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Sperm sexing in bovine: Focus on methods and conception rate

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Abstract
Sperm sexing is an Assisted Reproductive Technology (ART) that involves the sorting of “X” and “Y” chromosome-bearing live sperm cells of semen samples. It is the method of splitting spermatozoa into two subpopulations of spermatozoa-bearing X-chromosome and Y-chromosome with higher than average concentrations (up to 90%) of either the ‘X’ or ‘Y’ sperm to produce offspring of the desired gender. Sperm sexing technology has 80-90 % accuracy.
The earliest record of historical development is of ancient Greek philosophers like Democritus, around 470–402 BC, who proposed that the right testicle produces males, whereas the left testicle produces females. Afterward many scientist researchers had tried to separate X and Y bearing spermatozoa with variable success rate on the basis of the major differences between the X and Y chromosomes include DNA content, size, motility, surface charges and cell surface antigens, including the albumin gradient procedure, identification of H-Y antigen, free-flow electrophoresis, swim-up procedure, percoll density gradient, volumetric difference, flow cytometry, laser ablation, immunological, and microfluidic based sperm sorting. But among all major breakthroughs in sperm sexing was production of live offspring from sex-sorted, living rabbit sperm using Hoechst 33342 stain at the USDA Beltsville research center group. The flow cytometry is the most successful methods of sperm sexing till date. TLR 7/8 receptor expressed by 50% of round spermatid encoding X chromosomes.
Fertility is generally 10 to 12 % low when using normal concentrations of sexed semen compared with the use of normal concentrations of conventional semen. The production of sexed semen in India is primarily carried out by Sexing Technology and ABS, with these two companies dominating the market. Currently, the sole commercially accessible validated technique for sperm sexing is flow cytometry; however, it is not without its drawbacks, including cost, speed, and the fertility of sorted semen. Advancements in sperm sexing technologies may reduce costs and improve sorting efficiency, making sexed semen more accessible to farmers. Standardization of alternative sperm sorting methods such as immunological, microfluidic, and Raman spectroscopy based techniques is essential to ensure consistent accuracy, sperm viability and scalability for practical use in bovine. Optimization of sperm sorting protocols to minimize cellular damage and improve post-sorting viability can significantly enhance conception rates under field conditions.

Keywords: Sperm sexing, methods, conception rate, bovine

1. Introduction

The global human population is expected to reach 9.7 billion by 2050, placing immense pressure on livestock production to meet the rising demand for milk, meat, eggs, and hides (Gilland, 2002) [26]. Livestock already number over 26 billion worldwide and play a dual role by ensuring nutritional security and supporting the livelihoods of millions of rural households (FAO, 2017) [18]. With increasing incomes, particularly in developing countries, demand for animal products is projected to surge, with meat consumption expected to rise by nearly 80% by 2030 and over 200% by 2050. To meet this challenge, enhancing productivity through selective breeding and advanced reproductive technologies is crucial (Stear *et al.*, 2001) [64]. Artificial insemination (AI) is now widely practiced in large-scale farms, where the preferred sex of offspring is of economic importance female calves are favoured in dairy herds for milk production, while male calves are more profitable in beef industries.

Sperm sexing, an assisted reproductive technology, allows the separation of X- and Y-bearing spermatozoa, enabling production of offspring of predetermined sex with 80–90% accuracy (Joshi & Singh, 2005; Garner *et al*., 2013) [35,]. The use of sexed X-sperm increases the proportion of high-yielding dairy females, while Y-sperm is valuable for producing elite breeding males in beef herds. This manipulation of sex ratio improves herd structure, productivity, and reduces the costs of maintaining unwanted sexes (Seidel Jr., 2007) [57]. However, separation remains technically challenging because X- and Y-bearing sperm are morphologically similar, differing only at the nanoscale (Carvalho *et al*., 2013) [6]. Conventional sexing methods such as density gradient centrifugation, swim-up, Sephadex filtration, and HY antigen-based approaches have shown limited success due to sperm damage, low accuracy, and poor reproducibility. Despite these limitations, sperm sexing is increasingly recognized as a key biotechnological tool to meet the growing global demand for milk and meat, with applications expanding beyond cattle to other livestock species including sheep, pigs, and horses.

