Proceedings, Applied Reproductive Strategies in Beef Cattle
November 12 and 13, 2005, Texas A&M University, College Station

FROM WISHFUL THINKING TO REALITY: "SEX SELECTION OF SPERMATOZOA"

Juan Moreno
Genetic Resources International
Navasota, Texas

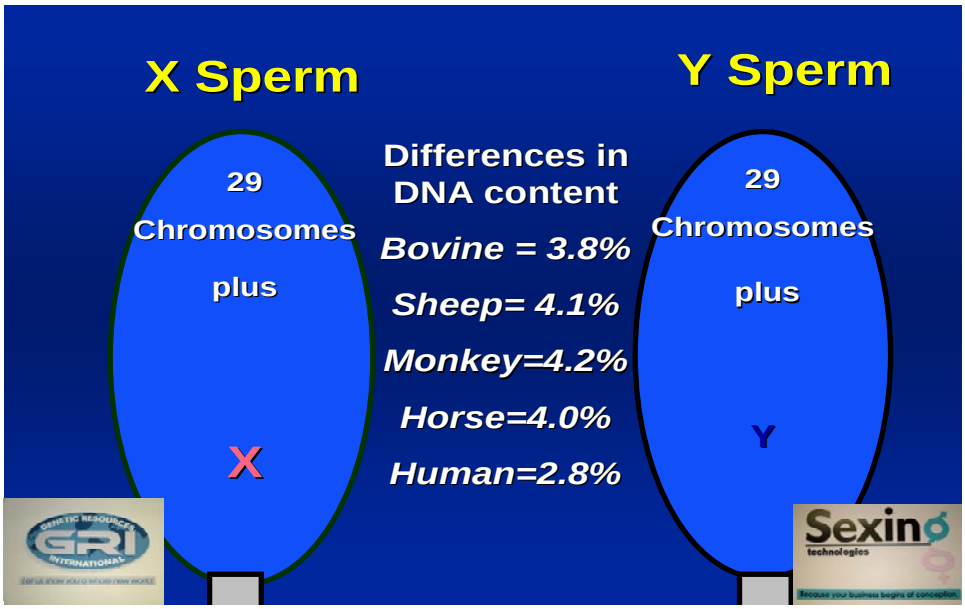
Historical perspective

For thousands of years, mankind has pursued the desire to have reliable, repeatable techniques for sex selection of sperm. In ancient Greece, the philosopher DEMOCRITUS OF ABADERA (460-370 BC) proposed that females originated in the left testicle and males in the right testicle. HIPPOCRATES (460-377BC) proposed the first description of sperm and the theory that strong sperm lead to the development of males and weak sperm to the development of females. In 1677, VAN LEEUWEHOEK used a microscope to describe sperm and new theories of sex determination arouse. It was not until the 1980's and 1990's that the development of sophisticated instruments allowed for an in depth study of the sperm and the scientific proof that sperm carry either the X chromosome for females or the Y chromosome for males. In the same period, the flowcytometer allowed for the separation of sperm based on the X- and Y- chromosome. Finally, in the new century commercialization of sexed semen became a reality.

Mechanisms of sexual differentiation.

The mechanisms of sexual differentiation vary according to species. In some reptiles, temperature depending enzymes regulate the sex of offspring (Dorizzi et al. 1996). While in other reptiles sex is determined chromosomically depending on the combination of the sex chromosomes (ZW vs ZZ) as is seen in lizards and turtles (Coriat et al. 1994). In mammals and avian species, sex determination depends on chromosomal information only. Mammalian sperm carry either the X or the Y chromosome (Jacobs and Strong 1959), and when combined with the X chromosome from the oocyte create an embryo that is ether male or female. Mourizzi in 1979 showed a difference in the size of the X and Y chromosome and Garner et al in 1983 showed on a quantitative basis the difference in DNA content between X- and Y- chromosome bearing sperm.

The simplest, most economical, and most efficient way to influence sex ratios in offspring is to determine the sex before fertilization and therefore to separate the populations of X and Y chromosome bearing sperm. Thousands of attempts and reports have been published regarding different methods to determine the sex of sperm and techniques to separate X- and Y- chromosome bearing sperm. Among the reported techniques were velocity, density, electric surface charge, and immunologically relevant structures. None of those techniques have proven repeatable and accurate. The only technology that has proven repeatable and accurate is the flow-cytometrical separation of X- and Y- chromosome bearing sperm based on the difference of DNA content.



