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Status and prospects of sex sorted semen in India

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Abstract
The dairy industry in India has experienced substantial growth, contributing significantly to the nation's economic development and rural livelihoods. Although artificial insemination (AI) has been instrumental in enhancing dairy herds and boosting milk production, persistent challenges remain, such as the surplus of low-value male calves and complications like dystocia. Sex-sorted semen technology presents a promising solution, allowing farmers to strategically choose the gender offspring. This technology empowers dairy farmers to focus on breeding superior female animals, optimizing their operations for milk production. The historical evolution of sex-sorted semen technology highlights significant milestones in genetics and reproductive biology, leading to its practical application in animal breeding. Various sperm sexing methods, both conventional and commercial, have been developed, with commercial approaches using advanced technologies like flow cytometry and microfluidics. However, the limitations in usage of sex-sorted semen are reduced conception rates and potential defects, such as DNA damage and membrane alterations in sperm. Strategies to enhance fertility, including higher insemination doses and synchronization programs, have shown promising results. The adoption of sexed semen in India, while growing, faces supply challenges and requires efforts to make it more accessible and affordable for farmers.

Keywords: Dairy industry, sex-sorted semen technology, artificial insemination, genetic advancement, conception rates

Introduction

The animal husbandry sector has transformed into a lucrative industry in numerous developing nations. It plays a vital role in both the economic growth of the nation and the creation of livelihood opportunities for rural communities. Artificial insemination (AI) has transformed the dairy sector by giving farmers a potent tool to enhance the genetics of their dairy herds while also sharply improving milk output and, consequently, farmers' income (Singh *et al.*, 2022) [62]. However, despite the overall growth and success in the dairy sector, dairy farmers still grapple with several on-farm challenges. Despite the overall growth and profitability of the dairy sector, dairy farmers continue to encounter several challenges on their farms. These challenges include male calves being born, which are of little value in the specialized dairy system, which is primarily centered on female cows for milk production. Moreover, the issue farmers confront is exacerbated by the high frequency of male calves, which might cause issues during calving. It's still difficult to find replacement heifers for the herd, deal with anti-slaughter laws for cattle, solve feed and fodder shortages, and deal with several other issues that impede the development of the dairy business. A recent breakthrough in sex-sorted semen technology offers a promising solution to these challenges. By combining sexed semen technology with artificial insemination, dairy farmers can selectively breed for desired offspring genders, potentially boosting milk production by focusing on valuable female animals. Sex-sorted semen provides farmers with the ability to significantly influence the gender ratio of their animal offspring, liberating herd reproductive management from the challenge of balancing the need for replacement heifers (De Vries *et al.*, 2008) [17]. Achieving sustainable and genetically optimized indigenous cattle requires a comprehensive strategy, including selection for adaptability, reducing unproductive or low-production individuals, improving infrastructure like AI and veterinary services in the field to reduce calving intervals, introducing economically viable technologies, enhancing farmers' and rural women's knowledge of improved practices through field/extension staff, and empowering rural women. Furthermore, sexed semen can be employed to produce desired *in vitro* sex embryos at lower doses, offering an alternative means of influencing offspring gender ratios.

Current status of semen sexing in India

The status of the use of sexed semen in India is indicative of an increasing tendency in the livestock industry towards its adoption. Sexed semen technology is growing in popularity in a number of Indian states, including Kerala, West Bengal, Punjab, Haryana, and others (Kumar *et al*., 2016) [35]. In India, there are 5 semen stations producing sexed semen. However, because of its high cost and low fertility, its wider use is constrained throughout the nation (Biswas *et al.*, 2013) [6]. The average cost of sexed semen in India varies based on factors such as the breed and pedigree of the bull used for producing the semen, as well as the quantity purchased. Sexed semen is available at the rate of Rs 900 to 2,000 per dose, however to promote the adoption of this technology among farmers, the government offers it at a subsidized rate ranging from Rs 100 to Rs 300. Recognizing the value of sexed semen in selective breeding, it is crucial to optimize its use to ensure cost-effectiveness and higher productivity. This necessitates the standardization of lower spermatozoa dosages and precise deposition sites for AI (Campanile *et al.*, 2011) [8], in order to achieve high pregnancy rates under Indian conditions. To attain higher efficiency and make the best use of sexing technology, excellent animal management is needed such as nutrition, disease control, estrus detection, handling semen, and insemination techniques.