History

The desire to control the sex of offspring has intrigued humans for centuries, with early records tracing back to ancient Greek philosophers such as Democritus (470–402 BC), who speculated that the right testicle produced males while the left produced females. In the late 19th century, Geddes and Thomson (1889) [24] published *The Evolution of Sex*, proposing that nutritional status influenced sex determination, with catabolic conditions favouring males and anabolic conditions favouring females. Scientific efforts toward practical sex control began in the early 20th century, when Lush (1925) [43] attempted to manipulate prenatal sex based on the presumed density differences between X- and Y-bearing spermatozoa in rabbits. A significant advancement was made by Gledhill *et al*. (1976) [27], who successfully separated X- and Y-bearing sperm using analytical flow cytometry. This work paved the way for a landmark achievement in 1989, when Johnson *et al*. at the USDA Beltsville Research Center reported the birth of live offspring from sex-sorted rabbit sperm (Johnson *et al*., 1989) [33]. Earlier, Johnson *et al*. (1987a) [34] had demonstrated the successful use of Hoechst 33342 staining in *Chinchilla laniger*, achieving separation of X- and Y-spermatozoa by flow cytometry with up to 95% purity. Advances in molecular biology further expanded the understanding of sex determination, with identification of the sex-determining region Y (SRY) gene as the pivotal factor in male development. Knockout studies of SRY confirmed its essential role, producing phenotypic females in mice (Bergstrom *et al*., 2000) [4] and rabbits (Kato *et al*., 2013) [37].

Sex preselection in mammals is feasible only when the inherent differences between X- and Y-chromosome-bearing spermatozoa are clearly understood. The most consistent and well-documented distinction lies in their DNA content, with X-sperm containing more DNA than Y-sperm. Additional distinctions reported between X- and Y-bearing sperm include differences in cell size, with X-sperm generally being larger (Cui, 1993; Moruzzi, 1979) [9, 46]; motility characteristics, where Y-sperm often display greater motility (Sarkar *et al*., 1984) [55]; variations in surface charge, with X-sperm carrying a negative charge and Y-sperm a positive one (Kiddy and Hafs, 1971) [38]; and the presence of unique cell surface antigens (Hoppe and Koo, 1984) [31]. Among these characteristics, the disparity in DNA content has emerged as the most reliable basis for sperm separation techniques.The differences in DNA content between X- and Y-bearing spermatozoa across different bovine breeds are presented in Table 2.

Table 1: Differences between X and Y Spermatozoa

Parameter	Difference	References
DNA content	X chromosomes contain approximately 3.8 %more DNA	Moruzzi *et al.*, 1979 [46]
Size	X sperm is larger	Cui, 1993 [9]
Motility	Y sperm has higher motility	Sarkar *et al.*, 1984 [55]
Surface charge	X sperm has a more negative charge	Kiddy and Hafs, 1971 [38]
Surface antigens	H-Y antigen on Y sperm	Hoppe and Koo, 1984 [31]

Table 2: DNA content differences between X- and Y-sperm in cattle and buffalo breeds.

Breeds	DNA Content Variation Between X- and Y-Bearing Spermatozoa	References
HF	3.98%	Garner *et al.*, 1983 [23]
Jersey	4.24%	
Angus	4.05%	
Hereford	4.03%	
Brahman	3.73%	
Murrah	3.59%	Lu *et al.*, 2006 [42]
Nili Ravi	3.55%	Lu *et al.*, 2006 [42]

Methods of Sperm Sexing

Different methods used for sexing semen in various animals are follows:

Conventional Methods

Conventional sperm sexing methods, including density gradients, swim-up, and immunological techniques, have been widely attempted, but their efficiency remains inconsistent. Most approaches relied on presumed motility differences between X- and Y-sperm (Shettles, 1960), though later studies showed no significant variation (Grant, 2006) [28]. While these methods are simple, low-cost, and cause less sperm damage, they lack reliability in separating X- and Y-sperm. Recent advances such as nanotechnology, microfluidics, and proteomics are being explored to improve these approaches, though their success is still limited.

