmente mas alta que en vacas lactantes, especialmente para ganado lechero. Se ha demostrado repetidamente que las tasas de preñez logradas en vaquillas inseminadas con semen sexado son solo ligeramente inferiores que las obtenidas con semen convencional. Sin embargo, las tasas de preñez con semen sexado son muy bajas con manejo marginal y con protocolos de sincronización del celo en los que las vaquillas son inseminadas a tiempo predeterminado, en lugar de ser inseminadas a calor observado. Las tasas de preñez en vacas lecheras lactantes inseminadas con semen sexado pueden ser similares a inseminaciones con semen convencional cuando se seleccionan únicamente aquellas vacas con características reproductivas completamente normales usando examen de ultrasonido, verificando registros, etc.; este tipo de selección es impráctico.

Normalidad de las crías

Una preocupación en el uso del semen sexado es que los becerros producidos podrían presentar anormalidades. Esta preocupación está principalmente relacionada con la unión de la tinción Hoechst 33342 a la molécula de ADN, además de la exposición del espermatozoide a la luz del láser de baja longitud de onda. Esto nos indujo, por lo tanto, a estudiar a los becerros nacidos en nuestras pruebas de campo usando semen sexado, incluyendo becerros nacidos de semen control proveniente de los mismos toros y usados en los mismos hatos (Tubman et al., 2004). No encontramos evidencia de anormalidades (Cuadro 1), ni pudimos documentar un incremento en las tasas de aborto. En algunos casos, se analizaron becerros producidos con semen sexado, hijos de madres provenientes también de semen sexado. Aunque estos estudios no pueden demostrar categóricamente que existe cero daño genético en el esperma como resultado del proceso de sexado de semen, estos indican que los becerros producidos son fenotípicamente normales. El daño genético es probablemente muy reducido o de hecho no ocurre.

Cuadro 1. Normalidad de becerros provenientes de semen sexado

	Sexado	**Control**
No.[a]	1158	787
Tasa de abortos (%)	4.5	5.0
Longitud de gestación (d)	279	279
Muerte neonatal (%)	3.5	4.0
Calificación facilidad de parto	1.22	1.23
Peso al nacer (kg)	33.9	34.1
Sobrevivencia al destete (%)	91.7	91.5
Peso al destete (kg)	239	241

[a] Estos números aplican para longitud de gestación, muerte neonatal y sobrevivencia al destete; las N fueron menores para las otras respuestas, debido a que no todos los datos fueron colectados en cada granja.

Aplicaciones del semen sexado en el ganado

En virtud de que el semen sexado ha sido usado para servir vaquillas exitosamente en múltiples ocasiones, una aplicación obvia es inseminar vaquillas para obtener crías hembras. Esto deberá resultar en reemplazos excelentes para hatos lecheros y de carne, ya que las hembras mas jóvenes en cualquier hato que lleve un programa de mejoramiento genético son genéticamente superiores que vacas mas viejas. Un beneficio importante adicional es que en promedio, las crías hembras pesan alrededor de 2 Kg. menos al nacimiento que las crías macho, por lo que la incidencia de dificultades al parto en vaquillas se reduce con esta aplicación.

Otra aplicación es obtener crías macho de las mejores vacas del hato para usarlos como toros reproductores. Un ejemplo son las vacas lecheras que las compañías de inseminación contratan para producir sus becerros. El semen sexado podría ser especialmente útil en los tratamientos de superovulación, en cuyo caso es frecuentemente deseable obtener crías de un sexo u otro para una cruza en particular. Se debe tener cuidado cuando se usa semen sexado para esta aplicación, debido a que típicamente se usa una dosis mayor de semen para inseminar ganado superovulazo, por lo que la producción de embriones será mas baja y mas variable que con semen convencional (Schenk et al., 2005). No obstante lo anterior, las crías producidas serán alrededor del 90% del sexo deseado, por lo que aún si la producción de embriones se redujera en un tercio, esta aplicación puede ser apropiada.