The production of sexed semen in India is primarily carried out by Sexing Technology (ST) and ABS, with these two companies dominating the market. In 2005, US commercial groups introduced sexed semen to India. The ABS India began producing sexed semen in 2017 under the "Sexcel" brand, utilizing its proprietary IntelliGen technology. On January 1, 2011, sexed semen was used in India to produce the first male calf, named Shreyas. As of now, there are a total of 56 semen stations across the country, collectively capable of producing approximately 81 million doses of semen annually. However, the demand for bovine semen in India has surged to nearly 119 million doses in 2019, and it is projected to exceed 150 million doses within the next five years. As of 2022, 27.86 lakh doses of sex sorted semen have been produced at government semen stations assisted under the Rashtriya Gokul Mission, and 31.12 lakh doses have been produced at milk federation, NGO, and private semen stations (Ministry of Fisheries, Animal Husbandry and Dairying Year End Review 2022). With a 30% sexed semen conception rate and a 90% accurate sex ratio for female calves, the project is expected to produce about 24.12 lakh high milk producing female calves. Sexed semen technology finds extensive use in specific regions of India, notably Punjab, Haryana, Telangana, and Andhra Pradesh, where imported semen from foreign breeds like Holstein-Friesian (HF) and Jersey is prevalent. The sexed semen production facilities in India are given in Table-1

Table 1: Sexed semen production facilities in India

State	Organization	Manufacturing company
Gujarat	State frozen semen station, Paltan Frozen semen station, Jagudhan	Genus -ABS India Genus -ABS India
Maharashtra	BAIF development research foundation, Pune BG Chitale dairy farm), Sangli	ST Genetics (Sexed ULTRA Genus -ABS India
Madhya Pradesh	Bhopal	ST Genetics (Sexed ULTRA
Uttar Pradesh	Livestock development Board, Babugarh	Genus -ABS India
Uttarakhand	Deep frozen semen production facility	ST Genetics (Sexed ULTRA

The Rashtriya Gokul Mission is a significant initiative aimed at developing indigenous breed semen straws such as Sahiwal, Gangatiri, Kankrej, and Gir. These breeds are well-suited to the Indian climate and exhibit resistance to tropical diseases (Rashtriya Gokul Mission, 2019) [49]. In India, certain organizations like Paschim Banga Go-Sampad Bikash Sanstha (PBGSBS) and the National Dairy Research Institute (NDRI) have initiated semen sorting using flow cytometry, achieving varying degrees of success. Reported pregnancy rates for Sexed ULTRA semen from Sexing Technologies stand at 52%, whereas conventional semen boasts a slightly higher pregnancy rate of 60%. Despite the considerable potential demand for sexed semen, the current supply in India falls short, prompting efforts to make it more affordable and accessible to farmers. While the adoption of sexed semen for AI in cattle has faced challenges related to cost and lower conception rates, recent technological advancements have improved its viability (Srivastava *et al.*, 2019) [66]. Further research is required to enhance the viability of this method for widespread implementation in our nation (Kumar *et al.*, 2016) [35].

Sperm sexing methods

Sperm sexing methods involve separating sperm into two subpopulations, one with a higher concentration of X-chromosome-bearing sperm and the other with a higher concentration of Y-chromosome-bearing sperm. This technique is valuable for selectively determining the gender of offspring, especially in agricultural and livestock breeding programs for milk and meat production, as well as in wildlife conservation efforts using assisted reproductive technologies. The journey of understanding and manipulating the role of sex chromosomes in determining the gender of mammalian offspring has been marked by significant milestones and advancements in the field of genetics and reproductive biology. It all began when there is groundbreaking discovery of sex chromosomes through microscopic identification (Guyer 1910) [23].