1) Albumin gradient

In the albumin gradient method, semen is layered over a discontinuous gradient prepared from human or bovine serum albumin. Due to their smaller size and greater ability to penetrate fluid interfaces, Y-bearing spermatozoa are presumed to migrate faster through viscous and dense layers than X-bearing spermatozoa. Consequently, fractions collected from different gradient layers are expected to be enriched in either Y- or X-sperm. Reported success rates with this approach are around 75% (Beerinik *et al*., 1993; Kumar *et al*., 2017) [3, 41].

2) Percoll Density Gradient (PGC)

In the PGC technique, a discontinuous gradient is generated using colloidal silica (Percoll) with progressively higher densities from the surface to the base (Promthep *et al*., 2016) [50]. During centrifugation, spermatozoa are separated based on density differences: X-bearing sperm migrate into the heavier bottom fractions, while Y-bearing sperm are recovered from the medium-density layers (Hindal *et al*., 2018) [30].

3) Swim up Method

In the conventional swim-up method, semen is placed at the bottom of a tube with culture medium layered above. The tube is inclined at 45° and incubated for about an hour, during which motile spermatozoa migrate into the upper medium. Y-bearing spermatozoa, owing to their lower mass and higher velocity, are reported to move upward more readily than X-bearing spermatozoa (Oseguera-López *et al*., 2019) [48]. A modified swim-up method employing a 30-cm pipette sealed with a rubber stopper has been reported, in which semen is introduced from the bottom, incubated for 45 minutes, and sequential 1-ml fractions are collected. This approach resulted in ~62% enrichment of Y-bearing bull spermatozoa (Azizeddin *et al*., 2014) [2]. Similarly, evaluation of Nili-Ravi buffalo semen using the modified swim-up technique showed enrichment of X-bearing spermatozoa in the bottom fractions and Y-bearing spermatozoa in the upper fractions (Awan *et al*., 2017) [1].

4) Free-flowelectrophoresis

X- and Y-bearing spermatozoa differ in their membrane surface charge characteristics. During electrophoresis, these differences cause distinct migration patterns: X-sperm, which possess a higher neuraminic acid content and thus a stronger negative charge, migrate toward the anode, whereas Y-sperm, carrying a relatively positive charge, move toward the cathode (Mohri *et al*., 1986) [45].

5) Identificationof H-YAntigen

H-Y antigen, a Y chromosome–encoded marker expressed on the surface of haploid cells, is widely observed in male mammalian tissues. This antigen provides a basis for distinguishing Y-bearing sperm (H-Y^+) from X-bearing sperm (H-Y^-) (Hoppe and Koo, 1984) [31]. The use of monoclonal antibodies targeting H-Y antigen has been explored for the immunological identification of Y-spermatozoa (Kumar *et al*., 2017) [41].

6) VolumetricDifferences

Variations in head volume between X- and Y-bearing spermatozoa can be evaluated through Differential Interference Contrast (DIC) microscopy, which allows measurements to be made without staining by using visible light at a wavelength of 550 nm (Van Munster, 2002) [68].

Commercial methods

Fluorescence-activated cell sorting (FACS) is the only commercially viable method for sperm sexing, as conventional techniques like Percoll gradients, albumin gradients, and swim-up have failed to produce consistent results (Espinosa-Cervantes & Córdova-Izquierdo, 2012) [16]. FACS exploits the validated difference in DNA content between X- and Y-bearing spermatozoa (Xie *et al*., 2020) [72], with fluorescent staining and flow cytometry enabling accurate separation (Garner, 2006) [20]. The development of the SX Moflo Nozzle to correct sperm orientation further enhanced efficiency, making FACS the global standard for producing sexed semen in livestock (Rens *et al*., 1998; Seidel Jr, 2007) [54, 57].

1) Flow Cytometry

Flow cytometers are the advanced cell sorters in which LASER is used to excite fluorescent dye, which binds to spermatozoa DNA. The primary principle for sperm sexing through flow cytometry is DNA content and DNA specific dyes. Flow cytometry differentiates X- and Y-bearing sperm by staining their DNA with the nucleic acid-specific dye Hoechst 33342, after which the labeled sperm are separated using a specialized high-speed cell sorter.

