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Technical **Article**

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Sexed sorted semen in Dairy Industry

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Introduction

For many years, animal scientists and livestock owners have dreamed of having a calf of their desired sex. In the case of cows, pre-conception selection for a particular sex is typically economically justified. Due to the mechanisation of agriculture, male cows are raised by livestock owners less frequently, and when they do, the animals are either slaughtered—which is illegal in the majority of Indian states—or left to starve to death. So, we can use sex-sorted semen technology to protect ourselves from this unusual predicament of having "unwanted" male calf born (Gaur *et al*., 2020). Sexed semen, also called Gender Enhanced Semen (GES) has been commercially available in the Indian dairy industry for approximately six year. This technology is being used on a limited basis, and there seems to be considerable interest in this new tool on dairy farms. There was no reliable way to identify the sex of sperm cells before the 1980s. At the Lawrence Livermore National Laboratory, Dr. Daniel Pinkel created the first sperm sorting technology. Additionally, Dr. Pinkel created the first flow cytometer. Researchers from Oklahoma State University and Lawrence Livermore National Laboratory showed that flow cytometry could distinguish between X and Y sperm based on changes in DNA content (Loggan, 2019). The system was later patented under the name "**Beltsville Sperm Sexing Technology**," and in 2001 Sexing Technologies (ST), Texas, acquired a licence to utilise it commercially in the United States. In many European states, the USA, Canada, Mexico, Brazil, China, and Japan, among others, Sexed semen now commercially produces sex-sorted semen.

Development of Sexed semen

By identifying differences between sperm that carries X and Y sperm are sorted. In cattle, the **X-chromosome (female)** has nearly 3.8% more DNA than the **Y-chromosome (male)**. The X- and Y-bearing sperm are separated using the difference in DNA content.

Table: Functional changes between X and Y sperm

Parameters	**Difference measurable**
DNA content	Less in Y sperm Size X sperm is larger
Fluorescence property	X chromosome is more fluorescence
Motility	Y sperm faster
Surface charge	X sperm migrate towards cathode
Cell surface	Antigen H-Y antigen on Y sperm

Johnson, 1995

Methods of sex sorting technique

1. Albumin gradient/Gradient swim-down method
2. Percoll density gradient method
3. Swim up procedure
4. Free flow electrophoresis
5. Identification of H-Y antigen
6. Sperm sexing based on the volumetric differences
7. Centrifugal counter current distribution
8. Immunological sorting of semen
9. **Flow-cytometric sorting of semen**

Out of all the above-mentioned methods, flow-cytometric sorting of semen is the most dependable and reproducible technique for producing sex-preselected animals is to sex X and Y chromosome containing sperm based on the difference in DNA content. Because the sperm exhibited random orientation in the flow-cytometer fluid stream, the previous investigations were unable to detect any differences in the DNA quantity between X and Y carrying sperm (Johnson and Welch, 1999). Pinkel *et al*. (1982) reported the first sperm flow sorting to separate X from Y-bearing spermatozoa

The most efficient way for semen sexing has been found to be flow cytometry-based sorting. Over the years, the technology has been improved to the point that sex sorting can now be done with a purity of more than 90%. The method has been successfully patented, standardised and commercialised in the USA, Europe, and other nations. The DNA content of the sperm is measure by fluorescent dye Hoechst -33342 (a DNA binding fluorochrome [2-(4-ethoxyphenyl)-5-(4-methyl-1- piperazinyl)-2, 5-bis-1H-benzimadazole-trihydrochloride). This dye penetrates the sperm membrane and then bind, the site where A-T region of nucleic acids are present. X-sperm binds more dye to their DNA than Y-sperm. When the low wavelength laser beam are exposed to these sperm cell then X-sperm cell gives off more fluorescence because it contain more 3-4% more DNA content as compared to Y-sperm. The charge is applied to the droplet having desired cells and these are subsequently deflected into the required population of the sexed cells resulting in high purity and viability.

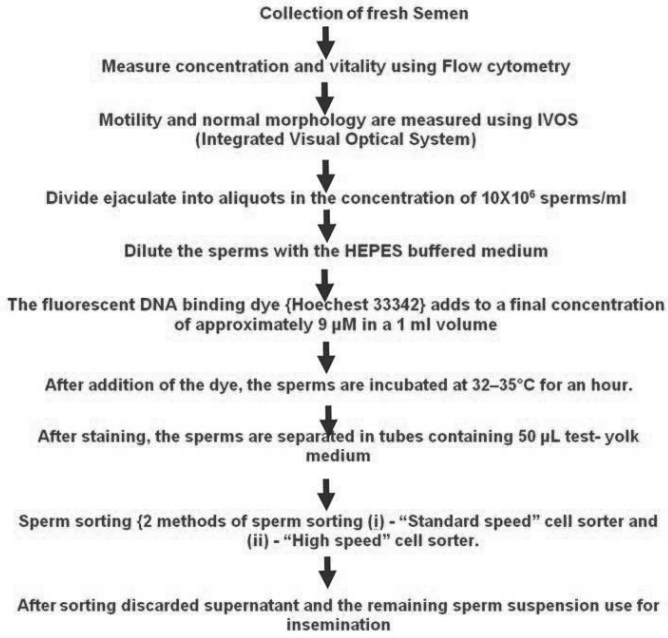

Fig 1. Flow chart showing step wise procedure of sex sorting using flow cytometry

