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Popular Article

Sexing of Semen in Bovine

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Introduction

Improving the genetic value of dairy and beef animals initially through traditional methods later improved by new technology. Artificial insemination plays a key role in improving dairy herds, as it requires the selection of an elite bull with good genetic potential and semen free of various diseases. A new technology has been introduced in artificial insemination, namely semen sexing. Sex determination of sperm depends on the amount of DNA. The DNA concentration in the X and Y chromosomes varies both within species and between species of different breeds. There are a number of methods for semen sex determination, of which flow cytometry compares favorably with other methods. Many researchers have attempted to manipulate the sex of offspring before conception (Garner et al., 2008). By sexing the semen, farmers can obtain the desired animals, such as dairy cattle or beef cattle, depending on their needs. Farm development depends on the genetic potential of livestock and genetic potential depends on several factors, of which desired sex may be one of the most important determinants. Recently, sex sorting of sperm using flow cytometry has enabled their inclusion in commercial reproductive management. The use of sex-sorted semen on farms presents several opportunities and challenges. Tubman et al. (2004) reported that sex-sorted semen showed no significant difference in reproductive and productive traits between calves produced with sexed and conventional semen. However, there are some limitations associated with sex-sorted semen: high cost of equipment, lack of skilled manpower, about half of the semen sample is waste, low sorting efficiency, low pregnancy rates, and the process is very slow. However, if the cost of sexing is low enough and fertility is close to normal, sexed semen programs will lead to more efficient milk and meat production.

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Table 1. Difference between X and Y spermatozoa

Parameter	Difference
DNA content	Less in Y sperm
Size	X sperm is larger
Motility	Y sperm is faster
Surface charge	X sperm is negative
Cell surface antigen	H-Y antigen on Y sperm

Basic principles of sexing of semen

Sex-specific sorting of sperm is based on information about the content of DNA genetic material in X and Y chromosomes. The DNA concentration in the X chromosome is higher compared to the Y chromosome. In this way, flow cytometry associates the laser, the different staining of viable and non-viable spermatozoa, and the hydrodynamic force that guides spermatozoa at the moment of reading during the process of separation of X and Y spermatozoa. In addition, the amount of DNA content in the X and Y chromosomes differs in different breeds of cattle (Garner, 2006). The percent difference in X-Y DNA content of sperm nuclei is 4.22 for Jersey, 4.07 for Angus, 4.01 for Holstein, 3.98 for Hereford, and 3.7 for Brahman. These differences are not able to determine fertility after sexing the semen, but they increase the speed and efficiency of sexing semen and must be considered when using flow cytometry. Day-by-day advances in the form of the tip of flow cytometry the positioning of sperm at the moment of passage through the laser, as well as changes in pressure and the type of staining cells, have significantly improved the separation process of gametes X and Y (Garner, 2006). The separation of X and Y spermatozoa is slow, i.e., it moves between 300,000 to 400,000 cells per minute. A sex-sorted sperm straw (0.25 cc) contains only 2.1 x 10^6 cells because sperm are damaged during sex sorting, which impairs fertilization (Garner, 2002).

During sorting, sperm are exposed to laser light and various physical forces, e.g., they exit the sorter at nearly 90 km/h before entering the collection medium. The sorting process results in an extremely dilute sample of 800,000 sperm /ml, and then the sperm is carefully centrifuged to obtain a concentrated sample suitable for packaging and cryopreservation.

