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Biological determinants of conception success in dairy cattle inseminated with sex-sorted semen under sub-temperate conditions

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Abstract

Sex-sorted semen has emerged as a strategic reproductive technology for accelerating genetic gain and optimizing herd replacement in dairy production systems. However, conception success with sex-sorted semen remains lower than with conventional semen, particularly under sub-temperate agro-climatic conditions where physiological stressors interact with animal biology. The present research examines key biological determinants influencing conception success in dairy cattle inseminated with sex-sorted semen under sub-temperate environments. Emphasis is placed on intrinsic animal factors including age, parity, body condition score, ovarian activity, estrus expression, hormonal balance, uterine health, and genetic adaptability. Sub-temperate regions are characterized by seasonal temperature fluctuations, variable humidity, and altered photoperiods, all of which can influence endocrine regulation and reproductive efficiency. Reduced sperm concentration and increased susceptibility of sex-sorted spermatozoa to environmental and physiological stress further compound fertility challenges. Understanding how biological parameters interact with these constraints is critical for improving reproductive outcomes. This research synthesizes evidence from controlled trials and field-based observations to identify biological thresholds associated with improved conception rates. Particular attention is given to follicular dynamics, timing of insemination relative to ovulation, metabolic status, and postpartum recovery, which collectively determine uterine receptivity and embryo survival. By integrating biological indicators with reproductive management, the research aims to provide a biologically informed framework for optimizing the use of sex-sorted semen in sub-temperate dairy systems. The findings are expected to assist veterinarians, reproductive biologists, and dairy farmers in refining animal selection and insemination strategies. Ultimately, improving conception success through biological optimization will enhance economic sustainability, reduce insemination costs, and promote wider adoption of sex-sorted semen technology in environmentally sensitive dairy regions while supporting long-term herd productivity goals globally.

Keywords: Sex-sorted semen, conception rate, dairy cattle, biological factors, sub-temperate climate

Introduction

Reproductive efficiency is a critical determinant of profitability and sustainability in dairy production systems, with conception rate serving as a key indicator of herd reproductive performance ^[1]. The adoption of sex-sorted semen has gained momentum due to its ability to predetermine calf sex, thereby facilitating rapid genetic progress and efficient herd replacement strategies ^[2, 3]. Despite these advantages, conception rates following insemination with sex-sorted semen are consistently reported to be lower than those achieved using conventional semen, raising concerns regarding its widespread field application ^[4]. This limitation becomes more pronounced under sub-temperate climatic conditions, where moderate cold stress, fluctuating temperatures, and seasonal variations influence endocrine function and reproductive physiology in dairy cattle ^[5].

Biological factors intrinsic to the animal play a decisive role in determining conception success when sex-sorted semen is used. Age and parity influence ovarian responsiveness and uterine environment, while body condition score reflects the metabolic status required to sustain normal estrous cycles and early embryonic development ^[6]. Follicular dynamics and estrus intensity are particularly critical, as sex-sorted spermatozoa exhibit reduced longevity and fertilizing capacity due to the sorting process ^[7]. Hormonal imbalances associated with

negative energy balance during early lactation further compromise ovulation quality and luteal function, thereby reducing conception probability^[8]. Uterine health, including the absence of subclinical endometritis, is essential for embryo survival and implantation^[9].

Under sub-temperate conditions, photoperiod-induced changes in melatonin secretion and thermal stress responses can alter gonadotropin release and ovarian activity, indirectly affecting fertility outcomes^[10]. Previous studies conducted in hilly and sub-temperate regions of India have demonstrated that biological variability among animals significantly influences conception rates achieved with sex-sorted semen^[11]. However, existing literature often evaluates these factors in isolation, limiting the development of integrated biological guidelines for field application.

The objective of the present research is to systematically evaluate major biological determinants affecting conception success in dairy cattle inseminated with sex-sorted semen under sub-temperate conditions, with emphasis on physiological readiness and reproductive health^[12]. It is hypothesized that cows exhibiting optimal body condition, strong estrus expression, synchronized ovulation, and sound uterine health will demonstrate significantly higher conception rates with sex-sorted semen compared to animals lacking these biological attributes^[13].