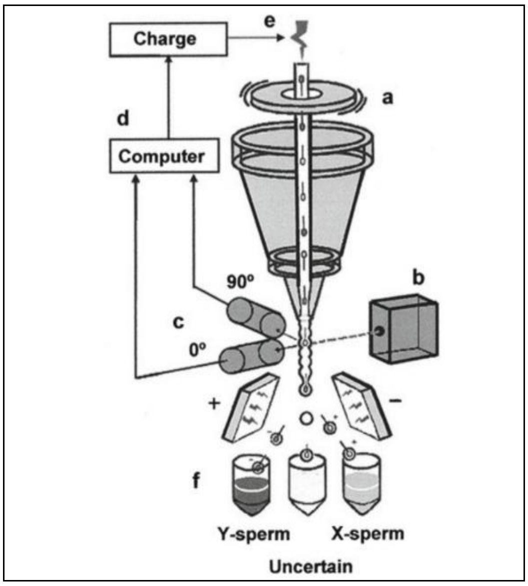

Fig 1: A. Piezoelectric crystal vibrator; b. Pulsed UV laser; c. Photodetectors; d. The signal is quantified; e. Charge is applied; f. Deflection of charged droplets. (Garner, 2001) [19].

A flow cytometer is an instrument used to analyse the physical properties and fluorescence characteristics of cells suspended in a fluid stream, and it operates through four core systems: fluidics, optics, electronics, and software. As the sperm suspension passes through a narrow fluid channel, it is exposed to a laser beam, and the resulting emissions are digitized for analysis. In sperm sexing, the Hoechst 33342 dye is widely utilized because it can penetrate intact membranes and selectively bind to A/T-rich regions of DNA. Upon excitation, it emits fluorescence in the range of 350–460 nm, which provides a reliable marker for assessing DNA content (Seidel & Garner, 2002; Garner, 2009) [58, 21]. Flow cytometry employs dual fluorescence detectors to measure these intensity differences, allowing discrimination between X- and Y-bearing sperm. The system streams sperm in single file, assigning an electrostatic charge to droplets containing individual cells, which are then deflected into separate collection containers. Fluorescence histograms are generated to distinguish sorted populations, while software gating excludes non-viable sperm, thereby enabling the recovery of highly enriched X- or Y-sperm populations (Vishwanath *et al*., 2018) [69].

2) Laser Ablation

Laser Ablation (LA) is a technique in which a focused laser beam removes material from a targeted surface. In semen sexing, Genus IntelliGen has developed the only commercially applied LA-based system, marketed as Sexcel, which took nearly seven years to optimize. In this approach, sperm cells suspended in a fluid stream pass individually through a laser detection zone, where their chromosomal sex is identified. Cells of the undesired sex are selectively inactivated using a high-powered laser, while those of the desired sex remain unaffected. Unlike flow cytometry, which subjects sperm to pressure, velocity, and electrical fields, LA technology minimizes physical stress, thereby enhancing post-processing sperm viability.

Recent method

Although FACS remains the only commercially successful sperm sexing technique, recent research has explored alternative methods to overcome its limitations. Identification of sex-specific proteins and generation of antibodies against them offer potential for sorting live spermatozoa with minimal physical damage (Rahman & Pang, 2020). Nanotechnology has also been applied, with nanoparticles acting as signal enhancers or carriers tagged to sex-specific sperm (Feugang *et al*., 2019) [17]. Microfluidic technology offers a promising alternative for sperm separation by utilizing inherent physical properties such as surface charge, motility velocity, and cell size to distinguish between X- and Y-bearing spermatozoa (Wongtawan *et al*., 2020) [71]. Raman spectroscopy is still at an early stage but has shown potential for differentiating sperm populations based on spectral signatures (De Luca *et al*., 2014) [11]. The integration of immunological, nanotechnological, spectroscopic, and microfluidic approaches may lead to novel sperm sexing methods with improved accuracy and reduced sperm damage.

1) Immunological approaches for sperm sorting

Hendriksen *et al*. (1999) [29] proposed that variations in gene expression could result in differences in protein composition between X- and Y-bearing spermatozoa. Immunological approaches for sperm sexing have been developed by targeting these proteomic differences.