Una preocupación que concierne al uso de semen sexado en ganado lechero, es que cuando su uso se generalice, las crías hembras serán producidas en números excesivos y su valor va a declinar. Esto puede ser solventado de alguna forma si el semen sexado es usado principalmente en vaquillas; en este caso, muchas vacas de mas edad y de menor valor genético podrían ser inseminadas con toros de carne (con semen sexado o no sexado) para producir animales cruzados lechero x carne para su uso como animales de engorda. Por ejemplo, animales cruzados Charolais x Holstein son excelentes para crecimiento y engorda, especialmente los machos y podrían fácilmente ser vendidos a mejor precio que un becerro Holstein. Otra aplicación obvia es incrementar el número de becerras cuando se desea aumentar el tamaño del hato. La expansión del hato podría ocurrir mas rápidamente y posiblemente sin la necesidad de comprar hembras. Esto puede ser muy valioso desde el punto de vista de la bioseguridad y posiblemente desde el punto de vista genético también. Se realizó recientemente un análisis económico de la tecnología de semen sexado (Seidel, 2003a) y algunas ideas sobre como esta tecnología podría encajar en la industria de la inseminación artificial han sido resumidas (Seidel, 2003b).

Una aplicación especial del semen sexado es en la fertilización in Vitro. Una dosis de semen puede ser usada para producir muchos embriones. Desde luego que esta aplicación es pertinente si un programa de fertilización in Vitro se justifica por otras razones. Una de ellas podría ser la de producir embriones para incrementar la tasa de preñez bajo condiciones cálidas y húmedas. Hansen and Block (2004) han demostrado que se obtienen mas elevadas tasas de preñez con transferencia de embriones que con inseminación artificial en vacas Holstein bajo esas condiciones en el sureste de los Estados Unidos.

Posibilidades futuras

Aunque no existen métodos alternativos promisorios de sexado de semen disponibles en la actualidad, es muy posible que eventualmente alguien va a desarrollar un método de sexado masivo de semen en paralelo, de manera que la inseminación con dosis bajas no sea una limitante. Esto haría también el sexado de semen menos caro.

Un desarrollo reciente es la fabricación de un separador de semen con múltiples boquillas, en lugar de una. Esto equivaldría a tener un motor de 8 cilindros en lugar de uno de un cilindro. Si esto se desarrolla a nivel comercial, ciertamente aceleraría el proceso del sexado de semen y muy probablemente reduciría los costos. El costo actual de una dosis reducida de semen sexado en la mayoría de los países en que se produce es usualmente mas del doble del costo de una dosis normal de semen congelado, proveniente del mismo toro.

Referencias

Den Daas, J.H., DeJong, G., Lansbergen, L.A.M. and Van Wagtendonk-DeLeeuw, A.M. 1998. The relationship between the number of sperm inseminated and the reproductive efficiency of individual dairy bulls. J. Dairy Sci. 81:1714-1723.

Garner, D.L. and Seidel, G.E. Jr. 2003. Past, present and future perspectives on sexing sperm. Can. J. Anim. Sci. 83:375-384.

Hansen, P.J. and Block, Jr. 2004. Towards an embryocentric world: The current and potential uses of embryo technologies in dairy production. Reprod. Fertil. Develop. 16:1-14.

Johnson, L.A., Flook, J.P. and Hawk, H.W. 1989. Sex preselection in rabbits: Live births from X and Y sperm separated by DNA and cell sorting. Biol. Reprod. 41:199-203.

Schenk, J.L., Suh, T.K. and Seidel, G.E. Jr. 2005. Embryo production from superovulated cattle following insemination with sexed sperm. Theriogenology 64:in press.

Seidel, G.E. Jr. 2003a. Economics of selecting for sex: The most important genetic trait. Theriogenology 59:585-598.

Seidel, G.E., Jr. 2003b. Sexing mammalian sperm – Intertwining of commerce, technology and biology. Anim. Reprod. Sci. 79:145-156.

Seidel, G.E. Jr. and Garner, D.L. 2002. Current status of sexing mammalian spermatozoa. Reproduction 124:733-743.

Tubman, L.M., Brink, Z., Suh T.K. and Seidel, G.E. Jr. 2004. Characteristics of calves produced with sperm sexed by flow cytometry/cell sorting. J. Anim. Sci. 52:1029-1036.