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Risk Factors Associated with Conception Rate in Holstein and Lai Sind Crossbred Cows in Small Holding Farms in Vietnam

Nguyen Van Thanh, Do Thi Kim Lanh, Nguyen Hoai Nam, and Bui Van Dung*

Department of Animal Surgery and Theriogenology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Trau Quy Town, Gia Lam District, Ha Noi, Viet Nam

*Corresponding author's Email: bvdung@vnua.edu.vn

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ABSTRACT

Reproductive efficiency is a critical factor in smallholder dairy systems, where management limitations often reduce fertility. Identifying factors influencing conception is essential to improving breeding performance in crossbred dairy cows raised in smallholder farms. The present study aimed to identify reproductive and management-related risk factors influencing conception rates in Holstein × Lai Sind crossbred cows at smallholder farms in Northern Vietnam. A retrospective analysis was conducted to evaluate the effects of ovarian disorders, estrus detection timing, age, parity, body condition score (BCS), calving-to-service interval, artificial insemination (AI) order, month of service, and age at first AI on conception rates in 433 cows. Farms were selected based on their willingness to participate, including a total of 40 smallholder farms in Northern Vietnam. Cows were included only if they had complete records on estrus detection, insemination, and pregnancy diagnosis; cows with missing or inconsistent data were excluded. Data was collected from the farm database and direct observation by trained personnel. Univariate analysis identified parity, age, age at first AI, month of AI, and BCS as significant factors influencing conception rates. The overall conception rate was 45.6%. Cows inseminated during December-January had a higher conception rate (52.9%) compared with those inseminated in September-October (37.7%). Heifers exhibited the highest conception rate (55.7%), while cows in parity 2 and parity 4-8 had significantly reduced fertility (37.6% and 42.0%, respectively). In the multivariate logistic regression model, parity and age at first AI were the most influential predictors of reproductive performance in Holstein crossbred cows. Cows first inseminated at 13-15 months, 16-17 months, and older than 17 months had significantly higher conception rates than those inseminated at 8-12 months. The present study demonstrated that both physiological factors (age, parity, and BCS) and seasonal management conditions (month of service) influence conception in Holstein × Lai Sind crossbred cows under smallholder conditions.

Keywords: Artificial insemination, Body condition, Month of service, Smallholder

INTRODUCTION

Reproductive performance is a fundamental factor of economic efficiency in dairy farming. Among several reproductive metrics, conception rate is a critical parameter influencing the number of calves born, the calving interval, and overall milk production, thereby affecting farm profitability (Ehsanollah et al., 2021; Ukita et al., 2022). Globally, dairy herds have experienced a decline in conception rates due to multifactorial influences, including increased metabolic stress associated with high milk production, suboptimal estrus detection, nutritional deficiencies, heat stress, and reproductive tract disorders (Lucy, 2007; Huang et al., 2009; Lee et al., 2024). Despite progress in reproductive management, achieving optimal conception rates is difficult because bovine fertility involves many factors (Kim and Jeong, 2018; Souames and Berrama, 2020). In Vietnam, fertility in dairy cows is strongly affected by smallholder production systems with limited access to advanced reproductive technologies such as estrus synchronization, sexed semen, and embryo transfer. Furthermore, dependence on visual estrus detection, inconsistent feeding practices, and seasonal heat stress substantially undermine reproductive efficiency (Nguyen-Kien et al., 2017). Understanding how these regional management conditions interact with genetic and physiological factors is therefore essential for improving reproductive outcomes in Vietnamese dairy herds.

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Fertility in dairy cows is highly sensitive to seasonal conditions, particularly in regions where ambient temperatures regularly exceed optimal thresholds (Tep et al., 2020; Ruelle et al., 2021; Otava et al., 2021). Heat stress disrupts the hypothalamic-pituitary-adrenal axis, leading to increased fetal cortisol release, reduced conception rate, higher embryonic loss, delayed ovulation, and impaired reproductive efficiency (O'Sullivan et al., 2020; Ferag et al., 2024). Additionally, high temperatures can compromise placental function and fetal development, intensifying reproductive losses (Davidson et al., 2021). Moreover, heat stress in dairy cows delays follicle selection, weakens dominant follicle development, and reduces progesterone concentrations, leading to abnormal oocyte maturation, implantation failure, and increased risk of early embryonic death (Khodaei-Motlagh et al., 2011). These findings highlighted the importance of considering environmental heat stress when assessing fertility limitations in Vietnamese dairy herds.

