Sexed semen efficacy at spontaneous and synchronized estrus in crossbred cows

A. Sharma[1], P. Sood[1], P. Kumar[1], P. Sharma[2]*, A. Sharma[1], A.K. Ahuja[1], H. Kumar[2], V. Negi[2]

[1]Dr. G.C. Negi College of Veterinary and Animal Sciences, CSK Himachal Pradesh Agricultural University, Palampur; [2]Department of Animal Husbandry, Himachal Pradesh, INDIA
*Corresponding author e-mail: pururavasharma@gmail.com

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Abstract

The present study investigated the complex dynamics between sexed semen utilization, estrus synchronization, and reproductive outcomes in crossbred cows under field conditions. The study, comprising apparently healthy crossbred cows, explored the efficacy of sexed semen straws in both spontaneous and synchronized estrus scenarios. Estrus detection methods included visual observation of behavioral signs, while synchronization was achieved through the Ov-synch protocol. Artificial insemination was conducted using sexed semen at appropriate timings based on estrus signs and hormonal synchronization. Gynecological examinations, including trans-rectal sonography, assessed factors like pre-ovulatory follicle diameter, genital discharge characteristics, and pregnancy progression. Statistical analysis revealed significant differences in Spinnbarkeit values between synchronized and spontaneous estrus, with tendencies toward higher conception rates in synchronized estrus scenarios. Additionally, higher pregnancy losses were observed in cows inseminated during spontaneous estrus, leading to lower overall pregnancy rates compared to synchronized counterparts. Remarkably, a higher proportion of female calves were born from cows inseminated during synchronized estrus. Future research endeavors aimed at elucidating the underlying mechanisms governing the interaction between sexed semen, estrus synchronization, and reproductive outcomes, will further enhance its understanding and utilization in cattle breeding programs.

Keywords: Ovsynch protocol, Sexed semen, Spontaneous estrus

Introduction

In India, limited and declining dairy herds expansion (Toor and Kaur 2023) and the percentage of progressive farmers has a key involvement for use of sexed semen under the field conditions (Sharma et al. 2024). The sexed semen technology was developed in United States Department of Energy's Lawrence Livemore Laboratory in 1980's at California and first insemination in cows was carried out in late 1990's (Seidel 2014). The integration of sexed semen technology with estrus synchronization protocols represents a promising approach for optimizing reproductive efficiency and genetic progress in bovine production systems (Patra et al. 2023). During spontaneous estrus, sexed semen allows breeders to capitalize on the cow's natural reproductive cycle, enhancing the chances of conception while also ensuring the desired gender of the offspring (Reese et al. 2021). Furthermore, the use of sexed semen during synchronized estrus, often employed in artificial insemination programs, maximizes reproductive efficiency (Tsiligianni et al. 2011). The sexed semen presents exciting opportunities, its efficacy depends on various factors such as uterine health, insemination technique, time of insemination and overall herd management practices (Chebel and Kunha 2020). Thus, its involvement in reproductive programs requires careful consideration and monitoring to ensure optimal results and long-term genetic progress in dairy cattle (Colazo and Mapletoft 2017). Keeping in view, the present study delves into the intricate interplay between sexed semen usage, estrus synchronization, and reproductive outcomes, drawing insights from findings under the field conditions.

Materials and Methods

The study population was apparently healthy crossbred cows (75% Jersey blood; Parity 1-3; Body Condition Score- 2.75-3.00; N=166) reared in the semi-loose housing system at Palampur, Himachal Pradesh (Fig 1). The experiment was designed to adjudge the efficacy of the sexed semen straws at spontaneous (n=81) and synchronized (n=85) estrus under field conditions. For detection of spontaneous estrus, visual signs were observed twice a day for 30 minutes for the expression of estrus signs. Estrus synchronization was achieved through application of Ov-synch protocol on day 7 after manifestation of estrus signs (Singh et al. 2019). In the Ovsynch protocol, a GnRH injection was administered on day 0, followed by an injection of prostaglandin $F_2\alpha$ on day 7. A second GnRH injection was given 48 hours later (day 9), and timed artificial insemination was performed 8-16 hours after the second GnRH injection (Singh et al. 2019). At estrus, cows were appropriately examined via trans-rectal examination and sonography (TRUS) (Mindray Z5 VET; Model 2017) for visualizing the ovarian structures i.e., diameter of pre-ovulatory follicle. The cows in estrus were appropriately secured in a crush before transrectal examination and sonography. Both the transducer and the sleeved arm were thoroughly lubricated with an obstetric lubricant to enable painless and easy insertion through the anal sphincter. The transducer was placed proximal to the genital tract and moved slowly toward the cranial side to visualize the ovarian structures, i.e., the diameter of the preovulatory follicle.