Sperm concentration for sex-sorted sperm in reproductive programs

There is commercial availability of sexed semen straw that contains 2.1 x 10^6 cells/dose which is much lower than that from conventional semen (~20 x 10^6 cells/dose). But there is no difference in conception rate in both the cases. However, there is an increase in conception rate when AI is performed with conventionally processed sperm (15 x 10^6 sperm/dose) as presented on table. 2

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Table 2. Sex-sorted sperm (dose) and non sex-sorted sperm (dose)

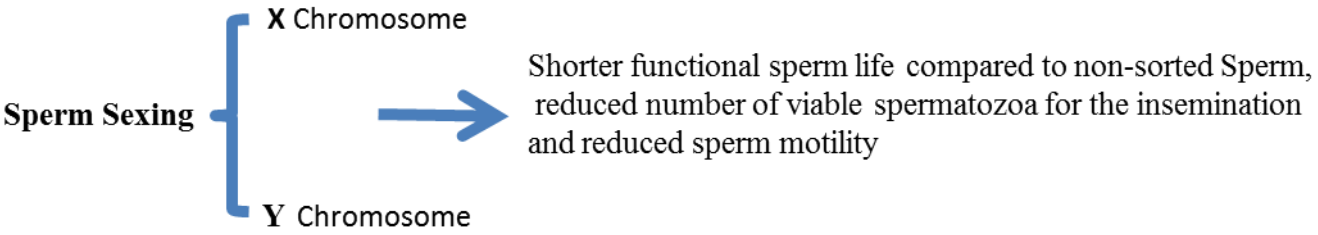
	Sex-sorted sperm (dose)		Non sex-sorted sperm (dose)
	2.1 x 106	3.5 x 106	15 × 106
Heifers (%)	43.9[a]	45.7[a]	60.7[b]
	(2,752/6,268)	(2,864/6,268)	(3,805/6,268)
Cows (%)	23.0[a]	25.4[a]	31.5[b]
	(1,257/5,466)	(1,388/5,466)	(1,722/5,466)

Different superscript letters ([a, b]) in row indicates statistical difference (P < 0.01), (Dejarnette *et al*., 2011) Conception rates Holstein heifers and cows after artificial insemination with 2.1 or 3.5 × 106 sex-sorted sperm or 15 × 10^6 non-sex-sorted sperm.

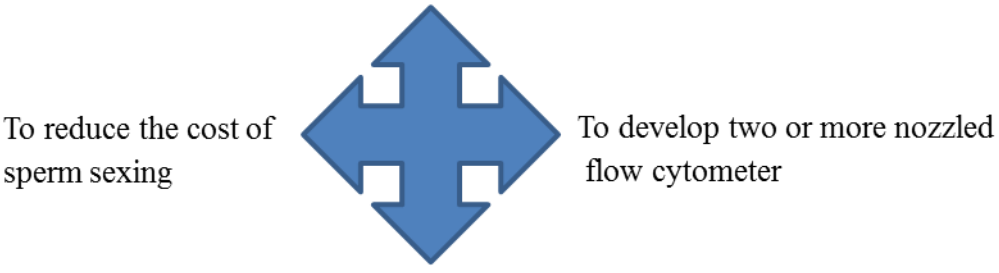
Constraints with Sperm Sexing

The cost of equipment and maintenance is high, and as well a number of skilled persons are required to handle it about half of the sperm sample is un-sexable, sorting efficiency is low, pregnancy rates are low, and the procedure is very slow. In the thermoresistance test, the result for sex-sorted semen was also not good compared to non-sorted semen, because motility decreases faster in sex-sorted semen compared to non-sorted semen.

In addition, part of the effect associated with samples from a particular bull and insemination dose is sex-sorted semen, and some samples from particular bulls may tolerate the stress of sorting in a desirable manner (Seidel and Schenk, 2008). During this process of separating X and Y chromosomes, shorter sperm longevity compared to non-sorted sperm, lower numbers of viable sperm for insemination, and lower sperm motility are the most common problems that can lead to lower conception rates.



