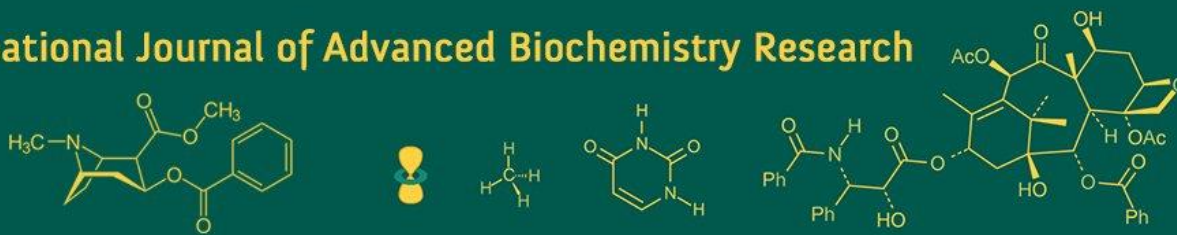


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Evaluation of sexed semen on conception rate in crossbred heifers and cows under field conditions

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Abstract

The present study was undertaken to evaluate the conception rate in dairy cows inseminated with sexed semen under field conditions. A total of 97 crossbred dairy heifers and cows, aged 16 months and above with a BCS 2.75-3.0 (5-point scale), were confirmed non-pregnant by transrectal palpation and assigned to different treatment groups. Cows (n=25) in estrus inseminated with conventional semen as Group I, heifers (n=30) in estrus inseminated with sexed semen as Group II, Cows (n=30) in their second lactation or beyond inseminated with sexed semen as Group III and heifers (n=12) in estrus inseminated with conventional semen as Group IV. Artificial insemination was performed on cows and heifers exhibiting estrus, characterized by the presence of clear cervico-vaginal mucus. Cows returning to estrus after insemination were re-inseminated according to their assigned group. Cows that did not return to estrus were subjected to pregnancy diagnosis at 30 days post-insemination using a Pregnancy Associated Glycoprotein (PAG) kit, with confirmation on the 45th day through rectal palpation and ultrasound scanning. The comparatively lower conception rate with sexed semen can be improved by combining its use with effective farm management practices and targeting high-quality heifers and cows with excellent reproductive and productive performance.

Keywords: Sexed semen, heifer, cow, conception rate, pregnancy associated glycoprotein, ultrasonography

Introduction

The livestock sector plays a crucial role in India's agricultural economy. To enhance livestock productivity, including milk, meat, wool, and hide, utilizing animals with superior genetic potential is key. The cattle population has shifted towards females, with female cattle numbers increasing from 102.98 million in 1992 to 145.12 million in 2017, while male numbers dropped from 101.59 million to 47.4 million. This trend reflects farmers' preference for female animals to meet the growing demand for milk, expand herds, and enhance profitability (Holden and Butler, 2018; Verma *et al.*, 2020) ^[11, 28].

Sexed semen technology, which sorts sperm bearing X and Y chromosomes to produce offspring of a desired sex, has achieved accuracy rates of 80-90% (Talokar *et al.*, 2017) ^[26]. In contrast, conventional AI results in a near-equal distribution of male and female calves, which can be economically disadvantageous for dairy farms (Karakaya *et al.*, 2014; Guner *et al.*, 2020) ^[14, 9]. The advantages of sexed semen are numerous, including increased production of female calves, improved milk yield, efficient herd expansion, reduced dystocia risk, genetic gains, and enhanced efficiency in progeny testing, embryo transfer (Seidel, 2014; Ettema *et al.*, 2017; Cottle *et al.*, 2018) ^[23, 7, 5]. Despite these benefits, its adoption in developing countries remains limited due to its higher cost and lower fertility rates compared to conventional AI (Holden and Butler, 2018; Neculai-Valeanu and Ariton, 2021) ^[11, 18]. However, combining sexed semen with effective farm management practices can improve the quality of female calves and support internal herd growth (Talokar *et al.*, 2017) ^[26].