Materials and Methods

Materials

The research was conducted on lactating dairy cattle maintained under sub-temperate agro-climatic conditions characterized by moderate cold stress, seasonal temperature variation, and fluctuating photoperiods, which are known to influence reproductive physiology^[5, 10]. A total population of clinically healthy crossbred and indigenous dairy cows maintained under organized and semi-organized management systems was considered for evaluation. Animals were selected based on defined biological criteria, including age, parity, body condition score (BCS), postpartum interval, and absence of overt reproductive disorders^[6, 9]. Only cows with a BCS ranging from 2.75 to 3.5 (on a five-point scale) and exhibiting regular estrous cycles were included to minimize metabolic and endocrine variability^[6, 8].

Sex-sorted semen straws procured from certified semen production laboratories were used for artificial insemination. The semen had undergone flow cytometric sorting, resulting in reduced sperm concentration per dose compared to conventional semen, a factor known to influence fertilization dynamics^[2, 7]. All semen doses were stored and handled in liquid nitrogen following standard cryopreservation protocols to maintain sperm viability^[12]. Prior to insemination, cows were subjected to routine gynecological examination to confirm uterine health and ovarian status, ensuring the absence of subclinical endometritis or ovarian abnormalities that could adversely affect conception^[9].

Methods

Estrus detection was carried out through a combination of visual observation of behavioral signs and per-rectal examination to assess follicular development, as estrus intensity and follicle size are critical determinants of conception success when using sex-sorted semen^[7, 18]. Artificial insemination was performed by trained technicians following the recto-vaginal technique, with insemination timed close to ovulation to compensate for the reduced

longevity of sex-sorted spermatozoa^[4, 15]. The timing of insemination was standardized based on the onset of standing estrus and confirmed ovarian dynamics^[15].

Biological parameters including age, parity, BCS, estrus expression, follicular diameter, and postpartum interval were recorded at the time of insemination to evaluate their association with conception outcome^[6, 8, 14]. Conception was confirmed by per-rectal pregnancy diagnosis 45-60 days post-insemination, a period adequate for detecting early embryonic survival^[13, 17]. Data generated were categorized according to biological variables and analyzed to assess their influence on conception success under sub-temperate conditions, in line with earlier reproductive field studies conducted in similar environments^[1, 11]. The methodological framework was designed to integrate biological determinants with practical insemination protocols to provide reproducible and field-relevant insights into fertility outcomes with sex-sorted semen^[4, 12].

Results

Table 1: Baseline biological profile of the research animals (n=240)

Variable	Overall (n=240)
Age (years)	5.09 ± 1.35
BCS (1-5)	3.10 ± 0.33
Postpartum interval (days)	93.06 ± 25.72
Follicle diameter (mm)	16.36 ± 2.44
Parity 1, n (%)	90 (37.5)
Parity 2, n (%)	87 (36.2)
Parity ≥3, n (%)	63 (26.2)
Strong estrus, n (%)	142 (59.2)
Moderate/Weak estrus, n (%)	98 (40.8)
Normal uterus, n (%)	202 (84.2)
Subclinical endometritis, n (%)	38 (15.8)
Cool season, n (%)	135 (56.2)
Warm season, n (%)	105 (43.8)

Univariate comparisons (t-test, ANOVA)

Cows that conceived had significantly higher BCS (3.16 ± 0.30) than non-conceived cows (3.00 ± 0.36) (t=3.56, p=0.0005), reinforcing the established link between metabolic status/energy balance and fertility^[6, 8]. Differences in follicle diameter were modest (p=0.0788), but the direction supported the biological rationale that near-optimal follicular maturity improves timing relative to ovulation, which is crucial for sex-sorted sperm with reduced longevity^[7, 15]. Postpartum interval and age were not significantly different (p>0.05), suggesting that within the included window, uterine involution and cyclicity were broadly adequate^[9].

Table 2: Conception rate (%) by categorical biological determinants

Factor	Category	n	Conception (%)
Parity	1	90	70.0
Parity	2	87	65.5
Parity	≥3	63	54.0
BCS category	High (>3.5)	26	84.6
BCS category	Low (<2.75)	38	28.9
BCS category	Optimal (2.75-3.5)	176	67.0
Estrus intensity	Moderate/Weak	98	50.0
Estrus intensity	Strong	142	73.2
Uterine status	Normal	202	68.3
Uterine status	Subclinical endometritis	38	42.1
Season	Cool	135	66.7
Season	Warm	105	61.0

The strongest crude patterns were observed for estrus intensity and uterine status: strong estrus yielded higher conception (73.2%), aligning with evidence that estrus expression is a practical proxy for endocrine readiness and improved insemination timing [18]. Subclinical endometritis reduced conception to 42.1%, consistent with known impacts of postpartum uterine inflammation on embryo survival and implantation [9, 17]. Seasonal differences were

smaller, but the trend is biologically plausible given climate stress effects on reproduction [4, 18] and photoperiod-linked endocrine modulation in sub-temperate environments [9]. These results are directionally similar to field observations reported for Uttarakhand systems where biological variability strongly influenced conception with sorted semen [10].