Conventional approches

One approach to sperm sexing is based on the swimming ability in a gradient solution. The Y-chromosome-bearing sperm, being smaller and more motile, tend to swim faster downwards in the solution compared to X-chromosome-bearing sperm. This difference allows for the separation of sperm into enriched fractions, with a success rate of approximately 75% (Sá Filho *et al*. 2010) [52]. Another method involves identifying surface proteins expressed in X or Y sperm, utilizing immunological techniques. Specific antibodies to the H-Y antigen (Found in Y-bearing sperm) are used to sort sperm with an efficacy of over 90% (Johnson 2000) [28]. Free-flow electrophoresis is based on the electrical charges present on the sperm surfaces, where X sperm have a negative charge and Y sperm have a positive charge (Johnson *et al.* 1989) [30]. These differences in surface charges allow for their separation in an electric field, with an 81% success rate reported. Size-mediated differences are also utilized, as Y-bearing sperm are smaller than X-bearing sperm. Various techniques can be used to sort sperm based on these size differences, although success rates may vary. Real-time PCR utilizes Y-chromosome-specific DNA sequences, including SRY gene-specific primers, to assess the separation efficiency of sex-sorting techniques and determine the percentage of Y-bearing sperm in semen samples (Parati *et al.*, 2006) [42]. This involves amplifying markers for X and Y sperm content in sexed semen. However, distinguishing X and Y sperm based on physical characteristics like weight, size, or density is challenging due to their subtle differences.

Commercial approaches: Commercial technologies for semen sexing employ advanced methods to separate sperm cells by their sex chromosome, offering significant benefits for animal breeding and reproduction. One such technology is the Beltsville sperm sexing method, utilizing flow cytometers with lasers to excite fluorescent dyes bound to sperm DNA. This method relies on DNA content and specific dyes, with stained spermatozoa individually exposed to a UV laser beam and the resulting fluorescence distinguishes between X and Y-chromosome-bearing sperm, enabling precise sex sorting (Prakash *et al.*, 2014) [48]. This technique builds upon earlier research, such as the work by Barlow and Vosa (1970) [5], who documented the difference between X- and Y-chromosome-bearing spermatozoa by observing the differential uptake of quinacrine stain, with the Y-chromosome fluorescing more brightly, providing an initial visual means of distinguishing between the two types of sperm. Flow cytometry facilitated the precise measurement of sperm DNA content, enhancing the understanding of sperm genetics significantly (Gledhill *et al.*, 1976) [21].

There are certain limitations that affect its commerciality (Table-2). The current sperm sorting techniques typically achieve an offspring-sex ratio accuracy ranging from 85% to 95%. Flow cytometry has been commercially applied to cattle, but the suitability of sperm for this method varies among species. Optimizing sperm dilution rates, changing dye types, and adjusting laser power were explored but did not significantly improve sorted sperm motility. Research aimed to improve hardware, optics, electronics, and handling for flow cytometry, enhancing post-separation sperm motility by reducing sorter sheath pressure and utilizing pulsed lasers for sperm transport. Yet, mechanical technology limits hinder further optimization, leading to a shift towards enhancing production speed and sperm quality through related technologies.

Table 2: Drawbacks of sex sorted semen by flow cytometry

Technological Limitation	High cost of sex sorting machines like flow cytometry.
	Low sorting efficiency and speed.
	Required skilled persons for handling.
	Little mishandling can cause sperm damage.
	Waste of approximately 50% of sperms.
	Reduced freezing potential of sorted sperms.
Implementation Limitation	More costly than conventional semen because it includes cost of intellectual property right.
	The conception rate with sex sorted semen is 10-15% less than conventional semen which is more adverse condition in our country where total coverage of AI is less than 25% of breedable population.
	Sexed semen contains only 2-4 million sperms/dose as compared to conventional semen which contains 20 million sperms/dose which will be challenge under Indian field condition.

Laser-killing-based sperm sexing, represented by LumiSort TM, is the next-generation solution. LumiSort TM employs flow cytometry and harmless DNA staining to identify sex-specific differences. Sperm cells bearing X-chromosome emit slightly stronger fluorescence due to their higher DNA content, enabling sex determination. Unwanted cells are then eliminated using lasers, leaving the desired cells intact.

Microfluidics-based sperm sexing, as developed by Engender Technologies, utilizes microfluidic and photonic chips. This innovative approach involves directing semen samples through a microfluidic chip, followed by laser-based determination of X or Y chromosomes in sperm cells. By nudging the cells into different outflows, this technology allows for the selection of the desired offspring's sex.