Identification of differentially expressed proteins between X and Y spermatozoa

Molecular studies have provided strong evidence of differential gene and protein expression between X- and Y-bearing spermatozoa. Chen *et al*. (2014) [8] reported 31 genes showing differential expression, of which 27 were upregulated in X-bearing sperm and 4 in Y-bearing sperm.Similarly, De Canio *et al*. (2014) [10], using label-free shotgun nUPLC-MS/MS, reported 15 proteins upregulated in X-sperm and 2 in Y-sperm. Scott *et al*. (2018) [56] applied SWATH-MS to flow cytometry-sorted sperm and identified eight proteins with differential expression. More recently, Shen *et al*. (2021) [59] profiled Holstein bull sperm and observed eight proteins upregulated and 23 downregulated in X-sperm, along with 81 proteins exclusively expressed in X-sperm and 151 in Y-sperm. These findings highlight the potential of proteomic and transcriptomic markers as targets for developing novel immunological or molecular sperm sexing approaches.

Development of antibodies for specific sperm surface proteins

Surface proteins or antigens that differ between X- and Y-bearing spermatozoa represent promising targets for antibody-based sperm separation (Quelhas *et al*., 2021) [51]. Soleymani *et al*. (2019) [62] produced polyclonal anti-rbSRY antibodies in goats, which specifically bound to Y-chromosome–bearing spermatozoa while showing no affinity for X-bearing counterparts. In a subsequent study, bovine monoclonal antibodies against rbSRY (mAbSRY2) were immobilized on a Sepharose column, enabling the retention of Y spermatozoa while X spermatozoa passed through (Soleymani *et al*., 2021) [61]. Similarly, developed the monoclonal antibody Whole Mom@ targeting Y-sperm surface epitopes and applied it to isolate Y-bearing cells from cryopreserved semen. More recently, engineered single-chain fragment variable (scFv) antibodies derived from the parental mAb-1F9. These scFv constructs, comprising VH and VL regions, demonstrated enhanced specificity for Y-bearing sperm and reduced cross-reactivity with X-bearing counterparts.

2) TLR7/8based method

Umehara *et al*. (2019) [67] demonstrated that Toll-like receptors TLR7 and TLR8, encoded on the X chromosome, are expressed in round spermatids and epididymal spermatozoa. Activation of these receptors with specific ligands selectively suppressed the motility of X-bearing sperm without affecting Y-bearing counterparts, thereby enabling separation based on differential motility. When the high-motility sperm fraction was used for IVF, approximately 90% of resulting embryos were XY, and embryo transfer yielded 83% male offspring. The functional disparity was attributed to TLR7/8-mediated signaling, supported by Western blot and immunofluorescence analyses confirming receptor expression, with spleen tissue serving as a positive control. Among the ligands tested, resiquimod (R848) activated both TLR7 and TLR8, whereas imiquimod (R837) was specific for TLR7. Motility assays revealed a dose-dependent reduction in the proportion of highly motile sperm following exposure to R848 or R837 (0.3–3 μM), with significant decreases in average path velocity (VAP) confirmed by computer-assisted sperm analysis.

An important advantage of the TLR7/8 ligand-based method is that it does not require specialized equipment or advanced technical expertise, making it suitable for laboratories already equipped for IVF procedures. In practice, semen is incubated with 0.03 μMresiquimod (R848) in calcium-supplemented human tubal fluid (mHTF) medium—1 mL for mouse sperm and 3 mL for bull sperm—for approximately 60 minutes. Following incubation, the motile sperm fraction is collected from the upper layer (400 μL in mice, 1 mL in cattle), washed by centrifugation, and resuspended in ligand-free IVF medium for fertilization. This simple separation produced striking results: upper-layer sperm generated nearly 90% XY embryos in both mice and cattle, whereas the precipitated fraction yielded more than 80% XX embryos. Notably, the entire process of separating X- and Y-bearing sperm could be completed within 2 hours (Umehara *et al*., 2019; Nakao *et al*., 2020) [67, 47].