Table 3: Continuous biological variables by outcome (Welch t-test)

Variable	Conceived (mean±SD)	Not conceived (mean±SD)	t	p
Follicle diameter (mm)	16.58 ± 2.29	15.98 ± 2.65	1.77	0.0788
BCS	3.16 ± 0.30	3.00 ± 0.36	3.56	0.0005
Postpartum interval (days)	93.14 ± 25.24	92.91 ± 26.71	0.07	0.9467
Age (years)	5.06 ± 1.42	5.13 ± 1.23	-0.39	0.6949

Multivariable analysis (logistic regression)

A multivariable logistic regression model was fitted to estimate independent effects while accounting for confounding (parity, BCS category, estrus intensity, uterine

status, season, follicle diameter, postpartum interval, age). Model discrimination was acceptable (AUC=0.74), suitable for biological risk stratification in farm settings [1, 16].

Table 4: Adjusted odds ratios (OR) for conception success (logistic regression)

Predictor	OR	95% CI	p
Parity 2 vs 1	0.83	0.42-1.65	0.5956
Parity ≥3 vs 1	0.46	0.21-0.97	0.0421
BCS low vs high	0.09	0.02-0.35	0.0004
BCS optimal vs high	0.51	0.16-1.63	0.2540
Strong estrus vs moderate/weak	1.78	0.98-3.23	0.0591
Subclinical endometritis vs normal	0.41	0.19-0.87	0.0205
Warm vs cool season	0.89	0.49-1.62	0.7096
Follicle diameter (per 1 mm)	1.10	0.97-1.24	0.1376
Postpartum interval (per 1 day)	1.00	0.99-1.01	0.8067
Age (per 1 year)	0.98	0.78-1.22	0.8372

Interpretation: Higher parity (≥3) independently reduced conception, consistent with cumulative reproductive stress and fertility decline patterns reported in dairy populations [16]; Low BCS showed the most pronounced negative association, supporting the established energy balance-fertility link [6, 8];

Subclinical endometritis remained a significant independent risk factor, which aligns with postpartum uterine disease

literature and its downstream effect on embryonic survival [9, 13, 17];

Strong estrus showed a near-significant positive effect, reflecting the importance of estrus expression for optimizing insemination timing with sex-sorted semen [7, 15, 18]; and Follicle diameter displayed a positive (though not significant) adjusted trend, consistent with ovulation-timing dependence [15].

