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# Sexing of semen in bulls: A mini review

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**Abstract**
Sexed semen has brought a revolution to the dairy industry. It can be used to produce offspring of the desired sex from a particular mating. Dairy farmers can use sexed semen to produce replacement heifers from genetically superior cows. The process of commercialization of sexed sperm has gained momentum recently. Sexing of semen is based on the principle of difference in DNA content between X and Y spermatozoa. X spermatozoa contains more DNA. But, this technology is hindered by many factors like high costs, complexity of operation and lower pregnancy rates than with traditional semen. Despite the odds, sexed semen will contribute to increased profitability of dairy and beef cattle production in terms of milk, meat and other essential products.

**Keywords:** Sexed semen, bulls, flow cytometer, DNA, traditional semen

## Introduction
The latest breakthrough in animal reproduction is sexing of semen. Predetermination of sex in livestock offspring is in great demand and is of great importance for profitable dairy industry. With the changes that have taken place in animal husbandry over the past generation, the application of sex pre-selection to production systems has become increasingly important. Semen from many mammalian species can be sexed by flow cytometry or cell sorting machine at about 90% accuracy without damaging them (Sidel, 2003) [13]. Sexed semen is now gaining popularity amongst the dairy and beef industries and is reaching a point of commercialisation (Seidel, 2012) [14]. Its commercial application is also seen in several other species (Seidel, 2012) [14]. Successful use of sexed semen requires good Managemental practices, proper preservation of semen and requires skilled manpower.

## Why Sperm Sexing?
1) To produce calves of desired sex in both dairy and beef cattle
2) Herd replacement and herd extension can be done very quickly
3) To ensure birth of heifers when progeny testing of young bulls is desired
4) Combination of super-ovulation and insemination with sexed semen further increases the desired calf crop
5) In in-vitro fertilization programmes, one dose of sexed sperms can be used to produce many embryos of desired sex

## 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 |

## Methods of Sperm Sexing
There are many methods of sperm sexing. Important amongst them are as follows:
1) Albumin gradient
2) Percoll density gradient

3) Free-flow electrophoresis
4) Identification of H-Y antigen
5) Flow- cytometry

**Principle of Flow Cytometry**

This is the best technique which is based on difference in DNA content between X and Y spermatozoa. X spermatozoa contains more DNA. Difference in DNA content for most mammals are in the range of 3-4.2% (Johnson *et al*., 2000) [6].

**Historical Perspective**

Gledhill in 1976 first attempted to separate X and Y sperm by analytical flow cytometry. First successful separation of sperm was made in mammalian sperm (Pinkel *et al*., 1982) [12]. Initial methods were found to be destructive because the sperm tails were removed by sonication leaving sperm biologically unusable. So, separation of living sperms are not possible with the initial methods. Modified cell sorter was produced by bevelling the sample injection tip within the nozzle of flow cytometer and developed it into a commercial cell sorter.

**How to measure DNA?**

The DNA content of the sperm is determined using a DNA specific fluorescent dye Hoechst -33342 (a DNA binding fluorochrome [2-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2, 5-bis-1H-benzimadazole-trihydrochloride). The stain penetrates the sperm membrane and binds to A-T region of nucleic acids. The X-sperm ends up with more dye bound to their DNA than Y-sperm. The dye fluoresces when exposed to low wave-length laser beam (5-watt water cooled argon laser).The fluorescence is measured by a detector and analysed by a computer. Since X-sperm has more DNA, it ends up with more dye than Y-sperm and it gives off more fluorescence.

**Speed of cell sorting**

The speed of cell sorting machine as reported by Seidel *et al*., 1999 are as follows:

1) Standard speed system: 35000 cells/h
2) High speed cell sorters : 15 million cells/h
3) Accuracy of sorting is 85-95%

**Pregnancy rate with sexed semen**

The use of sexed semen resulted in 45% conception in heifers and 28% in cows (Vries *et al*., 2008) [1]. Higher pregnancy rate was obtained in heifers for sexed cryopreserved semen with 7-20 times more sperm per dose when deposited in uterine body (Seidel *et al*., 1999) [15]. Pregnancy rate in heifers for 2×10 6 sexed sperm per insemination was 56% and in control with 10×10 6 unsexed sperm was 61% (Garner and seidel, 2003) [2]. In some cases pregnancy rate for sexed sperm was about 60-80% of those found with unsorted control sperm when sperm per dose were same for both. Conception rate of 69.7% (30/43) for sexed sperm and 66.5% (1545/2325) for unsexed sperm following AI was reported in china (Lu *et al*., 2010) [8]. Use of sexed semen has reduced dystocia cases in heifers. Offsprings from seven mammalian species primarily cattle have been produced after H33342-staining and flow sorting of fertilizing spermatozoa, but no gross abnormalities have been produced (Seidel *et al*., 1999) [15].

**Factors affecting the sorting efficiency of flow cytometer**

1) Orientation of sperm head
2) Angle of sperm presentation towards the excitation source, 450 is adequate
3) Orientation of fluid in the nozzle
4) Even with the correct orientation of cell and fluid 20-40% of live sperms are not measurable, passes directly to the waste tube
5) Optical techniques
6) Speed of computer processor

**Problem of sperm sexing**

Problem of sperm sexing is the high cost of equipment, high cost of maintenance, lack of skilled manpower, about half of the sperm sample is unsexable, low sorting efficiency, low pregnancy rates and the process is very slow.