Parity is another crucial factor affecting conception rate, as higher parity cows often exhibit reduced fertility due to accumulated physiological stress and reproductive tract changes (Vieira-Neto et al., 2017; Senbeta et al., 2024). Several studies have indicated that parity significantly affects different reproductive parameters, including calving interval, days open, services per conception, and milk production (Kim et al., 2009; Senbeta et al., 2024). Moreover, cow age plays a pivotal role in reproductive efficiency, with younger cows experiencing shorter postpartum anestrus and a faster resumption of ovarian cyclicity than older cows (Hossain et al., 2021). Additionally, body condition score (BCS) and nutritional status, including energy balance, protein intake, and micronutrient adequacy, directly influence gonadotropin secretion, luteal function, and follicular development, thereby significantly impacting reproductive performance (Horn et al., 2022; McCarthy et al., 2022; Briano et al., 2024; Claramunt et al., 2024). These factors emphasize the need for integrated management strategies that balance nutrition, parity structure, and reproductive physiology to optimize fertility in dairy herds.

Although extensive studies have been conducted globally on factors affecting conception rate in dairy cattle, studies focusing on Holstein × Lai Sind crossbred cows in tropical areas remain limited. In tropical regions such as Vietnam, declining conception rate in dairy cattle is influenced by ovarian disorders, environmental factors, and hormonal fluctuations (Long et al., 2022; Thanh et al., 2023). Cows with inactive ovaries have a lower pregnancy chance. Housing conditions, such as ventilation, stocking density, and bedding quality and mineral supplementation (selenium, zinc), can improve estrus expression, ovarian function, and conception rate (Long et al., 2022). In addition, semen quality and artificial insemination (AI) protocols are critical contributors to conception success. Recent evidence has shown that improving the post-thaw quality of cryopreserved bull sperm through extender optimization and antioxidant supplementation can enhance motility, reduce oxidative damage, and significantly increase *in vivo* fertility (Khaki et al., 2018; Borah et al., 2021; Afsar et al., 2024). These findings emphasized that both female reproductive status and semen characteristics should be considered when evaluating fertility outcomes.

This retrospective study aimed to investigate the effects of factors, including ovarian disorders, estrus detection timing, age, parity, BCS, calving-to-service interval (CSI), AI order, month of service, and age at first AI, on conception rate in Holstein × Lai Sind crossbred cows in Northern Vietnam.

MATERIALS AND METHODS

Ethical approval

This retrospective study was based on existing farm production and reproductive records from smallholder dairy farms in Northern Vietnam. No direct interventions, experimental procedures, or animal handling were performed during the present study. All procedures were conducted in accordance with the ethical guidelines for animal research established by the Vietnam National University of Agriculture (Approval No. CARE-2023/06).

Animals and sample size

The required sample size was calculated using G*Power version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) for a linear multiple regression model (fixed model, R^2 deviation from zero, Faul et al., 2009). An effect size of 0.06 was selected as the minimum detectable effect size. With a significance level of $\alpha = 0.05$, a desired statistical power of 0.95, and eight predictors in the model, the analysis indicated that a minimum of 387 animals were required (Faul et al., 2009). A total of 433 Holstein × Lai Sind crossbred cows with complete reproductive records from smallholder farms in Bavi, Vietnam, were included in the present study. Cows with incomplete or inconsistent records were excluded. Housing was provided in well-ventilated barns, either in individual or group sections. A total mixed ration consisting of grass silage, rice straw, and concentrate was provided to all cows, formulated to supply approximately 18% of crude protein and 2,550 kcal/kg of metabolizable energy (NRC, 2001), with *ad libitum* access to mineral blocks and clean drinking water. Estrus detection was performed by farm personnel based on the primary sign (standing to be mounted) and secondary signs such as increased activity, mounting, transparent vaginal discharge, sniffing, licking, or chin-resting (Michaelis et al., 2014). Insemination with frozen-thawed semen was performed according to the a.m./p.m. guideline; cows identified as being in estrus in the morning were inseminated that evening, whereas cows first detected in the evening were inseminated the following morning (Trimberger and Davis, 1943). This method ensures insemination occurs approximately 12 hours after the onset of estrus, optimizing fertility. Pregnancy examination was carried out on day 45 post-insemination using either an ultrasound fitted with a linear probe emitting 7.5 MHz (KX-5200; China) or rectal palpation.

Study design and data collection

Data collection was performed by trained personnel through direct observation and manual recording from farmers' record books. The collected information included cow identification, date of birth, time of estrus detection, cow age, parity, BCS, CSI, AI order (first, second, or ≥ third insemination), month of service (month of AI), and age at first insemination. The study used a purposive sampling approach to include all cows meeting the inclusion criteria, with the final sample size determined by the availability of complete records. The data collection period spanned from November 2023 to April 2024.