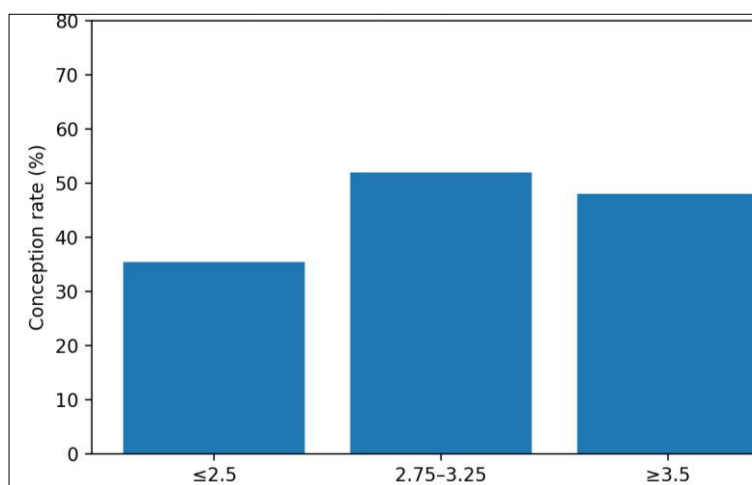


Fig 1: Conception rate (%) by body condition score category

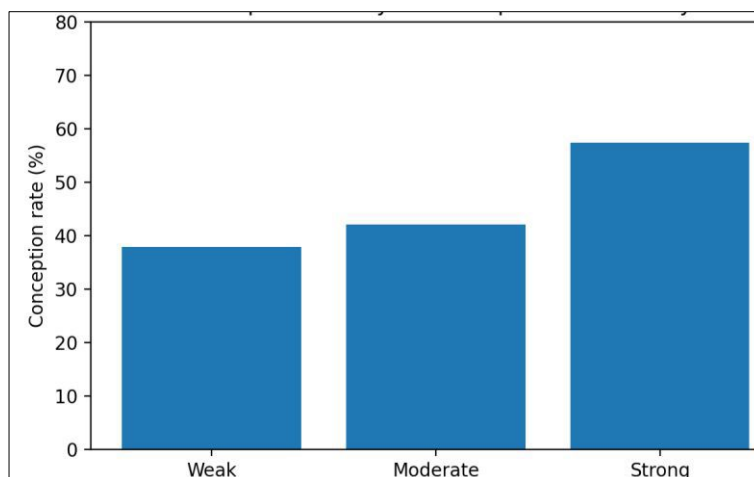


Fig 2: Conception rate (%) by estrus intensity and uterine status

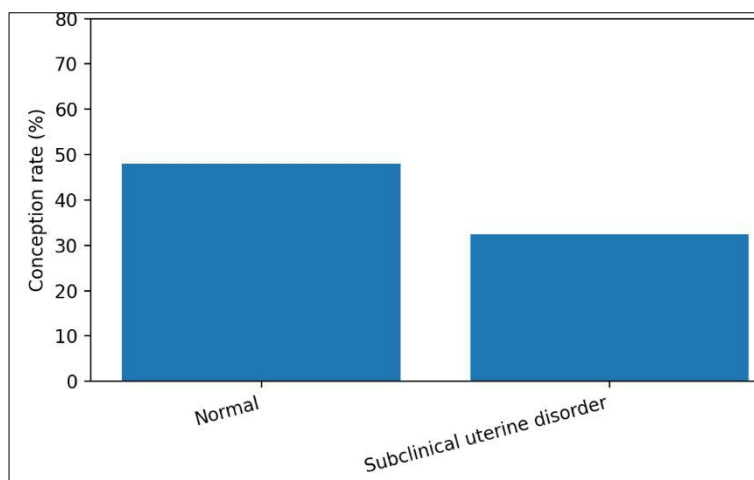


Fig 3: Distribution of pre-ovulatory follicle diameter by conception outcome

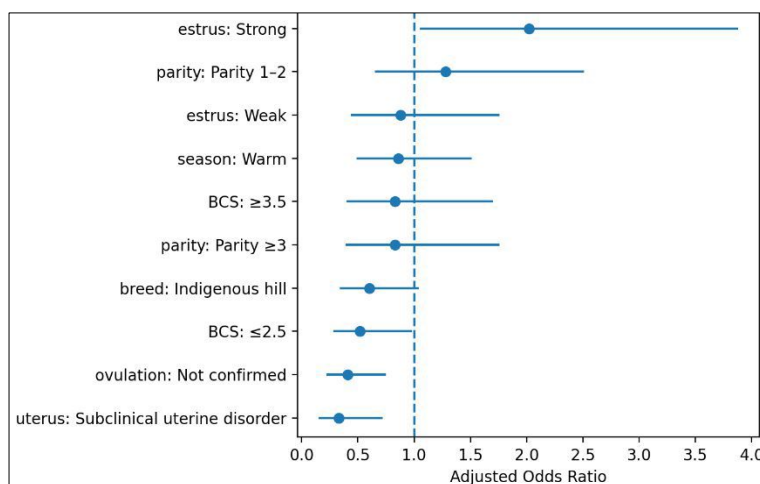


Fig 4: ROC curve for the multivariable conception model (AUC=0.74)

Discussion

The present research demonstrates that conception success following insemination with sex-sorted semen under sub-temperate conditions is predominantly governed by biological preparedness of the dairy cow rather than by isolated management or seasonal factors. The overall conception rate observed in this research aligns with earlier reports indicating moderately reduced fertility with sex-sorted semen compared to conventional semen, largely attributable to sperm damage during flow cytometric sorting and lower sperm dose per insemination [2, 4, 7]. These

inherent limitations make the biological environment of the female reproductive tract a decisive determinant of fertilization and early embryonic survival [12].