Genital discharge aspirated was subjected to pH and Spinnbarkeit value (SBK) examination. Two to three drops of the collected mucus sample were placed on a grease-free slide, and another slide was placed over it. The mucus was stretched between the two slides by moving the second slide away from the first slide until it broke. A plastic geometrical scale (cm) was used to measure the distance between the two slides immediately before the breakage of the mucus string.

Artificial Insemination was performed in cows with clear genital discharge, and were inseminated after 16-18 hours (mid-estrus) of onset of estrus signs and 8-16 hours after GnRH administration at spontaneous and synchronized estrus, respectively, with placement of sexed semen (Intelligen technology Sexcel semen straw; 2 million spermatozoa) in the uterine body. The standard thawing procedure i.e. 37ºC for 30 seconds, were adopted as recommended by Centre for Agriculture and Bioscience International (CABI).

Gynaecological examination for the assessment of late embryonic mortality i.e. every 72 hours from non-return to estrus till onset of period of the fetus, was performed through trans-rectal sonography. Pregnancy diagnosis was carried out at day 75 post-insemination via trans-rectal examination. In order to ascertain the reproductive efficiency, various reproductive indices were evaluated as depicted in Table 1. Numeric data for all the parameters have been expressed as Mean ± SEM and statistical analysis was carried out with Welch's t-test and Pearson's Chi-square test (for categorical data) on SPSS 21.0.

The absence of invasive procedures and the focus on routine diagnostics tended to no requirement of ethical approval committee. All the treatment guidelines have been followed during the research.

Results

Among results, the genital discharge Spinnbarkeit was significantly higher ($p<0.01$) at synchronized rather than spontaneous estrus but pH had no statistical difference. Interestingly, the pre-ovulatory follicle diameter tended to be significantly higher ($p<0.01$) on estrus synchronization and led to a numerically higher overall conception rate in fixed time artificially inseminated cows. The late embryonic mortality and abortions that constituted the pregnancy losses (%) were higher in spontaneous estrus cows and eventually led to a lower

overall pregnancy rate (%) as compared to cows inseminated at synchronized estrus. Similarly, the number of inseminations per conception were significantly higher ($p<0.01$) for spontaneous as compared to synchronized estrus (Table 1).

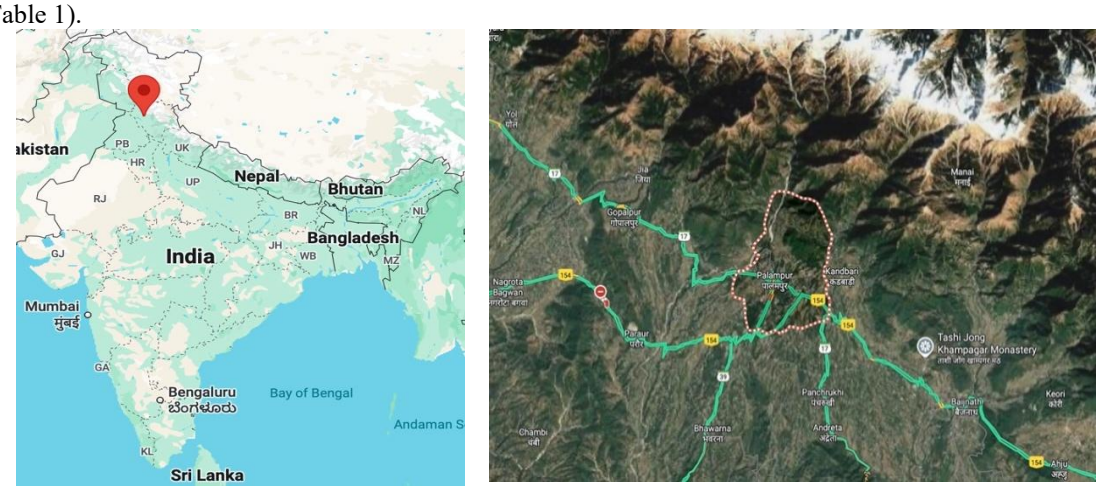

Figure 1: Geographical map of area under study (Palampur, Himachal Pradesh), Red Map Pin (Left image) and Red Dotted area (Right image); Source (Google Maps).