Techniques to identify sex-related characteristics of spermatozoa.

The path to the development of today's current sperm sexing technology

A chronological series of events lead to the development of today's proven, repeatable technology to separate sperm based on the size of the X- and Y- chromosome bearing sperm by flowcytometry.

a. Difference in DNA content of X- and Y- chromosome bearing sperm

Moruzzi 1979

Showed that the X chromosome carries more DNA than the Y chromosome and autosomal cells have identical DNA content.

b. Flowcytometry and sperm sorting

Sprenger et al 1971 and Gledhill et al 1976

First experiments of flowcytometric analysis of sperm with no success.

Pinkel et al 1982

Modified the injection tubes in a flowcytometer to allow for better orientation in front of a laser beam.

Garner et al 1983

First report of detecting DNA content differences in sperm with a flowcytometer.

Bull 3.8% ; Boar 3.7%
Ram 4.1% ; Rabbit 3.9%

Johnson and Pinkel 1986

Added a second detector to a flocytometer and developed a beveled tip for the injection tube.

Johnson et al 1987 and Johnson et al 1987b.

Start using Hoescht 33342 as the stain of choice to selectively bind to the chromosomes and be able to measure DNA differences with a flocytometer. Process very slow 55 sperm/second.

Johnson and Clarke 1988

Show sperm decondensation and pronuclear formation of sex selected sperm by flowcytometry. Do sperm injection in Hamster oocytes.

Morrell et al 1988

Reports the births of the first animals with sorted spermatozoa.

Johnson et al 1989

Report the birth of offspring and gender selection accuracy of 94% and 81% for animals inseminated with X- and Y- chromosome bearing sperm.

Cran et al, 1993

Reports the first use of sex sorted sperm in in-vitro fertilization

Seidel et al 1997

Reports the use of low dose insemination with sex sorted semen

Rens et al 1998

Creates an improved orientation nozzle, high speed flowcytometers are introduced and speed goes up to 30,000 evens per second with a 90% plus degree of accuracy.

Schank et al 1999

Reports the freezing of sex sorted semen with pregnancy results.

COMMERCIALIZATION

XY, Inc 1996

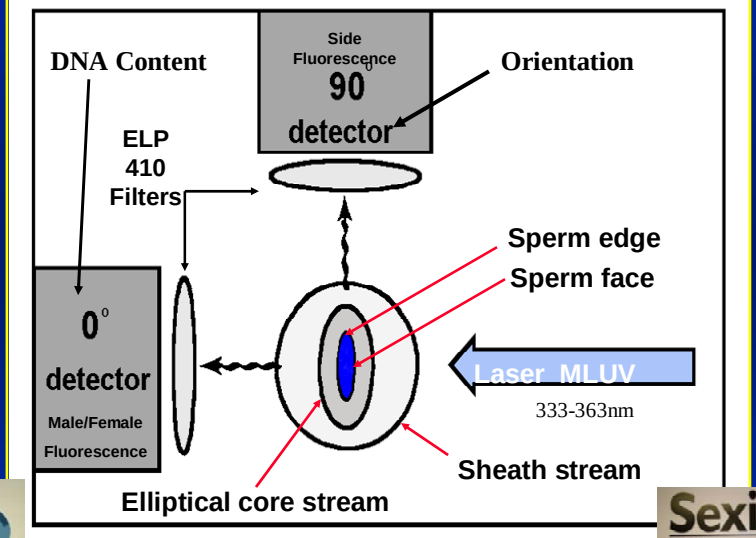
Leads the development of new sperm preparation procedures, handling, medias, and freezing that lead to the use of sex sorted semen in AI, IVF and ET with a high degree of success.