Immunological approaches for semen sexing

The application of Fluorescence-Activated Cell Sorting (FACS) in sperm sex-sorting has opened new avenues for studying X- and Y-sperm populations and exploring molecular properties that can inform the development of novel sex selection methods. Immunological techniques have emerged as a promising approach for sexing sperm, capitalizing on differences in protein expression between X and Y-sperm (Katigbak *et al.*, 2019; Yadav *et al.*, 2017) [34, 75]. This approach relies on the assumption that genomic distinctions between X and Y-sperm may lead to differences in protein composition and function. Notably, a sperm-sexing method based on Toll-like receptor 7/8 (TLR7/8) activation in X-sperm has been developed (Umehara *et al.*, 2019, 2020) [72-73]. The TLR7 and TLR8 were detected exclusively in the tail and midpiece of the X-sperm, respectively, and activation with resiquimod (R848) resulted in reduced motility and X-sperm enrichment. However, further optimization is required for the application of this method to freshly ejaculated sperm and AI. Efforts to identify surface-specific antigens distinguishing X and Y-sperm have been explored (Sang *et al.*, 2011; Yadav *et al.*, 2017) [55, 75]. Initially, HY antigen was considered a potential immunological marker due to its exclusive presence in mammalian tissues (Wachtel *et al.*, 1975) [74]. While some studies suggested a correlation between anti-HY antibody binding and Y-chromosome-bearing sperm, conflicting results emerged regarding the antigen's presence in both X and Y-sperm (Bradley, 1989; Hendriksen *et al.*, 1993) [7, 24]. As such, anti-HY antibody-based sexing methods proved inappropriate for differentiating between X and Y sperm. Proteomics has played a pivotal role in the search for specific proteins in X and Y-sperm (Katigbak *et al.*, 2019; Yadav *et al.*, 2017) [34, 75]. Recent studies have identified differentially expressed proteins between bull X and Y-sperm, shedding light on factors related to energy metabolism, cytoskeleton, and serine protease inhibition (Chen *et al.*, 2012; De Canio *et al.*, 2014; Scott *et al.*, 2018)

[11, 14, 57]. These findings hold the potential to advance immune-sexing techniques, offering a less aggressive and economically viable alternative to current methods.

Immunological approaches show promise in separating X and Y-sperm, with surface-specific antigens serving as potential candidates for antibody-based selection (Yadav *et al.*, 2017) [75]. Antibodies against the surface antigen sex-determining region Y (SRY), specific to bovine Y-sperm, have shown the capability to selectively bind Y-sperm and holds potential for sex separation (Soleymani *et al.*, 2019, 2021) [64, 63]. Other studies have also explored monoclonal antibodies targeting Y-sperm plasma membrane epitopes, such as WholeMom®, for the production of cattle embryos with pre-selected sexes (Chowdhury *et al*., 2019) [12]. These innovative approaches hold promise for improving sperm sex-sorting techniques, offering alternatives that merit further exploration and development (Soleymani *et al*., 2021; Chowdhury *et al*., 2019) [63, 12].

Effect of sex sorted semen on morphological and functional integrity of sperm

A range of studies have delved into the effects of various factors on sperm during sex-sorting procedures. It was observed that the transfer of molecular energy from DNA to fluorochromes could trigger chromatin decondensation, impacting the sperm's genetic material (Johnson 1999) [29]. The extent of damage caused by stains hinges on the stability of chromatin, and the use of high dye concentrations during sorting may adversely affect sperm motility parameters. In a study on pigs, it was revealed that exposure to dyes could disrupt HSP70, resulting in changes in sperm membrane characteristics similar to capacitation (Spinaci *et al.*, 2006) [65]. The staining and incubation processes may lead to DNA damage, raising concerns about the genetic integrity of sperm used in sorting techniques (Sá Filho *et al.*, 2010) [52]. The sorting pressure and speed could impact sperm quality even before capacitation occurs, underscoring the need for meticulous control of sorting conditions. It was observed that UV-laser sorting could cause significant DNA damage, which could have implications for the genetic quality of sorted sperm (Silva *et al*., 2006) [61]. Beyond this, the influence of electrical charging and electrostatic deviation on sperm membranes, particularly in the mid-piece and tail regions, can lead to depolarization. As described by this depolarization can diminish mitochondrial activity, primarily due to the presence of reactive oxygen species (ROS) generated by electric forces (Rath *et al.*, 2008) [50]. A substantial presence of ROS in seminal plasma could result in spermatozoa with damaged DNA, raising concerns about their genetic viability (Gosálvez *et al.*, 2011) [22]. Besides, the spermatozoa harbouring damaged DNA might potentially compete with those with normal DNA, thereby diminishing embryo viability in assisted reproductive processes. These collective findings emphasize the importance of understanding and mitigating the various challenges associated with sperm sex sorting techniques to ensure the optimal quality of sorted spermatozoa (Tesarik *et al.*, 2004) [69].