Materials and Methods

The present investigation was conducted from May 2023 to June 2024, involving dairy cows presented for insemination at the Department of Veterinary Gynaecology and Obstetrics, Hebbal, Bengaluru, as well as cows maintained by dairy farmers in and around Bengaluru, Karnataka.

A total of 97 crossbred dairy heifers and cows, aged 16 months and above with a BCS 2.75-3.0 (5-point scale), were confirmed non-pregnant by transrectal palpation and assigned to different treatment groups.

Group I (n=25): Cows in estrus inseminated with conventional semen.

Group II (n=30): Heifers in estrus inseminated with sexed semen.

Group III (n=30): Cows in their second lactation or beyond inseminated with sexed semen.

Group IV (n=12): Heifers in estrus inseminated with conventional semen.

Frozen sexed semen straws for artificial insemination were procured from the Department of Animal Husbandry and Veterinary Services, Karnataka, supplied by ABS GENUS Semen Straws, Uruli Kanchan, Pune, Maharashtra, using "Sexcel" technology, with each straw containing 2×10^6 sexed spermatozoa. Conventional semen straws were obtained from the Central Frozen Semen Production & Training Institute, Hesserghatta, Bengaluru, Karnataka, each containing 20×10^6 unsexed spermatozoa from a Holstein Friesian bull. Artificial insemination was performed on cows and heifers exhibiting estrus, characterized by the presence of clear cervico-vaginal mucus. Sexed frozen semen from ABS Global or conventional frozen semen was used for insemination (Fig 1). Due to the shorter viability of sexed semen in the female reproductive tract, insemination was conducted 12-18 hours after the onset of standing heat behavior (Thomas, 2021) [27], with the semen deposited in the body of the uterus, just anterior to the internal os of the cervix.

Cows returning to estrus after insemination were re-inseminated according to their assigned group. Cows that did not return to estrus were subjected to pregnancy diagnosis at 30 days post-insemination using a Pregnancy Associated Glycoprotein (PAG) kit, with confirmation on the 45th day through rectal palpation and ultrasound scanning. Conception rate for heifers and cows inseminated with both sexed and conventional semen were recorded.

Statistical Analysis

Data related to conception rate, was calculated for both types of semen employed for insemination. Statistical analysis (chi-square test) of data and results was performed using GraphPad Prism version 10.0.0.

Results and Discussion

The conception rate using sexed semen was evaluated in both heifers and cows (Table 1). Among the 30 heifers inseminated with sexed semen, 9 (30%) were positive for pregnancy. In cows, 12 (40%) out of 30 were positive for pregnancy. Similarly, the conception rate using conventional semen was assessed in both heifers and cows (Table 2). Out of 12 heifers inseminated with conventional semen, 5 (41.7%) were positive for pregnancy. In cows, 12 (48%) out of 25 were positive for pregnancy. A comparative analysis of conception rates between sexed and conventional semen is presented in Table 3. In heifers, the conception rate with sexed semen (30%) was lower than with conventional semen (41.7%), though the difference was non-significant ($p>0.05$). Likewise, in cows, the conception rates with sexed and conventional semen were 40% and 48%, respectively, with no significant difference ($p>0.05$). The overall conception rate with sexed semen (35%) was lower than with conventional semen (45.9%), but the difference was not statistically significant ($p>0.05$).

Similar to the current study, conception rates ranging from 31.60% to 39.53% following the use of sex-sorted semen in cattle have been reported by Healy *et al.* (2013) [10], Patel and Jethva (2019) [20], and Guner *et al.* (2023) [8], with rates of 31.60%, 39.53%, and 34.20%, respectively. In contrast, lower conception rates of 23.70%, 21.00%, 25.00%, and 20.70% were observed by Bodmer *et al.* (2005) [4], Andersson *et al.* (2006) [2], Norman *et al.* (2010) [19], and Biswas *et al.* (2013) [3], respectively. On the contrary, higher conception rates compared to the current study using sex-sorted semen were reported by Hutchinson *et al.* (2013) [13], Remmik *et al.* (2016) [21], Drake *et al.* (2020) [6], and Guner *et al.* (2023) [8], with rates of 53.00%, 56.30%, 51.00%, and 48.90%, respectively.