Future challenges and possibilities



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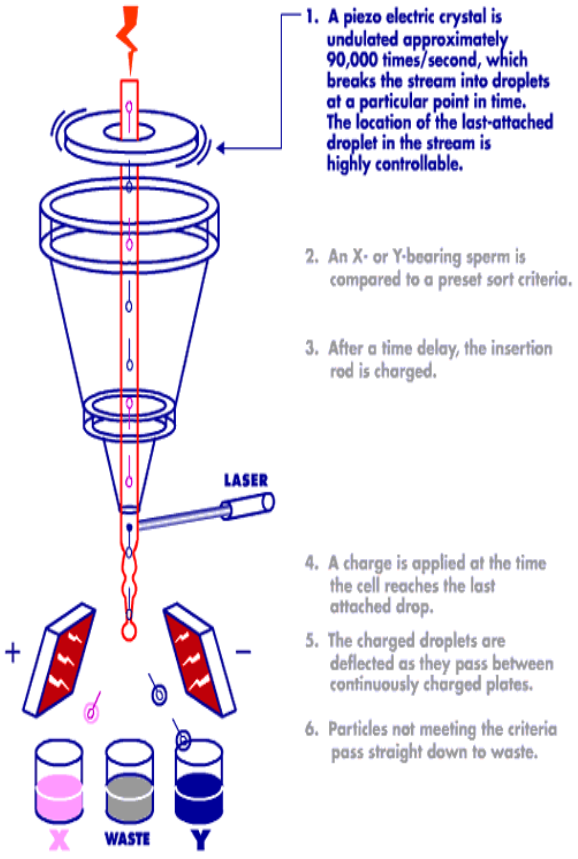
Economical aspect of sexed semen

Marketing of sexed semen has been already started in number of the country. Khalajzadeh *et al*. (2012), have hypothesized that sexed semen may be used to accelerate the rate of genetic gain in dairy herds by selecting only the highest-ranking cows to breed for replacements. Mc Cullock *et al*. (2013) reported economic effects of sexed semen can be used round the year whereas also in seasonal production systems (Hutchinson *et al*., 2013a and 2013b). The economic advantage of using sexed semen depends on function of interactions among the market environment, management practices and technological efficiency (Mc Cullock *et al*., 2013).

Applications sexed semen in Dairy Cattle

Sex-sorted semen increases number of female calves which is helpful to expand the herd or herd replacement as well as increases selection intensity by choosing genetically superior dams of replacement. It reduces the dystocia cases as it produces more number of female calves. For maintaining genetic gains in breeding farm, it is helpful in production of young proven bull. It also uses in *in-vitro* fertilization, superovulation, and embryo transfer programs. The first calves produced with accurately sexed semen resulted from *in-vitro* fertilization (IVF), which requires many fewer sperm than artificial insemination.



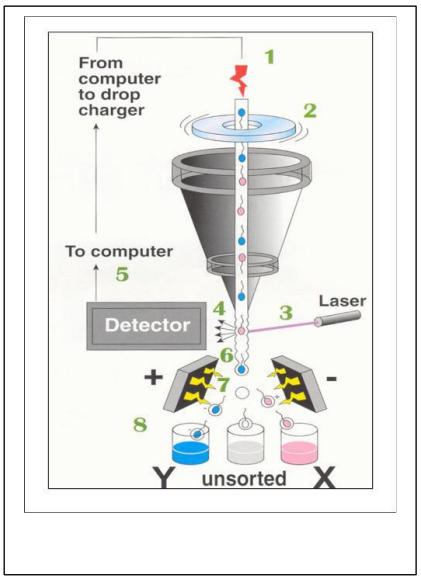


Fig 1 & 2: Flow cytometer (Source: Internet)

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Conclusion

In India, there are a large number of cattle and buffaloes, but not all animals have good genetic value, so the good genetic material is not passed on to the next generation in an appropriate way. Therefore, it is necessary to identify and breed animals with higher genetic potential. Now, there are a number of methods that can be used to maintain good genetic potential on a farm, with sex-sorted semen being the predominant method. Flow cytometry is currently the most reliable and validated method for sexing semen. Sexed semen should preferably be used in heifers because they have higher fertility compared to cows. Sexed semen can be routinely used for embryo transfer and IVF to produce more calves of the desired sex. If used systematically and judiciously, this technique can revolutionize animal husbandry and lead to greater success.

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