**Future challenges and possibilities**

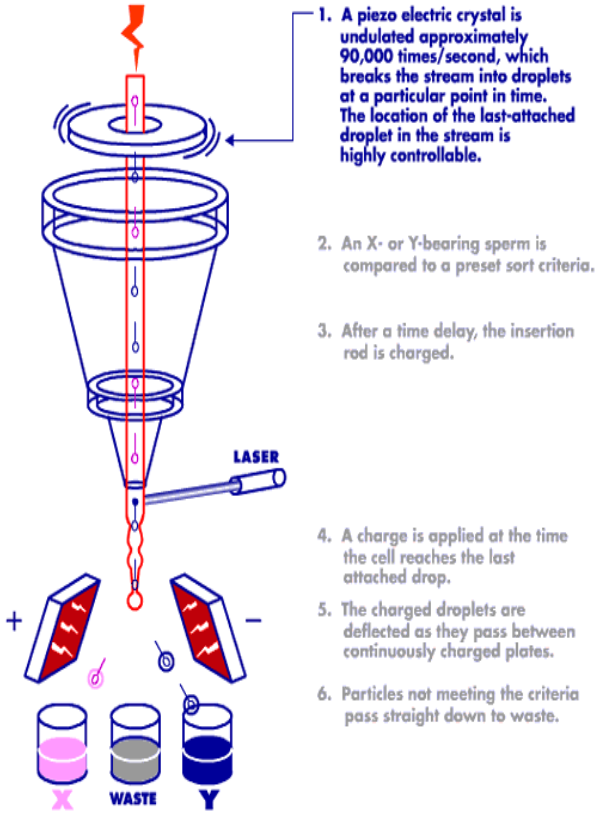
1) To reduce the cost of sperm sexing
2) To develop two or more nozzled flow cytometer
3) Isolating a protein marker characteristic of X or Y sperms

**Utility of sexed sperm in Indian context**

Utility of sexed sperm in Indian context has tremendous scope. Production of unwanted dairy cattle males can be eliminated or minimized as they cannot be slaughtered in India. It can facilitate production of required number of daughters for progeny testing programme in shortest time, thus it will increase genetic gain. Production of breeding bulls can be conserved as country has limited elite cattle and buffaloes (< 0.1% of total).

**Economics aspect sexed semen**

Commercialisation of sexed semen has already been started in many parts of the world. Khalajzadeh *et al*., 2012 [7] have hypothesised 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. The economic effects of sexed semen can be used round the year (Seidel, 2003; Olynk and Wolf, 2007; McCullock *et al*., 2013) [13, 11, 9] and also in seasonal production systems (McMillan and Newman, 2011; Hutchinson *et al*., 2013a and 2013b) [10, 4, 5]. The economic advantage of using sexed semen is the function of interactions among the market environment, management practices and technological efficiency (McCullock *et al*., 2013) [9].



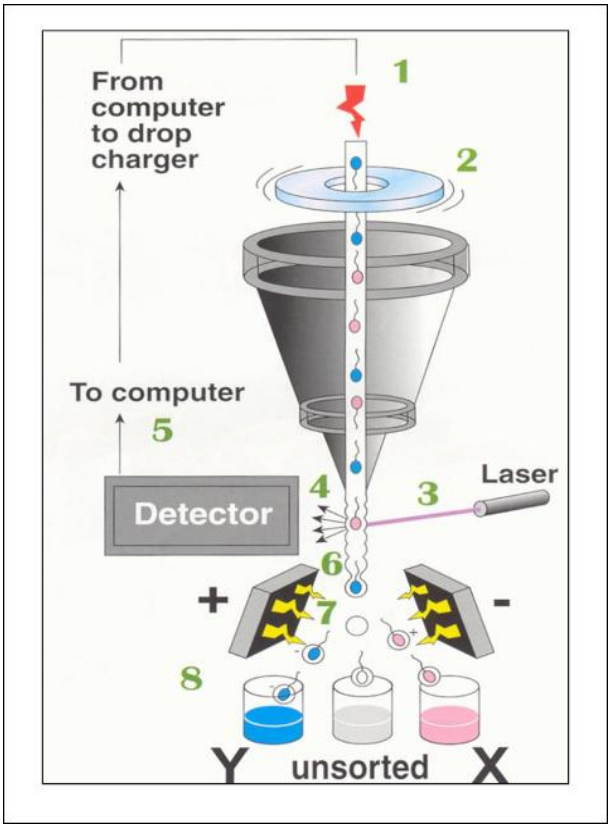




**Fig 1 & 2:** Flow cytometer (Source: Google image)

## Conclusion

Sexed semen is obtained by the application of many sorting techniques. Of these, Flow Cytometry technique is the most reliable and fully validated method at present for sperm sexing. Beside cows, sexed sperm should preferably be used on heifers which have inherent high fertility. Sexed semen can be routinely used for embryo transfer and IVF to produce calves of desired sex. This technique if used judiciously in a systematic manner can bring revolution in the field of animal husbandry to a greater height.

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