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Use of Sexed and Conventional Semen in Dairy Animals

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ABSTRACT

The use of sexed semen has become an important reproductive biotechnology in the dairy industry, allowing producers to predetermine the sex of offspring and increase the production of female calves for herd replacement and genetic improvement. Sex-sorting technologies utilize physical and biochemical differences between X- and Y-chromosome-bearing sperm, such as variations in size, motility, surface proteins, and electrical charge, with flow cytometry being the most reliable method, achieving about 90% accuracy. However, sexed semen has certain limitations, including lower sperm concentration per insemination dose, reduced sperm lifespan, and lower conception rates compared to conventional semen. The sorting process may also cause physiological and molecular stress to sperm due to staining, laser exposure, and mechanical forces, potentially affecting membrane integrity, mitochondrial function, and DNA stability. Despite these challenges, improved estrus detection, precise timing of artificial insemination, hormone supplementation, and optimized sperm doses can enhance fertility outcomes. Overall, sexed semen offers significant benefits for dairy breeding by increasing the proportion of female offspring, improving genetic progress, and enhancing herd productivity.

Keywords: Dairy Operations, Artificial Insemination, Sperm.

INTRODUCTION

Artificial insemination (AI) has been the most successful breeding technique in accelerating genetic gains, increasing productivity and reducing diseases of livestock [1,2]. Breeding methods evolved consistently over time and currently AI with sexed semen has become an essential reproductive technology in the dairy industry, as it allows control over the sex ratio of offspring. This assisted reproductive technology acts based on the inherent difference in DNA content of X and Y bearing sperms. Obtaining an ideal proportion of males and females using sex sorted semen could improve productive efficiency. Moreover, efficient selection of superior replacement heifers would be possible after the use of sex sorted semen [3]. In addition, more efficient progeny testing programmes, MOET (multiple ovulation and embryo transfer) and IVP (*in vitro* embryo production) are expected from the use of sex sorted semen [4]. Consequently, the ability to predetermine the sex of offspring promotes the efficient production of females and enhances profit margins in dairy operations.

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METHODS OF SPERM SEXING

Traditional sperm sexing techniques rely on physical and biochemical differences between X- and Y-chromosome-bearing sperm. One such method utilizes a gradient solution to separate sperm based on swimming ability. Since Y-bearing sperm are generally smaller and more motile, they migrate faster through the gradient than X-bearing sperm, resulting in two enriched fractions with an approximate accuracy of 75% [5]. Immunological methods, particularly those targeting surface antigens like H-Y, have been extensively researched for sorting X and Y sperm, but their reported efficiency and ability to specifically separate Y-bearing sperm are inconsistent across studies [6]. Additionally, free-flow electrophoresis distinguishes sperm according to their surface electrical charge, where X-bearing sperm exhibit a more negative charge and Y-bearing sperm a relatively positive charge. These variations in surface charge enable sperm separation when subjected to an electric field, achieving a reported success rate of around 81% [7]. Differences in size are also exploited, as Y-bearing sperm are typically smaller than X-bearing sperm. Several techniques have been developed to sort sperm based on these size disparities, though their efficiency varies. Real-time PCR can also be used to evaluate the effectiveness of sperm sexing by amplifying Y-chromosome–specific DNA sequences, such as the SRY gene, to determine the proportion of Y-bearing sperm within a sample [8]. Despite these methods, distinguishing X and Y sperm based solely on physical traits like weight, size, or density remains difficult due to the minimal variations between them. Sperm sorting using flow cytometry remains the most reliable method for selecting X-chromosome-bearing sperm. This technique relies on staining sperm DNA with a nucleic acid–specific fluorescent dye, Hoechst 33342, which allows differentiation between X- and Y-bearing sperm based on their DNA content. The stained sperm are then passed through a specialized flow cytometer for sorting, after which they are collected in supportive media, concentrated, and cryopreserved in sufficient numbers for use in artificial insemination or in vitro fertilization, depending on the species [9].

Comparison Between Sexed and Conventional Semen

Sperm sorting using flow cytometry achieves approximately 90% accuracy, however, it may adversely affect sperm viability [10]. Additionally, lower number of spermatozoa can be packaged into each sexed-semen straw. As a result, the conception rate after artificial insemination (AI) using sexed semen is lower and is about 70–80% of that achieved with conventional semen in heifers and cows [11]. Owing to this reduced efficiency, the use of sexed semen is primarily recommended for the first and second inseminations of nulliparous heifers, as they generally exhibit higher conception rates [12]. Moreover, because of the lower conception rates, sexed semen is commonly used in fertile females that show clear estrus signs and possess desirable genetic traits for breeding [13].