Table 1. Comparison of genital discharge attributes (Mean±SEM), pre-ovulatory follicle diameter (Mean±SEM) and reproductive indices at spontaneous and synchronized estrus.

Parameters	Spontaneous estrus	Synchronized estrus	*p-value*
pH	7.86±0.10	7.73±0.09	0.33
Spinnbarkeit (cm)	11.45±0.21	12.88±0.18	0.00
Pre-ovulatory follicle diameter (mm)	12.00±0.24	13.07±0.20	0.00
35-day First insemination conception rate (%) (FICR)	55.56 (45/81)	52.94 (48/85)	0.90
Overall conception rate (%)	48.50 (81/167)	50.30 (85/169)	0.73
Pregnancy losses (%)	8.64 (7/81)	4.71 (4/85)	0.31
Overall pregnancy rate (%)	46.25 (74/160)	47.92 (81/169)	0.76
Number of inseminations per conception	2.08±0.05	1.69±0.06	0.00
Percent female calves born (%)	89.19 (66/74)	91.35 (74/81)	0.64

Discussion

The observed differences in genital discharge, spinnbarkeit, pre-ovulatory follicle diameter, and conception rates between synchronized and spontaneous estrus cycles underscore the multifaceted influence of estrus synchronization protocols on reproductive parameters. The significantly higher spinnbarkeit at synchronized estrus suggests a potentially favorable cervical mucus environment conducive to sperm transport, albeit without a discernible effect on pH levels (Layek et al. 2013). This finding aligns with previous research indicating the beneficial impact of synchronization on cervical mucus quality, which can enhance sperm survival and migration (Tsiligianni et al. 2011). Moreover, the tendency towards larger pre-ovulatory follicle diameter following estrus synchronization corroborates to the improved follicular dynamics and ovulatory synchrony achieved through hormonal manipulation (Singh et al. 2019). This, in turn, may contribute to enhanced oocyte quality and subsequent embryo development, as evidenced by the numerically higher conception rates observed in cows subjected to fixed-time artificial insemination (FTAI) protocols (Lopez Gatius et al. 2022). The comparison of conception rates between cows inseminated with sexed semen at synchronized versus spontaneous estrus yields intriguing insights. While the 35-day first insemination conception rate did not exhibit a statistically significant difference between the two groups, the incidence of late embryonic mortality and abortions was notably elevated in cows experiencing spontaneous estrus. This disparity reflects the potential influence of estrus synchronization on embryo viability and pregnancy maintenance, possibly mediated by variations in uterine receptivity and endocrine milieu during the peri-conception period (Szelenyi et al. 2023). Notably, the sex skewing efficacy of sexed semen remains a focal point of interest, with a higher proportion of female offspring observed across both synchronized and spontaneous estrus groups (Colazo and Mapletoft 2017). Similar results in female percentage have been observed in various studies world-wide with 87% in German Holsteins (Diers et al. 2020), 89-91% in U.S. dairy herd (Norman et al. 2010) and 91.7% in Tharparkar cows in India (Patra et al. 2023). This reaffirms the reliability and consistency of sexed semen technology in achieving desired sex ratios, thus offering

producers greater control over herd composition and genetic selection strategies in dairy industry (Maicas et al. 2019).

Conclusion

Synchronized estrus improved cervical mucus quality, pre-ovulatory follicle development and reduced the number of inseminations per conception. Pregnancy losses were lower in synchronized cows, contributing to better overall reproductive efficiency. While conception rates were comparable between synchronized and spontaneous estrus; synchronization offered practical reproductive advantages.

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