

## Semen Sexing: Technique, Scope, and Constraints

**Prachi Sharma<sup>1</sup> and Anjali Arya<sup>2</sup>**

<sup>1</sup>Department of Veterinary Gynaecology and Obstetrics, <sup>2</sup>Department of Livestock Production and Management, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand -388001, Gujarat  
<https://doi.org/10.5281/zenodo.7315692>

### *Abstract*

The practice of sexed semen in livestock production and genetic improvement has shown to be achievable with variable degrees of efficiency during several species and proved to be economically viable in cattle. Sexed semen is a reproductive technology intended to change the sex ratio of the offspring toward a preferred gender. Though along with various advantages, this technology has some constraints also, as discussed in this article, these can be overcome by using suitable practices for optimizing conception rates of sex-sorted semen.

**Key Words-** Sexed semen, flow cytometry, DNA content, sperm, conception rate

### **Introduction**

Since the beginning of written history, there has been a great deal of interest in pre-selecting the gender of progeny in both humans and animals. Theories for controlling the sex of offspring have been established since the era of the Greek philosophers when Democritus, 470-402 BC, suggested that the right testis produced males, whereas the left testis produced females. Some people thought that males and females formed on the right and left sides of the uterus, respectively. Scientific efforts in the right direction started early in the 20th century when the first documented microscopic identification of sex chromosomes was reported by Guyer. One of the first sensible scientific studies to be conducted to control prenatal sex was reported in 1925. The basis for this research was the possible differential density of X- and Y- bearing sperm in the rabbit. Later, many physical methods of separation of X- and Y- chromosomes based on differences in the mass and motility, swimming patterns, surface charges,



volumetric differences, centrifugal countercurrent distribution, and immunologically relevant properties were reported but failed to convincingly demonstrate the result of distortion in the sex ratios of animal offspring in a practical sense. The only method that has proven to be commercially viable with promising results to date is flow cytometry /cell sorting depending upon the DNA content difference between X-Y sperm difference.

### **A. Technique**

The sperm sorting procedure involves staining the sperm with a dye (Hoechst 33342) that binds specifically to the DNA. The diluted mixture passes through a flow cytometer in a fine stream, and a vibrating crystal breaks the stream into droplets. The stained sperm are illuminated by 351 and 364 nm lines of an argon laser beam for fluorescing. X-sperm shines more brightly than Y-sperm. A computer measures the sperm's fluorescence and categorizes each sperm droplet as either X, Y, or unsure. The sperm progressively travels through an electromagnetic field where, depending on their allocated collection into distinct test tubes, they are pulled to either the positive, negative or no charge side. The current sorting accuracy is about 90% for each sex. XY LLC and Sexing Technologies' unique technology is used to make commercially accessible sexed (sorted semen) straws, and then separate businesses offer bull studs sexing of semen services.

### **B. Scope of semen sexing technology in India**

Scope of semen sexing technology in India in the Indian context, sexed semen has the following potential applications: 1. To realize the increasing demand for milk production. To meet the increasing demands, it is necessary to substantially increase the number of elite females which can be achieved by shifting the sex ratio towards females. The sexed semen bearing the X chromosome could be used in elite cows to produce superior high-yielding cows at a faster rate than the conventional unsexed semen. 2. In our country there is a shortage of superior breeding bulls. The few available elite cows might be used to make better bulls by adding sexed male sperm. But the superior male can be produced by sex-sorted spermatozoa from the superior dam, which will be a great boost for the semen station which is the need of the hour for increasing the frozen semen productivity in the country. 3. Sexed semen technology ensure a required number of progenies per bull under the progeny testing program, thereby increasing the accuracy of bull testing. The sexed female sperms could be used in test mating to ensure the production of the required number of daughters in the shortest time, thus increasing the genetic gain. 4. Reducing the number of unproductive young bulls. Male calves born by artificial insemination are of limited value as future breeding bulls or bullocks in the agricultural fields as a source of farm power because cow slaughter is prohibited almost everywhere in India. The use of sexed semen can solve the problem of the production of unwanted male progenies. 5. Sexed semen is an excellent way to expand



the dairy herd without spending a large amount on replacement and virtually a breeder need not buy new heifers. Combining sexed semen with assisted reproductive technologies such as multiple ovulation embryo transfer technology, in-vitro fertilization, gamete intra-fallopian transfer, and sperm intra-fallopian transfer might increase the advantages of sexed semen even further. The reduced fertility issue might be solved with the use of these technologies.