3) Microfluidics for Sperm Sexing

Physical properties such as electrical charge and motility have also been explored for sperm sexing. Koh (2015) [39] reported that dielectrophoretic forces could differentially influence the velocities of X- and Y-bearing spermatozoa under varying electric field strengths and viscoelastic conditions, with non-uniform dielectrophoresis in a viscoelastic medium improving sorting efficiency. Zeta potential analysis further revealed that Y-bearing spermatozoa possess a comparatively higher negative surface charge than X-bearing spermatozoa. Interestingly, these charge differences were more pronounced in TALP buffer than in alternative media such as HEPES or TRIS (Wongtawan *et al*., 2018) [70].

Further refinement of electrophoretic approaches revealed that under conditions of 4 V at 1 MHz, positively charged spermatozoa adhered to the electrode surface, while negatively charged cells migrated freely and were collected at the outlet. This technique resulted in a ~30% reduction of Y-bearing sperm in mixed samples, indicating selective separation (Wongtawan *et al*., 2020) [71].

More recently, microfluidic technologies have been adapted for sperm sexing. A New Zealand–based startup developed a device that utilizes radiation pressure within a microfluidic system to alter the trajectory of individual spermatozoa. This platform incorporates a DNA-specific fluorescent dye to distinguish X- and Y-bearing cells, functioning on principles similar to fluorescence-activated cell sorting (FACS), but in a miniaturized and potentially more efficient format.

4) Raman Spectroscopy (RS) in Sperm Sexing

Raman spectroscopy (RS) has emerged as a promising label-free and non-invasive approach for discriminating between X- and Y-bearing bovine spermatozoa (De Luca *et al*., 2014) [11]. This technique relies on detecting variations in the spectral signatures of cellular components such as DNA, proteins, lipids, and other macromolecules. While the nucleus is considered the primary site of biochemical differences, no significant peak variations were observed across specific nuclear regions including the head, neck, and acrosome. Instead, the spectral differences were attributed mainly to variations in DNA content and membrane composition between X- and Y-bearing sperm. Efforts are currently underway to translate this method into practical application, with Jiva Bioscience Pvt. Ltd. (Bengaluru, India) working toward its commercialization.

Reproductive performance of sexed semen

Reproductive efficiency is a key determinant of profitability in dairy farming (Howley *et al*., 2012) [32]. Numerous field trials have evaluated the reproductive performance of sexed semen by examining parameters such as conception rate, sex ratio, incidence of dystocia, and calf mortality, and comparing them with outcomes from conventional artificial insemination (AI). Evidence consistently indicates that conception rates achieved with sexed semen are lower than those with unsexed semen (Chebel *et al*., 2010) [7]. This reduction in fertility has been primarily attributed to the lower sperm concentration per dose and the detrimental effects of the sorting process on sperm quality (DeJarnette *et al*., 2008; De Vries, 2009) [14, 12].

Studies consistently report that conception rates with sexed semen are significantly lower than those obtained with conventional semen. Average conception has been estimated at 40.8% for sexed semen compared with 52.35% for conventional AI. Reported ranges further highlight this disparity, with conception rates varying between 25–51% for sexed semen versus 30–62% for conventional insemination. When analyzed by parity, heifers show conception rates of 44.35% (32–56%) with sexed semen compared to 59.03% (40–70%) with unsexed semen (Thakur and Birthal, 2023) [65]. In contrast, conception rates in lactating cows are notably lower when sexed semen is used, indicating a significant gap between the two groups. From an economic standpoint, these findings suggest that the use of sex-sorted semen is most advantageous in heifers rather than cows (Fetrow *et al*., 2007; De Vries and Nebel, 2009; De Jarnette *et al*., 2009) [12, 13].

Sexed Semen Status in India

The adoption of sexed semen in India reflects a growing trend in the livestock sector toward precision breeding and herd improvement. Several states, including Kerala, West Bengal, Punjab, and Haryana, have witnessed increasing use of this technology (Kumar *et al*., 2016) [40]. The prices of sexed dose ranging from Rs. 900 to 2,000 per dose, depending on factors such as bull breed, pedigree, and purchase volume. Now, a days to encourage uptake among farmers, government subsidies reduce the cost to as low as rs.100-300 per dose. But Optimizing the use of sexed semen under Indian conditions requires refinement of techniques, such as reducing sperm dose per insemination and ensuring accurate deposition sites during AI (Campanile *et al*., 2011) [5], along with robust herd management practices including balanced nutrition, disease prevention, efficient estrus detection, and proper semen handling.