(Source : Sexed semen: challenges and opportunities for Indian dairy industry **By Dairy Planner**

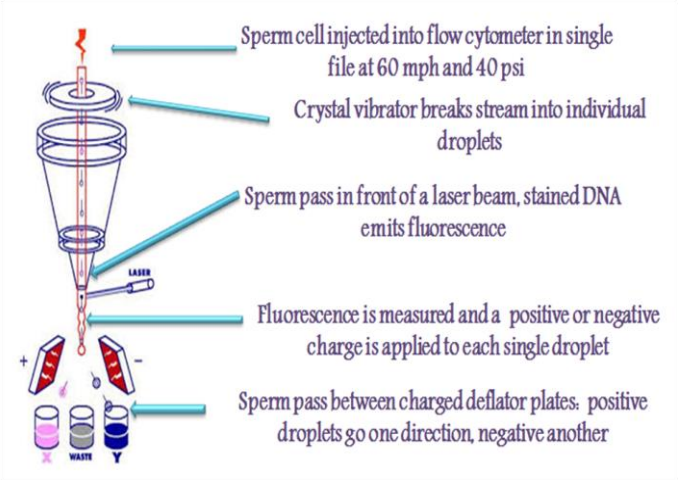

Fig.2 Schematic Diagram of Sex Sorting using Flow Cytometry. (Source: Dairy Knowledge portal, NDDB)

Application of sex sorting technology

1. Farmers are able to conserve resources by producing exclusively female calves rather than undesired males.
2. **Improved biosecurity**: Utilising sexed semen to produce more replacement heifers may be a benefit for herd expansion and also for improved biosecurity (Weigel, 2004). Importing heifers from outside is a cost-effective way to strengthen the herd without running the threat of spreading diseases.
3. **Increased rate of genetic gain**: The use of sexed semen should increase the rate of genetic progress, if fewer cows are required to breed the next generation of heifers. There could, however, be increased problems with inbreeding. Therefore, there is a need to calculate changes in annual genetic progress with varying use of sexed semen for a range of traits including those pertaining to health and welfare.
4. The dairy farmers can raise the herd more quickly and internally with the application of sexed semen. The amount of undesired male dairy calves is also reduced owing to this technology.
5. Heifers are known to benefit from the technology more. Compared to heifer calves, dairy male calves are more likely to have dystocia. Dystocia cases can be reduced with the use of this technology. Dystocia expenses in first-calving heifers may be 20% lower when female sexed semen is used (Seidel, 2003)
6. Only viable sperm are available because dead, dying, or damaged sperm cells are eliminated during the sorting process, allowing the sexing of semen to be successful even at low concentrations than conventional semen.
7. **Shortening gestation length**: A cow that calves late in the spring will have a longer dry period (higher cost) and a shorter lactation (lower income) than a cow that calves at the beginning of the calving period in late winter/early spring. Lactation length is one of the major factors influencing the profit generated per individual cow in seasonal calving systems. Depending on geographical variations in the price of milk and beef, there may be a relative economic benefit to either shortening the gestation period or increasing the beef yield from the dairy herd. (Ashutosh Mishra *et al*., 2023)

Limitations of using sexed semen

1. Sex-sorting equipment is expensive
2. Inadequate sorting speed and efficiency
3. Operate sex sorting equipment only with highly skilled professionals.
4. Shear force, electrostatic charge, droplet production, and abrupt stop all cause harm to the sperm. Approximately 50% of sperm is wasted
5. Decreased ability of the sorted sperm to be frozen
6. Excessive price for the product, which also includes the cost of the IP (Rs. 1500-4500 per dose verses Rs. 15-20 per dose for conventional semen).
7. Sex-sorted semen has a 10–15 percent lower conception rate than conventional semen. Given the poor artificial insemination coverage (20–25 %) and low artificial insemination conception rate in India, this element will be much more important (25-35%).
8. The process of insemination with sexed semen is not standard. This is a further cause for concern because conventional semen contains 20 million sperm each dosage, whereas sexed semen has 2 to 4 million sperm per dose. Under Indian conditions, managing lower sperm concentration will be challenging.

Availability of sexed semen in India

The first and only bovine semen sexing lab in India is the Sex Sorted Semen produced by the Brahma Genetics Facility. Sexed ULTRA technology, BAIF is available and is manufacturing and distributing sexed semen for Holstein, Jersey, Sahiwal, Gir, Red Sindhi, Crossbreeds, and Murrah and Mehsana buffaloes. In 2017, ABS India implemented Genus IntelliGenTM technology at Brahma, near Pune in Maharashtra.

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