## **F. Constraints**

There are certain limitations of sexed semen in India such as 1. The machine's cost is in crores and their royalty should be paid for each dose of semen produced. 2. The technology is not fully commercially available. The firm holding patent for orienting the nozzle does not sell it and it is a major hindrance. 3 Reduced efficiency and speed of sorting. The amount of semen harvested from a bull with good genetic merit will be reduced by 70% thanks to this technology because 30% of the sperms in an ejaculate will be rejected during the sexing process because they can't be detected precisely for the difference in DNA content, and 50% of the sperms that are detected will be Y bearing. 4. The conception rate (CR) is 10-20% lower in sorted sexed semen compared to conventional semen. Numerous variables may contribute to the reduced conception rate in sexed semen, but the main ones are the low dosage rate and physical or chemical stress on sperm during the sorting process. The high dilution rate, dye staining, mechanical pressures, UV laser light beam, greater fluidic pressure during projection into the collecting tube, and centrifugation are some of the sorting stressors. Additionally, the CR in sexed semen is influenced by the location of semen deposition in the uterus. The conception rate is more when sexed semen is deposited in the body of the uterus rather than the horn of the uterus in buffaloes heifers. 5. The *Bos taurus* dairy and beef breeds are the principal beneficiaries of the standardization and manufacture of the sexed semen technology, which is patented and used in western nations. Although it is stated that the technology has been tried and used in *Bos indicus* breeds like Gir in Brazil and that some states have adopted the use of sex semen, its use under the tropical conditions of India needs to be verified since the effects of implementing the technology for the genetic improvement of cattle have not been reported. There is a need to standardize the lower dosage of spermatozoa and site of deposition for AI with a good conception rate under Indian conditions. Moreover, the form of the head is another characteristic that varies across animal sperm. The capacity to correctly orient these gametes at the moment of measurement in the flow cytometer/cell sorter is just as important as the relative variations in DNA content between sperm carrying X and Y chromosomes. As a result, sperm are sorted by sex differently depending on the breed as well as the type of mammal. Hence, standardization of the technique concerning different breeds of indigenous cattle and buffaloes is also required. 6. The alternatives for semen sexing among genetically superior bulls will be restricted by the dearth of exceptional bulls. 7.



For inseminations, skilled and reliable AI technicians are needed. Furthermore, developing such a scheme for tropical developing countries is constrained by small flock size, indiscriminate mating, and absence of pedigree and performance recording. 8. Lack of high-quality ejaculates from local buffalo and cattle.

### **G. Practices for Optimization of the Conception Rate on Using Sexed Semen**

Following practices can be used to improve the conception rate: i. Use sexed semen only in herds where conventional semen routinely has an AI pregnancy rate of 60% or above. ii. Use exclusively with healthy female cyclists (heifer and cows). Make sure that a significant portion of animals was in heat before doing AI while utilizing fixed-time AI. iii. Use only trained AI professionals. When handling, storing, and thawing the straws, use the utmost caution. Above all, skilled and cautious animal management is necessary for the best application of sexing technology (nutrition, disease control, oestrus detection, semen handling, and insemination technique).

### **Conclusion**

In Indian conditions, there is a need to standardize the lower dosage of spermatozoa, the site of deposition for AI with a good conception rate in our conventional system. To make this technology practical in India, more research must be conducted in partnership with other laboratories. Additionally, its widespread usage across the nation is constrained by its high cost and fertility-related issues. So, the main target should be focused on to use of sex-sorted spermatozoa in good quality heifers and cows with excellent reproductive and productive performance to achieve good results. There is also an immense requirement to develop instruments to transfer sex-sorted spermatozoa non-surgically and to train the skilled manpower in the above area to achieve good results. The fact that this technology has so many advantages has led to its enthusiastic acceptance in several Indian regions, and with costs likely to fall, it is also anticipated to flourish further in other places.