Impact of sex-sorted semen on conception, embryo development and molecular alterations

The use of sex-sorted semen in AI has traditionally resulted in about 25% lower conception rates compared to conventional sperm (Hutchinson *et al*., 2013; Andersson *et al*., 2006) [27, 1]. However, the introduction of advanced technologies like Sexed ULTRATM, involving an increase in the number of sperm per insemination from 2 to 4 million, has resulted in improved pregnancy rates, with values reaching 60% to 80% of those achieved with conventional sperm (Lenz *et al*., 2016; Thomas *et al*., 2019) [37, 70]. *In vitro* applications, such as fertilization (IVF) and embryo production (IVP), show variable outcomes with blastocyst rates of 36% to 52% and pregnancy rates of 26% to 40% (Pontes *et al*., 2010; Pellegrino *et al*., 2019) [47, 45]. *In vivo* studies in superovulated cows have demonstrated fewer recoverable embryos with sex-sorted sperm compared to conventional (Schenk *et al*., 2006; Kaimio *et al*., 2013) [56, 33]. Calves born from sex-sorted sperm generally exhibit no abnormalities in 89% of cases, and lower rates of dystocia, but higher stillbirth rates when the sex of the calf is incorrectly sorted (Norman *et al*., 2010; Mikkola *et al*., 2015) [41, 38]. Molecular studies have linked sperm sorting to increased levels of reactive oxygen species (ROS), which contribute to decreased sperm motility and viability due to elevated membrane permeability and decreased ATP levels (Tvrda *et al*., 2016; Carvalho *et al*., 2010; Balao *et al*., 2013; Holden *et al*., 2017) [71, 10, 4, 25]. Additionally, sorted sperm shows more DNA damage than conventional sperm, as evidenced by sperm chromatin structure assays (Baker *et al*., 2005) [3]. Post-thaw, sorted sperm exhibits decreased motility and higher capacitation, leading to more acrosome-reacted sperm (Hollinshead *et al*., 2003; Moce *et al*., 2006) [26, 40]. Moreover, sex sorting can impair gameto-maternal interactions, notably affecting sperm binding to ciliated cells in tubal explants (Carvalho *et al*., 2018) [9], which may contribute to the challenges in achieving higher conception and successful pregnancy rates.

Strategies to prevent sorting defects

Researchers have explored various strategies to mitigate the potential damage inflicted on sperm during sex sorting processes. One notable approach involves the use of SYBR-14/propidium iodide staining, which has been found to cause considerably less damage compared to staining with Hoechst 33342. Additionally, it was reported that a reduction in sorting pressure, from 50 psi to 40 psi, led to improvements in the quality of spermatozoa in both bulls and stallions (Suh *et al.*, 2005) [68]. Also, there is significance of utilizing UV lasers with argon or state solid lasers to minimize damage (Rath *et al.*, 2008) [50]. The addition of 10% seminal plasma into the staining medium not only improved viability and motility but also reduced capacitation-like changes in boar and ram spermatozoa (De Graaf *et al*., 2007) [15]. Moreover, efforts to lower DNA damage have involved decreasing the concentration of stains, laser strength, sorting pressure, and the use of spermatozoa extenders. In cattle, it was found that a protamine supplement, combined with bovine sheath fluid containing specific components, contributed to reducing DNA damage (Gosalvez *et al.*, 2011) [22]. Another valuable technique involves gradient centrifugation before sperm sexing, which has been shown to enhance resolution and sorting rates. Employing sperm sorters with two or more nozzles, as well as opting for an eight-cylinder engine instead of one, can increase sorting doses and reduce costs, potentially improving the efficiency of the sex sorting process (Seidel, 2007) [58].

