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# Sexed/Sorted Semen Technology in Dairy Cattle Breeding

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# ABSTRACT

Sexed semen technology favours offspring of a desired sex. Dairy farmers prefer female calves to male calves. Production of more female calves would increase the profitability of the dairy farmers. Artificial insemination with sexed semen rather conventional semen restricts birth of male calves and its associated dystocia. Sperm sex sorting by flow cytometry is one of the most efficient methods. Breeding with sexed semen has a positive correlation with the profit of the dairy farm, as it maintains replacement heifers and increases milk production. In developing countries, application of sexed semen is limited due to its high price, less accuracy and low fertility.

Key words: sexed semen, sorted semen, breeding, flowcytometry, reproduction

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# INTRODUCTION

The selection of desired sex of calf to be born is one of the determining factors in increasing the milk production and profitability of dairy farmers [1]. In dairy farms, female calves are more preferable than male calves in each calving. Production of more number of female calves would increase the profitability of the farmers[2]. Sexed semen is a new reproductive technology that helps to alter the sex ratio of the offspring toward a desired gender[3]. About 90% of the newborn will be from the desired sex[4]. Sexed semen may be used to produce replacement heifers[5]and to improve the annual genetic gain by production of superior replacement heifers[6,7].

Breeding of cows by using conventional frozen semen straws result in surplus production of male calves [8]. Male calves increase the risk of dystocia compared with heifer calves and they have a low economic value. Incorporating sexed semen into the breeding programme can minimise the number of unwanted male dairy calves and reduce dystocia[1].Sexed semen can reduce the occurrence of dystocia by 20% as heifer calves are smaller and easier to calve[9,10]. Moreover, dystocia associated mortality is 57% greater with male calves than with female calves[11].

Artificial insemination of farm animals with sex sorted semen is approved by Food and Agricultural Organization to meet out the food requirement of growing population. It provides opportunities to increase the number of female calves born that ultimately leads to an increased milk production[12] and income of dairy farmers[13]. Predetermination of calf's sex with 90% accuracy rate has benefits both to the dairy and beef industry[14]. The female calves may be sold as calves or heifers or used as replacement to the cows in the future. Recently, much interest was shown across the globe in adopting sexed semen technology. There are both opportunities and challenges to this technology at farm level[15].

# **BOVINE SPERM SEXING/ SORTING**

Sex sorting is the process of separating the desired sperm cell (X) from bovine semen to fertilize the egg cell. The basic differences such as less DNA content in Y sperms, larger size of X sperm, faster motility of Y sperm, negative surface charge of X sperm, H-Y antigen in Y sperm cell surface are exploited to separate the desired sperms [16]. The separation of X chromosome bearing sperm (X-sperm) from Y chromosome bearing spermatozoa (Y-sperm) is done based on the difference in DNA content [17]. The X -sperm bears 3.8% more genetic material (DNA) than Y- sperm[18], since X chromosome is larger than Y chromosome [19] it is utilised to quickly identify X- and Y-chromosome bearing sperm in cattle. The DNA content of X-Y

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sperms varies according to breeds with 4.22% in Jersey and 4.01% in Holstein. These breed variations did not affect the fertility of sexed semen [20].

# SPERM SEX SORTING BY FLOCYTOMETRY

Sperm sex sorting by flow cytometry is one of the most efficient method for separation of X and Y spermatozoa [21]. The flow cytometric sperm sexing technology is also called "Beltsville Sperm Sexing Technology". It was first developed by Johnson and his colleagues at Beltsville Agricultural Research Centre, USDA[22]. At present, by manipulating the relative abundance of viable X and Y-chromosome bearing sperm can reliably pre-determine the offspring sex. This is typically carried out via a specialized type of flow cytometry called fluorescence-activated cell sorting[23] while using laser to split the unwanted X or Y-chromosome bearing sperm have recently been reported[24]. Flow cytometry is an established method that has been commercially used in cattle for sexing sperms[25]. Sex sorting is possible at 90% accuracy by this method [26]. Other alternative methods for sex sorting are by nutritional, genetic, physical, immunological and swim up method in buffalo semen[27].