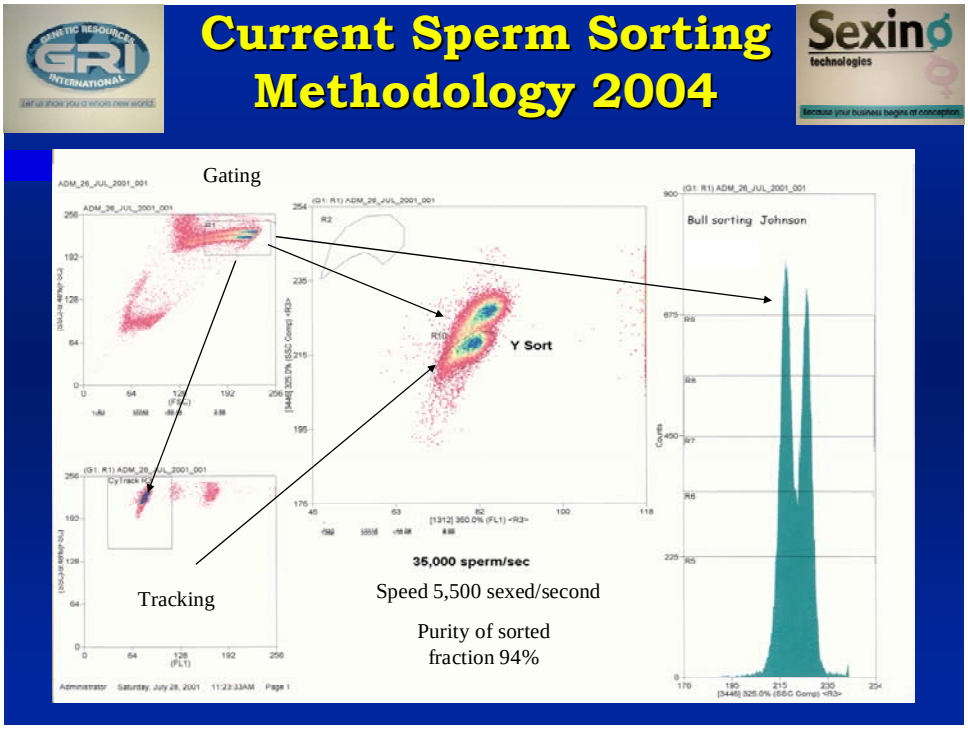
Introduces licensing of its patented technology to the market and leads to the first commercial applications of sex sorted semen:

1. Cogent in England
2. Goyaike in Argentina
3. Sexing Technologies in the US and Brazil

MoFlo® SX

Fluorescence detectors





RESULTS

PREGNANCY RATES

In Artificial Insemination

BULL	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***Total***
DAIRY									
A	***38%***	***30%***	***41%***	***43%***	***42%***	***42%***			***39%***
B	***46%***	***15%***	***58%***	***78%***	***25%^***	***50%***			***50%***
C	***51%***	***51%***	***54%***	***53%***	***12%***	***31%***			***42%***
D	***51%***	***53%***	***56%***	***48%***	***62%***	***47%***	***55%***		***53.5%***
E	***39%***	***62%***	***27%***	***57%***	***52%***	***55%***	***51%***	***48%***	***54%***
F	***10%***	***20%***	***17%***	***32%***	***25%***	***33%***			***24%***
G	***64%***	***62%***	***71%***	***58%***	***49%***	***71%***	***66%***	***55%***	***61.5%***
Total									***52.5%***

All artificial Inseminations were performed with straws of 2 million sperm in virgin dairy heifers. The data se contains more than 10,300 AI's

Sexed Semen In Embryo Transfer

Embryos (1245) were produced with female sexed semen from Brahman and Nelore females during the spring- summer of 2005. Different concentrations of semen and times of artificial inseminations were tested to determine the best protocol. The Heat Watch system was used for heat detection. Embryo average per donor rangeed from 3.2

embryos to 7.5 embryos depending on treatment. Embryos (1032) were transferred fresh for an average pregnancy rate of 68%. Ultrasound fetal sexing on a random group of recipients provided an average of 91% female fetuses.

Sexed Semen in In Vitro Fertilization

During the Spring and Summer of 2005, sexed semen was used in in vitro fertilization trials in Brazil and the United States. Sexed semen samples from 23 different bulls were used. Breeds represented included Brahman, Nelore, jersey, Holstein, Brown Swiss, Gyr, Guzerat.

4324 oocytes were matured
2248 oocytes divide to two cells
1513 embryos were produced

Development to Blastocyst/transferable embryos was 35.2%. Pregnancy rate of the embryos was 48.3% and 93.1 % of the embryos were female.

Economic Analysis

Advancements in sexing procedures have allowed the commercialization of sexed semen at commercially viable prices. Straws of sexed semen in dairy bulls sell for between $35.00 and $60.00 per straw, depending on the bull. For beef sires, sexed semen prices range from $30.00 to $450.00 per straw, depending on the bull.