Fertility of sex sorted semen

The fertility outcomes of sexed semen compared to conventional semen exhibit significant variation and are influenced by multiple factors. However, a consistent finding is that the conception rate with sexed semen is consistently 10 to 15% lower than that achieved with conventional semen (Norman *et al.* 2010) [41]. These include compromised sperm quality resulting from mechanical and shear stress during sorting, a decrease in antioxidant defence mechanisms leading to increased lipid peroxidation, and damage to the DNA and plasma membrane of sperm.

Due to higher fertility in heifers than lactating cows, sex-sorted semen is recommended for use in first and second AI services in virgin heifers (Seidel and Schenk, 2008, Garner and Seidel, 2008, De Vries and Nebel, 2009) [68, 20, 16]. The overall conception rate for sexed semen within a herd averaged approximately 45%, with a range spanning from 27% to 70%, whereas the conception rate for conventional semen in the same study averaged around 56%, with a range from 34% to 83% (DeJarnette *et al.*, 2009) [18]. The total conception rate with sexed semen was 44.7% in tharparkar cattle at an organized cow and buffalo farm (ICAR-IVRI Bareilly), which is 8.9% lower than the rate with conventional semen with female sex ratio of 91.7%. Also with sex-sorted sperm, the successful conception usually happened 16-41 hours following the commencement of estrus. The double AI technique also showed a 20% increased conception rate (Patra *et al.*, 2023) [43]. A field study conducted in 940 AI centers of Bharatiya Agro Industries Foundation (BAIF, Pune) across 147 districts in seven states of India reported an overall conception rate (CR) of 39.92% using sex-sorted semen (Joshi *et al.*, 2021) [31].

To successfully produce sexed semen doses using indigenous cattle and buffalo bulls, it is crucial to ensure that field conception rates across different herds at not less than 92% of the rates achieved with conventional semen under similar conditions. This emphasizes the need for continuous improvement and optimization of sexed semen technology, particularly when applied to native cattle and buffalo breeds (Norman *et al.*, 2010; DeJarnette *et al*., 2009) [41, 18].

Effect of sexed semen in pregnancy: The application of sexed semen technology in animal reproduction has yielded varying conception rates, as highlighted in multiple studies conducted by various researchers. It was observed that the conception rate ranged from 70-80% and 45% in heifers and 50-60% in lactating cows when sexed semen was utilized for insemination (Seidel. 2003) [59].

The pregnancy rate of 56% in heifers when using 2×10^6 sexed sperm per insemination, while the control group, receiving 10×10^6 unsexed sperm, achieved a slightly higher rate of 61% (Garner and Seidel, 2003) [19]. Several factors play a crucial role in determining the success of sexed semen technology. These factors encompass low insemination doses, the manifestation of weak heat symptoms (Yoshida and Nakao, 2005) [76], and variations in the number of inseminations and insemination doses required between cows and heifers (Peippo *et al.*, 2009) [44]. It is essential to acknowledge that the use of sexed semen may lead to a substantial increase in the costs associated with producing sex-sorted straws. These findings underscore the intricacies involved in utilizing sexed semen and emphasize the necessity for careful consideration within reproductive management strategies.

Strategies to improve conception using sexed semen

The likelihood of achieving pregnancy following AI, whether using conventional or sex-sorted semen, is influenced by several factors (Fig.1). These factors encompass the quantity of sperm per straw, semen quality, the site of semen deposition, and the timing of AI concerning the estrous cycle (Dalton *et al*., 2001; Andersson *et al*., 2004; Kurykin *et al*., 2007; Saacke., 2008; Roshan Krishna *et al*., 2022) [13, 2, 36, 53, 51]. Improving fertility through the administration of exogenous gonadotrophins, either at the onset of estrus or concurrently with AI, has shown promise, but the outcomes have been inconsistent (Kaim *et al.*, 2003, Stevenson *et al.*, 2007; Perry and Perry, 2009) [33, 67, 46]. As a result, strategies aimed at enhancing fertility, such as the use of exogenous hormones, refining estrus detection methods, or optimizing the timing of AI relative to estrus detection, could serve to enhance the application of sexed semen in dairy cattle breeding.