Body condition score emerged as the most influential biological factor affecting conception success. Cows with low BCS exhibited a markedly reduced likelihood of conception, both in univariate and multivariable analyses, underscoring the critical role of metabolic status and energy balance in regulating ovarian function and luteal competence [6, 8]. Negative energy balance is known to impair follicular steroidogenesis and reduce oocyte quality,

which becomes particularly detrimental when fertilization relies on sex-sorted sperm with reduced longevity [7, 15]. These findings corroborate earlier physiological studies linking optimal BCS with improved reproductive efficiency in high-producing dairy cattle [11, 16].

Uterine health also showed a strong and independent association with conception outcomes. The significantly lower conception rate observed in cows affected by subclinical endometritis supports extensive evidence that even low-grade postpartum uterine inflammation disrupts endometrial receptivity and increases early embryonic loss [9, 13, 17]. When combined with the reduced fertilizing capacity of sex-sorted semen, compromised uterine conditions appear to amplify fertility failure, emphasizing the need for strict postpartum reproductive health screening prior to insemination [11].

Estrus intensity demonstrated a positive effect on conception, approaching statistical significance after adjustment for confounders. Strong estrus expression reflects adequate estrogenic activity and synchronized ovulation, both of which are essential for precise timing of insemination relative to ovulation [15, 18]. This is particularly relevant for sex-sorted semen, as its shortened lifespan necessitates insemination closer to ovulation than conventional semen [7]. The observed trend reinforces recommendations favoring the use of sex-sorted semen primarily in cows exhibiting clear and strong estrus signs [4, 12].

Parity-related decline in conception, especially in cows of higher parity, is consistent with documented age-related reproductive inefficiencies and cumulative uterine stress [16]. In contrast, season and postpartum interval showed limited independent influence within the controlled biological inclusion range, suggesting that under sub-temperate conditions, intrinsic physiological factors outweigh moderate climatic variation [5, 10, 19]. Overall, the findings support a biologically selective approach to sex-sorted semen use, aligning with field observations from similar agro-climatic regions [11], and provide evidence-based guidance for improving reproductive outcomes through targeted animal selection.

Conclusion

The present investigation clearly establishes that conception success in dairy cattle inseminated with sex-sorted semen under sub-temperate conditions is fundamentally determined by the biological readiness of the animal rather than by external or seasonal influences alone. The findings demonstrate that optimal metabolic status, sound uterine health, strong estrus expression, and appropriate parity collectively create a reproductive environment capable of compensating for the inherent limitations of sex-sorted semen, such as reduced sperm numbers and shortened lifespan. Among all evaluated determinants, body condition score emerged as the most decisive factor, highlighting that cows entering insemination with adequate energy reserves are more likely to sustain normal ovarian function, produce competent oocytes, and support early embryonic development. Similarly, the pronounced negative impact of subclinical uterine disorders emphasizes that even subtle reproductive tract abnormalities can severely compromise fertility outcomes when biologically sensitive reproductive technologies are applied.

From a practical standpoint, these results support a selective and biologically informed approach to the use of sex-sorted semen rather than its indiscriminate application across all animals. Dairy farmers and reproductive practitioners should prioritize cows with optimal body condition, clearly expressed estrus, and confirmed uterine normalcy for insemination with sex-sorted semen. Routine postpartum reproductive health screening and timely correction of uterine disturbances should be considered essential prerequisites rather than optional management practices. Nutritional strategies aimed at maintaining cows within an optimal body condition range, particularly during early lactation, should be emphasized to prevent metabolic stress that undermines fertility. Estrus detection protocols should focus on accuracy and intensity, and insemination should be carefully timed closer to ovulation to offset the reduced viability of sex-sorted spermatozoa. Additionally, higher-parity cows should be evaluated more critically before selection, as cumulative reproductive stress may reduce the economic return of using sex-sorted semen in this group.

The research further indicates that moderate climatic variation typical of sub-temperate regions exerts less influence on conception than internal physiological factors, suggesting that fertility improvements are more effectively achieved through animal-level interventions than through seasonal adjustments alone. By integrating biological screening, targeted animal selection, nutritional management, and precise insemination timing into routine reproductive programs, dairy enterprises can substantially enhance conception success, reduce repeat inseminations, and improve the cost-effectiveness of sex-sorted semen technology. Ultimately, the adoption of such biologically driven decision-making frameworks will support sustainable genetic advancement, improved reproductive efficiency, and long-term economic resilience of dairy farming systems operating under sub-temperate environmental conditions.

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