The investigated reproductive factors were classified into different categories before analysis. The time of estrus detection was categorized as morning or afternoon. Cow age was divided into three groups, including < 3 years, 3-6 years, and > 6 years. Parity was classified into five groups, including heifer, parity 1, parity 2, parity 3, and parity 4-8. The BCS was categorized into three groups, including ≤ 2.5, 2.75-3, and > 3 (Randall et al., 2015). The CSI was classified into four groups, including < 2 months, 2-3 months, 4-5 months, and >5 months. The AI order was classified into three groups, including first service, second service, and third service onward. The month of service was divided into three categories, including September-October, November, and December-January. Age at first insemination was grouped into four categories, including 8-12 months, 13-15 months, 16-17 months, and >17 months.

Statistical analysis

Logistic regression was performed to examine the potential risk factors for conception rate. At first, univariate analysis was performed to identify the association between different single potential risk factors and conception rate. The independent risk factors included time of estrus detection, age of the cows, parity, BCS, CSI, AI order, month of service, and age at first service. Second, a multivariate logistic regression analysis was conducted to build the final multivariable model that best explained the variation of conception rate. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, 2013). For all analyses, including one-way ANOVA and Student's t-test, a P-value of less than or equal to 0.05 was considered statistically significant.

RESULTS

The overall conception rate was 45.6%. The present results revealed that parity, age, age at first AI, month of AI, and BCS had significant effects on conception rate ($p < 0.05$; Table 1). Conception rates were higher in heifers and cows with a BCS ≤ 2.5 and those inseminated from December to January. However, lower conception rates were observed in cows with parity 2 and 4-8, those older than 6 years, and those inseminated from September to October. Parity and age at first AI had significant effects on conception rate ($p < 0.05$). Fertility significantly declined from the second parity compared to the heifers and first-parity cows ($p < 0.05$). Cows inseminated at older ages (≥ 13 months) exhibited higher conception rates (43.8%) than those inseminated at 8-12 months (24.4%; Table 1). Additionally, age was a contributing factor, with cows over six years of age showing significantly lower fertility than younger cows ($p < 0.05$). The BCS indicated a significant relationship with fertility ($p < 0.05$). Cows having a BCS ≤ 2.5 achieved the highest conception rate (54.3%). In contrast, cows with a moderate BCS (2.75-3.0) demonstrated reduced fertility compared to those having either a lower (≤ 2.5) or higher (> 3.0) BCS. The month of service had a significant impact on conception rate ($p < 0.05$), with cows inseminated from December to January (52.9%) exhibiting markedly higher conception rates than those inseminated in September-October (37.7%). Other variables, including time of estrus detection, CSI, and AI order, did not demonstrate a significant association with conception rate in the univariate analysis ($p > 0.05$).

The multivariate analysis indicated that parity and age at first AI were the most significant factors associated with conception rate ($p < 0.05$; Table 2). Heifers exhibited the highest conception rate (55.7%, 59/106). In comparison to heifers, conception rates were significantly lower in parity 2 (OR = 0.45, $p < 0.05$) and parity 4-8 cows (OR = 0.50, $p < 0.05$). No significant differences in conception rate were observed between parity 1 and parity 3 ($p > 0.05$). Cows inseminated at 13-15 months, 16-17 months, and >17 months had significantly higher conception rates than those inseminated at 8-12 months ($p < 0.05$).

Table 1. The potential risk factors for conception rate in Holstein crossbred cows raised in Northern Vietnam, 2024

Factors	Conception rate	OR (95%CI)	P-value
Time of estrus detection			
Morning	49.8 (137/275)	1	
Afternoon	46.2 (73/158)	0.865(0.59-1.18)	0.469
Age			
<3 years	53.1 (102/192)	1	
3-6 years	48.2 (93/193)	0.821 (0.55-1.22)	0.333
>6 years	32.6 (15/46)	0.43(0.22-0.84)	0.014*
Parity			
Heifer	55.7 (59/106)	1	
Parity 1	46.7 (42/90)	0.70 (0.40-1.23)	0.21
Parity 2	37.6 (35/93)	0.48 (0.27-0.85)	0.012*
Parity 3	60.0 (45/75)	1.20 (0.66-2.18)	0.561
Parity 4-8	42.0 (29/69)	0.58 (0.31-1.07)	0.079
Body condition score			
≤2.5	54.3 (119/219)	1	
2.75-3	40.9 (76/186)	0.58 (0.39-0.86)	0.007*
>3	53.6 (15/29)	0.97 (0.44-2.13)	0.939
Calving to service			
<2 months	48.5 (16/33)	1	
2-3 months	43.2 (38/98)	0.81 (0.36-1.80)	0.602
4-5 months	47.4 (54/114)	0.96 (0.44-2.08)	0.91
>5 months	46.2 (43/93)	0.91 (0.41-2.02)	0.824
AI order			
First service	51.2 (85/166)	1	
Second service	49.3 (75/152)	0.93 (0.60-1.44)	0.74
From the 3rd service onward	43.5 (50/115)	0.73 (0.45-1.18)	0.203
Month of service			
September-October	37.7 (40/106)	1	
November	47.2 (25/53)	1.47 (0.76-2.87)	0.255
December-January	52.9 (145/274)	1.86 (1.17-2.93)	0.008*
Age at first AI			
8-12 months	24.4 (10/41)	1	
13-15 months	43.8 (63/144)	2.41 (1.10-5.29)	0.028*
16-17 months	55.6 (89/160)	3.89 (1.79-8.46)	0.001*
>17 months	54.5 (48/88)	3.72 (1.63-8.51)	0.002*