Increasing the quantity of sperm used for AI has been identified as a potential avenue for enhancing fertility (Schenk *et al.* 2006) [56]. This suggests that higher insemination doses may positively impact the likelihood of achieving successful pregnancies. Furthermore, the implementation of synchronization programs alongside AI has emerged as a pivotal factor in fertility improvement (Sales *et al.*, 2011) [54]. These synchronization programs are expected to play a substantial role in optimizing the timing and coordination of AI procedures, potentially leading to increased fertility rates. A substantial increase in embryo production among superovulated cows inseminated with 20 million sex-sorted sperm, compared to the conventional dose of 2 million (49 embryos versus 28 embryos). This noteworthy observation highlights the potential benefits of adjusting insemination doses to achieve more favorable outcomes in reproductive processes (Schenk *et al*., 2006) [56].

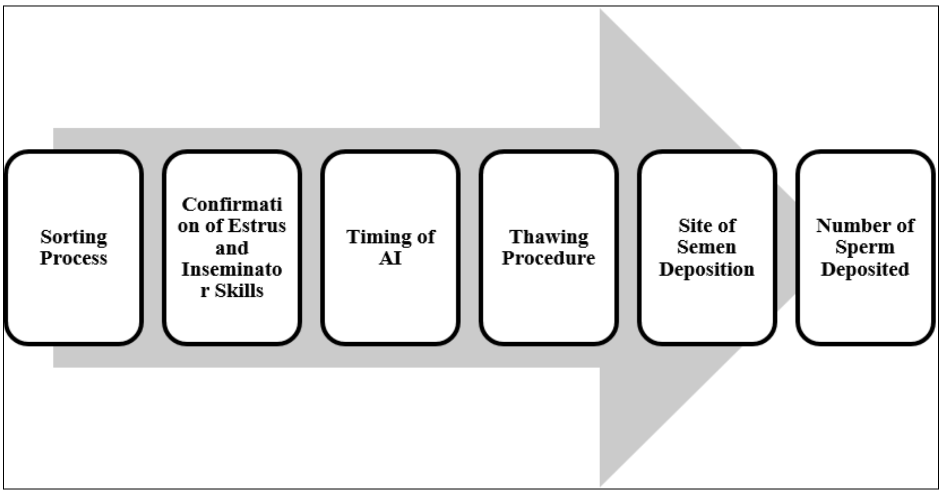

Fig 1: Factors affecting conception rate of sexed semen

Prospects of semen sexing in India

Even though India has been a pioneer in crossbreeding programs and has seen considerable advances in milk production, semen sexing technology has become more significant in the country for several reasons (Table-4). Crossbred female cattle have produced more milk than native cattle, but this advantage does not extend to crossbred male cattle, as they do not produce milk. Moreover, with the increasing mechanization of agriculture, the demand for male cattle for labor has significantly decreased, making them less economically viable in mechanized farming environments.

It has become difficult to manage the male progeny from crossbreeding operations because of this performance disparity. It's critical to preserve economic efficiency in the dairy sector. Maintaining underperforming crossbred males is not cost-effective since they use resources while making minimal milk contributions. Semen sexing technology makes it possible to selectively produce superior females, which may result in more effective resource management. Without semen sexing technology, many of these underperforming crossbred males would likely be left to die or would frequently have to be culled. This is not just a waste of resources, but it is also unethical. An alternative to lowering the number of undesirable male animals is semen sexing. By employing semen sexing, Indian dairy farmers can concentrate on raising superior female animals, which could eventually result in genetic advancement. The dairy business becomes more competitive because of this selective breeding strategy's assistance in preserving and developing the desirable features for milk production. Sexed semen has the additional benefit of reducing the occurrence of dystocia, or difficult calving, thus providing another advantage. As India's population grows, so does the demand for milk and dairy products. To meet this demand sustainably, dairy production must be optimized. By using sexed semen, farmers can increase the production of female calves, thereby enhancing milk output efficiently.

Table 4: Advantages of using sexed semen

Benefits of ensuring female offspring with sexed semen	Increases milk production. Provides replacement heifers, boosting livestock farming's economy.
Addressing the issue of cattle slaughter restrictions	Helps control the birth of male calves, reducing unproductive bulls. Optimizes feed resources for livestock.
Meeting demand for elite male offspring in assisted breeding programs	Y-chromosome sorted semen can be used for AI to produce male offspring.
Considerations for meat production, particularly in goats	Male offspring desired due to better feed conversion efficiency and lean-to-fat ratio
Reducing disease introduction and procurement of new heifers	Minimizes the need for new heifer procurement. Decreases the risk of introducing diseases like brucellosis, Johne's disease, and FMD.