Dr. Bob Everett of Cornell University has developed an economic analysis spread sheet calculation for the value of sexed semen. The producer can input all of his costs, pregnancy rates, value of their calves, etc and determine if sexed semen is economically viable for his or her operation. We will provide for those interested a copy of that calculation at the time of the conference.

REFERENCES

Coriat, A.M., Valleley, E., Ferguson, M.W., and Sharper, P.T. Chromosomal and temperature-dependent sex determination: the search for a conserved mechanism. J. Exp Zool. 1994; 270:112-116

Cran DG, Johnson, LA, Miller NGA, Cochrane D, Polge C. Production of bovine calves following separation of X- and Y- chromosome bearing sperm ad in vitro fertilization. Vet Rec. 1993; 132:40-41

Dorizzi, M., Richard-Merceier, N., and Pieau, C. The ovary retains male potential after the thermo sensitive period for sex determination ion the turtle Emys orbivularis. Differentiation 1996; 60: 193-201

Garner DL, Glendhill BL, Pinkel D, Lake, S, Stephenson D, Van Dilla MA, Johnson LA. Quantification of the X- and Y- chromosome bearing spermatozoa of domestic animals by flow cytometry. Biol Reprod. 1983; 28:312-321

Jacobs, P.A., Strong, J.A. A case of human intersexuality having a possible XXY sex-determining mechanism. Nature. 1959; 348:452-454

Johnson, L.A., Pinkel, D. Modification of a laser-based flow cytometer for high resolution DNA analysis of mammalian spermatozoa. Cytometry. 1986: 268-273

Johnson, L.A.., Flook, J.P., Look, M.V., Pinkel, D. Flow sorting of X and Y chromosome-bearing spermatozoa into two populations. 1987b. Gamete Res. 16, 1-9.

Johnson, L.A., and Clarke, R.N. Flow sorting of X and Y Chromosome-bearing mammalian sperm: activation and pronuclear development of sorted bull, boar, and ram sperm microinjected into hamster oocytes. Gamete Res. 1988; 21: 335-343

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

Morrell, J.M., Keeler, K.D., Noakes, D.E., Mackenzie, N.M., and Dresser, D.W. Sexing of sperm by flow cytometry. 1988. Vet Rec 122: 322-324

Moruzzi, J.F. Selecting a mammalian species for the separation of the X- and Y-Chromosome-bearing spermatozoa. J Reprod Fertil. 1979; 57: 319-323

Pinkel, D., Lake, B.L., Gledhill, B.L., Van Dilla, M.A., Stephenson, D., and Watchmaker, G. Cytometry. 1982 3: 1-9. High resolution DNA content measurements of mammalian sperm.

Rens, W., Welch, G.R., and Johnson, L.A. A novel nozzle for more efficient sperm orientation to improve sorting efficiency of X and Y chrosome-bearing sperm. 1988. Cytometry 33: 476-481.

Schenk JL, Suh TK, Cran DG, Seidel GE Jr. Cryopreservation of flow sorted bovine sperm. THERIOGENOLOGY. 1999; 52:1375-1391

Seidel GE Jr. Sexing mammalian spermatozoa and embryos- state of the art. J Reprod Fertil 199a;(suppl 54):475-485

Seidel GE Jr. Commercializing reproductive biotechnology- the approach used by XT,inc. Theriogenology 1999b;51:5

Seidel GE Jr, Johnson LA, Allen CH, Welch GR, Holland MD, Brink Z, Catell MB. Artifitial insemination with X- and Y- bearing bovine sperm. Theriogenology 1996a;45:309

Seidel GE Jr, Allen CH, Johnson LA, Holland MD, Brink Z, Welch GR, Graham JK, Cattell MB**.** Uterine horn insemination of heifers with very low numbers of nonfrozen and sexed spermatozoa. Theriogenology. 1997;48:1255-1264

Seidel GE Jr, Allen CH, Brink Z, Holland MD, Cattell MB. Inseminations of heifers with very low numbers of frozen spermatozoa. J Anim Sci. 1996b;74(supple 1);235

Sprenger, E., Bohm, N., Sandwritter, W. Flow fluorescence cytophotometry for ultra-rapid DNA measurements on large. Histochemie. 1971. 26:238-257