Commercial production in India is dominated by Sexing Technologies (ST) and ABS. The technology was first introduced by U.S. based companies in 2005, and in 2017 ABS India began producing sexed semen under the brand “Sexcel” using its proprietary IntelliGen platform. The first male calf born in India through sexed semen technology, named *Shreyas*, was produced in 2011. At present, India hosts 56 semen stations with a combined capacity of 81 million doses annually, though national demand already exceeded 119 million doses in 2019 and is projected to surpass 150 million within the next five years. Under the Rashtriya Gokul Mission, production has scaled up: as of 2022, government semen stations generated 27.86 lakh doses of sexed semen, while private, NGO, and milk federation stations produced an additional 31.12 lakh doses (Ministry of Fisheries, Animal Husbandry and Dairying, 2022). With conception rates averaging around 30% and a 90% accuracy in producing female calves, these initiatives are expected to yield approximately 24.12 lakh high-yielding dairy heifers.

Notably, the application of sexed semen in India is concentrated in states such as Punjab, Haryana, Telangana, and Andhra Pradesh, where imported germplasm from high-yielding breeds like Holstein-Friesian and Jersey is widely used. This regional focus underscores both the potential and challenges of scaling up sex-sorting technology to meet the rising demand for elite dairy replacements in the country. The sexed semen production facilities in India are given in Table 3.

Table 3: Sexed semen production facilities in India

Semen Station	States	Category
ABC MACS	Andhra Pradesh	Pvt.
ARDA-Ode (Mobile Van and Stationary)	Gujarat	Coop.
DURDA, PSK, Jagudan	Gujarat	Coop.
SAG, Bidaj (Mobile Van)	Gujarat	NDS
SFSS, GLDB, Patan	Gujarat	Govt.
CSS, Bhopal	Madhya Pradesh	Govt.
BAIF, Pune	Maharashtra	NGO
Rahuri (Mobile Van)	Maharashtra	NDS
Chitale	Maharashtra	Pvt.
Alamadhi (Mobile Van)	Tamil Nadu	NDS
DLF, FSPS, Ooty (Mobile Van)	Tamil Nadu	Govt.
ULDB, Rishikesh	Uttarakhand	Govt.
ABC, Salon (Mobile Van)	Uttar Pradesh	NDS
DFSS, Babugarh	Uttar Pradesh	Govt.

The Rashtriya Gokul Mission represents a major national effort to promote indigenous cattle breeds such as Sahiwal, Gangatiri, Kankrej, and Gir, which are well adapted to Indian climatic conditions and demonstrate resilience against tropical diseases (Rashtriya Gokul Mission, 2019) [53]. Several research and development organizations, such as the Paschim Banga Go-Sampad Bikash Sanstha (PBGSBS) and the National Dairy Research Institute (NDRI), have undertaken semen sorting initiatives using flow cytometry, although the outcomes have shown variable success rates. Commercially, Sexed ULTRA semen produced by Sexing Technologies has reported conception rates of around 52%, which remain slightly lower than the ~60% achieved with conventional semen. Although demand for sexed semen is growing steadily, widespread adoption in India continues to be limited by cost constraints and reduced fertility outcomes. However, recent technological refinements have improved its practicality and field performance (Srivastava *et al*., 2019).
Field evaluations have provided valuable insight into the performance of sexed semen under Indian conditions. A large-scale investigation carried out across 940 AI centers of the Bharatiya Agro Industries Foundation (BAIF, Pune), spanning 147 districts in seven states, reported an overall conception rate of 39.92% with the use of sex-sorted semen (Joshi *et al*., 2021) [36]. At the ICAR-IVRI farm in Bareilly, Tharparkar cattle inseminated with sexed semen achieved a conception rate of 44.7%, which was 8.9% lower than that of conventional semen, but importantly, yielded a female sex ratio of 91.7% (Patra *et al*., 2023). These findings highlight that while conception rates with sexed semen remain modestly reduced compared to conventional AI, the technology offers substantial advantages in achieving female-biased calf production, particularly in indigenous breeds.

Conflict of Interest
Not available

Financial Support
Not available

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