A study by Seidel (2022) [24] reported conception rates for heifers inseminated with sexed semen to be between 25% and 40%, depending on factors such as the timing of AI and the quality of semen used. An *et al.* (2010) [1] suggested that the decreased conception rates are mainly a result of this low sperm count. Additionally, factors associated with the flow cytometric sex-sorting process, such as UV-laser exposure (Lubart *et al.*, 1997) [15], pressure effects (Suh *et al.*, 2005; Schenk and Seidel, 2009) [25, 22], and dilution (Maxwell and Stojanov, 1996) [17], contribute to reduced sperm quality. These factors can induce capacitation, cause membrane damage (Maxwell *et al.*, 1998) [16], and lead to a rapid loss of motility (Hollinshead, 2003) [12].

Table 1: Conception rate in animals inseminated with sexed semen

| Parameter | No of animals inseminated | No of animals positive for pregnancy | Conception rate (%) | χ^2 value, df |
|-----------|---------------------------|--------------------------------------|---------------------|-------------------------|
| Heifers | 30 | 9 | 30.00 | 0.428 ^{ns} , 1 |
| Cow | 30 | 12 | 40.00 | |

Note: ns=non-significant

Table 2: Conception rate in animals inseminated with conventional semen

| Parameter | No of animals inseminated | No of animals positive for pregnancy | Conception rate (%) | χ^2 value, df |
|-----------|---------------------------|--------------------------------------|---------------------|-------------------------|
| Heifers | 12 | 5 | 41.67 | 0.129 ^{ns} , 1 |
| Cow | 25 | 12 | 48.00 | |

Note: ns=non-significant

Table 3: Comparison of Conception Rates - Sexed vs. Conventional Semen

| Sl. No. | Parameter | Sexed | Conventional | χ^2 value, df |
|---------|-----------|---------------|---------------|--------------------------|
| 1. | Heifers | 30.00 (9/30) | 41.67 (5/12) | 0.5250 ^{ns} , 1 |
| 2. | Cows | 40.00 (12/30) | 48.00 (12/25) | 0.3548 ^{ns} , 1 |
| 3. | Overall | 35.00 (21/60) | 45.95 (17/37) | 1.151 ^{ns} , 1 |

Note: ns=non-significant

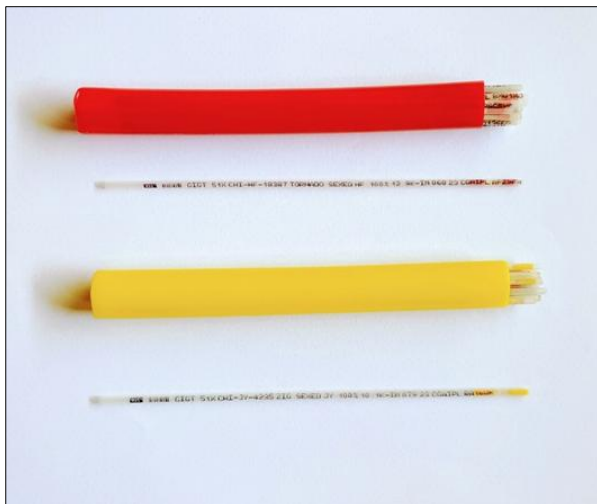


Fig 1: Sex sorted semen straws

Conclusion

The results of this study indicate that the conception rate with sexed semen was lower than with conventional semen, although the difference was not statistically significant. These findings suggest that, while sexed semen may have a slightly lower fertility potential than conventional semen, integrating its use with effective farm management practices and targeting high-quality heifers and cows with excellent reproductive and productive performance may enhance conception rates in both heifers and cows.

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