# **MECHANISM OF FLOW CYTOMETRY**

Flow cytometry separates the X bearing sperm (female) from Y bearing sperm (male) based on the principle that the former contains more DNA than the latter [28]. The sperm cells are stained with fluorescent dye and are passed through the flow cytometer in the form of droplets containing a single sperm cell. Once illuminated with a laser beam the X-bearing sperm shine brighter than Y-bearing sperm. Then both positive and negative charges are applied to the droplets of sperm. This result in charged droplets being deflected in opposite directions and uncharged droplets would pass straight through. Multiple sperms, damaged cells and cells that were not aligned in proper direction get collected in the uncharged droplets. The final collection has 30% X sperm, 30% Y sperm and 40% unidentified or non-viable sperm[16]. Recently, the accuracy of separation has improved due to advancements in sperm cell positioning, tip of flow cytometry, pressure changes and staining techniques. In flow cytometry, nearly 3, 00,000 to 4, 00,000 cells are separated per minute and the average sperm concentration is 2.1 x 10<sup>6</sup> per 0.25 ml straw[20].

# ECONOMIC BENEFITS OF SEXED SEMEN

Use of sexed semen increases the number of calves of desired sex. Artificial insemination using sexed semen is positively correlated with the profit of the farm as it maintains replacement heifers and increases milk yield[28]. In Japanese dairy herd the use of sexed semen has increased the agricultural income[29]. A study in Brown Swiss cattle revealed that production cost for female calves is influenced by pregnancy rate and rate of calves. Pregnancy rate was lowered but the birth rate of heifers was improved. Artificial Insemination using sexed semen is profitable as it produces offspring to increase the herd [30,31].

The extra cost for sexed once, sexed twice and the wide sexed strategies were 1.7, 2 and 2.7 times, respectively that in unsexed semen strategy. The cost of insemination required for achieving 88% calving in the sexed once, sexed twice and the wide sexed were 2, 4.4 and 6.9 times as that in the unsexed. There was an increase in the number of heifer calves from 44 in the unsexed semen strategy to 56, 64 and 80 in sexed once, sexed twice and sexed wide strategies, respectively[32]. Sexed semen with high fertility has the potential to accelerate herd size, improve animal welfare and increase profitability compared with conventional semen[1].

# LIMITATIONS

In developing countries sexed semen has limited applications due to its high price and low fertility [9]. The price of one sexed insemination dose is four times as the price of the unsexed one [32]. Various studies showed that the success rate of sexed semen is lower than that of unsexed one [33,34,and 35]. About two-thirds of sexed semen fertility reduction might be due to lower sperm number in the insemination dose (2-4 million), and to the adverse effects of the sorting procedures[35]. Staining and exposure to UV during flow sorting tended to increase the incidence of sperm chromosome aberrations[36] that affects its fertilizing ability and embryonic survival.

The sperm concentration in sexed semen straw is lesser (2 million) than in conventional semen straw (20 million), loss of spermatozoa due to damage is more, conception rate is 10 to 20 % less, costlier and the processing time is 3-4 times more than conventionally processed semen [16]. Studies conducted in the US[10], Denmark [33], Australia[37] and Japan [38]imply that the conception rate was lower with sexed semen. The conception rate after artificial insemination with conventional semen was 56.9 % whereas with sexed semen it was 47.3%[39]. The conception rate in Holstein heifers was 67-82% with

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conventional sperm, and it was 40-68% with sexed semen [40]. The overall fertility of sexed semen decreases due to less number of sperms / dose / insemination and damages that occur to sperms during the sorting procedure[35]. The rate of pregnancy per AI was low with sexed semen compared with conventional semen[41]. The lesser pregnancy rate with sexed semen was due to decreased lifespan of spermatozoa in uterus, low semen concentration in straw and due to fertility related issues in bulls[42]. A small decrease in fertility of sexed semen relative to conventional semen can negate much of the economic benefit[1]. Other technological limitations of sexed semen are its low sorting efficiency, wastage (50%) during sorting procedure and low freezing potential. In India, the cost of sexed semen straw is from Rs.1500 to 4500/ dose which is not affordable to the farmers. Another shortfall is that the artificial insemination procedure is not standardized for sexed semen[43, 44].

# CONCLUSION

The application of sexed semen technology in dairy cattle breeding is widespread around the world. The concerns of slaughtering male calves, shortage of dairy replacements and beef cattle have led to a greater demand for sexed semen. It is a profitable tool to the dairy farmers as it assures the production of more female calves. In India, implementation of this technology on a large scale would propagate the population of desired indigenous breeds of cattle and enhance milk production. Current technology has 90% of accuracy, but the cost is more than conventional semen. Although the price of sexed semen is higher and fertility is lower, its application provides greater net profit than the unsexed semen. In the future, further advancements in sexed semen technology would lead to improved fertility, precise sexing accuracy and reduction in its cost.

## **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest

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