Sexed semen generally exhibits a shorter lifespan within the female reproductive tract and contains fewer spermatozoa per insemination dose compared to conventional semen. Furthermore, embryos produced from sexed semen show reduced developmental competence relative to those derived from conventional semen, both under in vivo and in vitro conditions. Consequently, artificial insemination using sexed semen demands more accurate timing and meticulous handling to achieve optimal fertility outcomes. Sex-sorting technologies, particularly flow cytometry, face several practical constraints. These systems are highly expensive and operate with relatively low sorting efficiency and speed. Skilled and trained personnel are essential for handling the equipment, as even minor mishandling can lead to significant sperm damage. Additionally, in flow cytometry, there is unavoidable loss of approximately 50% of sperm during sorting and it often reduces the freezing potential and post-thaw viability of sorted spermatozoa [14].

The use of sexed semen is considerably more costly than conventional semen due to the high operational expenses and inclusion of intellectual property rights. Moreover, conception rates achieved with sexed semen are typically 10–15% lower, in some finding more decrease has been observed, than those obtained with conventional semen. This is a critical limitation, especially in the countries where artificial insemination covers breedable population [15]. Reduced sperm concentration per dose, as sexed semen generally contains only 2–4 million spermatozoa per straw [16] compared to approximately 20 million in conventional semen, also decreases the conception rates in dairy herds [11].

Physiological and Molecular Damage During Sex-Sorting

Several studies have investigated the impact of sex-sorting procedures on sperm membrane integrity and function. It has been speculated that the transfer of molecular energy from DNA to fluorochromes during the staining process can induce chromatin decondensation, potentially affecting the genetic material of sperm. The extent of this damage largely depends on chromatin stability, and the use of high dye concentrations during sorting has been associated with adverse effects on sperm motility parameters [17]. Research on animal models has also shown that exposure to fluorescent dyes can disrupt heat shock proteins such as HSP70, leading to alterations in sperm membrane properties resembling capacitation-like changes [18].

The staining and incubation steps involved in sperm sex-sorting procedures may cause DNA damage, raising concerns about the genetic integrity of the processed sperm. Factors such as sorting pressure and speed can also influence sperm quality even before capacitation, highlighting the importance of maintaining precise control over sorting conditions. Additionally, exposure to UV-laser light during sorting has been shown to induce notable DNA damage, potentially compromising the genetic quality of sorted spermatozoa [19].

Furthermore, the electrical charging and electrostatic deviation applied during sorting can disrupt sperm membrane stability, particularly in the mid-piece and tail regions, leading to depolarization. This depolarization can reduce mitochondrial activity, primarily due to the generation of reactive oxygen species (ROS) under electric stress. Elevated ROS levels in seminal plasma are known to cause oxidative damage to sperm DNA, further affecting genetic viability [15]. Spermatozoa with compromised DNA integrity may also compete with healthy ones during fertilization, potentially reducing embryo viability in assisted reproduction [20].

Strategies to improve fertility of sexed semen

The success of achieving pregnancy following artificial insemination (AI), whether using conventional or sex-sorted semen, depends on multiple factors. These include the number of spermatozoa per straw, semen quality, the site of semen deposition, and the timing of insemination relative to the estrous cycle. Efforts to enhance fertility through the administration of exogenous gonadotrophins, either at the onset of estrus or at the time of insemination, have shown some promise, although results have been variable [21]. Therefore, strategies such as hormone supplementation, improved estrus detection, and precise timing of AI may significantly enhance the effectiveness of sexed semen in dairy breeding programs.

Increasing the number of sperm per insemination dose has been identified as another approach to improving fertility, suggesting that higher insemination doses can increase the likelihood of conception [22]. Additionally, the integration of estrus synchronization programs with AI has proven to be an effective method for improving fertility by optimizing the timing and coordination of insemination procedures [23]. Some researchers have investigated strategies to minimize this potential damage, including one effective method involving the use of SYBR14/propidium iodide staining, which has been shown to cause significantly less harm than conventional Hoechst 33342 staining [24]. Reducing the sorting pressure from 50 psi to 40 psi has also been associated with improved sperm quality in both bulls and stallions [25]. Similarly, employing UV lasers equipped with argon or solid-state sources can help minimize cellular damage during sorting [26,27].

CONCLUSION

Sexed semen represents a transformative tool in dairy herd management, enabling increased proportion of female offspring for milk production, replacement stock generation, enhanced genetic gain and productivity. However, its widespread adoption is constrained by reduced fertility, high costs, and technical limitations associated with sperm sorting. Strategic use in genetically superior and reproductively sound females, along with advancements in sorting technologies and fertility-enhancing protocols, can significantly improve outcomes. Future integration with genomic selection and precision reproductive management is expected to further enhance its efficiency and applicability in dairy production systems.

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