Factors affecting adoption of sexed semen

The adoption of AI is influenced by various factors within the realm of dairy farming. Owner education, herd size, information accessibility, and knowledge pertaining to AI positively contribute to the adoption of this reproductive technology (Fig. 2). Challenges such as the absence of AI centers and suboptimal conception rates contribute to a dependence on natural breeding methods. Economic factors, including the cost of AI services and geographical distance to service providers, exert a negative influence on adoption. In India, the adoption of sexed semen has also been influenced by its availability. Currently, production facilities for sex-sorted semen are operational at four government and three private semen stations. The government has launched an accelerated breed improvement program utilizing sex-sorted semen, with 44.37 lakh doses produced so far.

Higher returns serve as a positive incentive for the adoption of AI. Moreover, the successful integration of sexed semen technology emphasizes the significance of efficient management practices, incorporating considerations such as conception rate and sex ratio. Dairy farm producers achieving elevated conception rates are more predisposed to integrate sexed semen into their breeding initiatives. It is noteworthy that sexed semen frequently exhibits reduced conception rates in comparison to conventional semen.

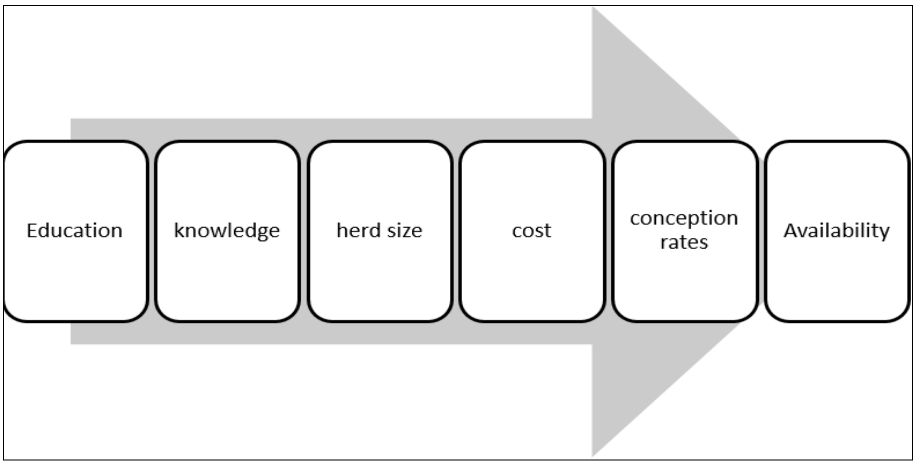

Fig 2: Major factors affecting adoption of sexed semen

Conclusion

The utilization of sexed semen technology is anticipated to have a beneficial effect on farm economies by preserving replacement heifers and enhancing the overall productivity of dairy herds, ultimately resulting in increased milk production. However, it's important to acknowledge that there are challenges associated with sexed semen, particularly in the sorting process, bull fertility, timing and location of insemination, estrus detection, and the proficiency of inseminators. These challenges have a significant impact on the conception rates when using sexed semen in dairy cattle. Ideally, sexed semen should be preferentially employed for inseminating heifers, as they naturally possess higher fertility rates, increasing the likelihood of successful conception. Furthermore, sexed semen can be regularly employed in techniques like embryo transfer and IVF to produce calves of the desired sex, offering more options for controlled breeding and herd management.

Future prospects

Sexed semen technology has the potential to revolutionize dairy herd management and genetic selection. It offers several future prospects, including enhanced genetic selection, improved herd management, market expansion, research and development, and sustainable livestock production. Sexed semen can be used to selectively breed animals for specific traits, leading to improved herd health, productivity, and profitability. It also reduces the need for external procurement of animals, thereby decreasing the risk of disease introduction. Moreover, sexed semen technology can lead to cost savings, as farmers can raise replacements at lower costs than purchasing them. The demand for sexed semen is growing, both domestically and internationally, providing opportunities for market expansion. Research and development in sexed semen technology is ongoing, with a focus on improving conception rates and reducing costs. Overall, the future outlook for sexed sorted bull semen in India appears promising, with potential benefits for both the agricultural sector and the wider economy.

Conflict of interest

Authors do not have any conflict of interests to declare.

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