* Significant difference at the level of $p \leq 0.05$. OR: Odds ratio, CI: Confidence interval

Table 2. The multivariate analysis of potential risk factors for conception rate in Holstein crossbred cows raised in Northern Vietnam, 2024

Factors	OR (95%CI)	P value
Parity		
Heifer	1	
Parity 1	0.69 (0.40-1.24)	0.217
Parity 2	0.45 (0.25-0.81)	0.008*
Parity 3	1.19 (0.64-2.22)	0.575
Parity 4-8	0.5 (0.27-0.94)	0.031*
Age at first AI		
8-12 months	1	
13-15 months	2.41 (1.10-5.29)	0.028*
16-17 months	3.89 (1.79-8.46)	0.001*
>17 months	3.72 (1.63-8.51)	0.002*

* Significant difference at the level of $p \leq 0.05$. OR: Odds ratio, CI: Confidence interval

DISCUSSION

The present investigation noted a marked reduction in conception rates in cows over six years of age, suggesting an age-associated decline in reproductive efficiency. These findings are consistent with several studies conducted by Shorten et al. (2015), Samkange et al. (2019), and Otava et al. (2021). Shorten et al. (2015) reported a notable quadratic effect of cow age on pregnancy rate, with fertility increasing during the early reproductive years and declining as age progressed beyond physiological maturity. Similarly, a decline in conception rates among older dams has been documented in beef cattle by Samkange et al. (2019), as well as in dairy buffalo (Otava et al., 2021). Reproductive efficiency declines more starkly in multiparous cows compared to heifers or primiparous cows, a trend likely attributable to cumulative reproductive stress, heightened metabolic demands, and previously experienced calving-related challenges (Otava et al., 2021). Moreover, age-related reproductive issues have been reported by Hossain et al. (2021), including longer CSI in older cows, further compromising reproductive performance. Beyond physiological aging, genetic background interacts with reproductive performance. In crossbred Holsteins, breed composition and heat-stress adaptability can modify estrous expression and ovarian activity, thereby affecting conception probability. Nutritional interventions, including general supplementation with antioxidants or methyl donors, have been demonstrated to improve thermotolerance and sperm quality in bulls under heat stress (Ijab et al., 2022). These trends are probably driven by biological factors such as decreased oocyte quality (Walters et al., 2002), changes in the uterine environment (Thurmond et al., 2005; López-Gatius, 2012; Figueiredo et al., 2023), accumulating metabolic stresses (Lean et al., 2023), and physiological stresses (López-Helguera et al., 2016), with a notable increase in parity chance. Structural and functional changes in the uterine environment, including reduced uterine tone, increased uterine size, and delayed involution, have become more noticeable with higher parity (López-Helguera et al, 2016), where maternal age is a key factor linked to pregnancy loss (Thurmond et al., 2005; López-Gatius, 2012). These findings are consistent with those of Iwata et al. (2016), who attribute reduced conception rates in cows older than six years to declining oocyte quality.

The current results indicated that parity remarkably affected conception rate, with cows in parities 2-8 having a lower conception chance than heifers and first-parity cows. This pattern suggested that parity affects fertility by altering physiological maturity, metabolic status, and reproductive tract condition throughout the cow's productive lifespan. Jayawardana et al. (2022) reported that first-parity cows, such as Holstein-Friesian × Jersey crossbred, exhibited lower fertility metrics and conception rates. In contrast, third-parity cows demonstrated the highest fertility and conception rates under tropical and subtropical production systems. While the first and third parity groups did not exhibit considerable deviations from heifers, notable reductions were observed in the second and 4-8 parity groups, suggesting a threshold effect. Similarly, conception rates did not increase steadily with higher parity. However, Bhagat and Gokhale (2016) demonstrated that conception rates peaked around the third lactation and then gradually declined in later parities, thereby indicating a physiological threshold effect. The threshold effect describes the point where increasing parity initially enhances fertility up to an optimal level, after which further increases in parity result in a decline (Bhagat and Gokhale, 2016). This pattern supports the present finding that fertility is not linearly related to parity and may peak at intermediate parities. These findings underscored the complex relationship between parity and fertility. Heifers and young cows may require nutritional and hormonal support to achieve optimal reproductive readiness, while older cows may benefit from enhanced monitoring for uterine health, metabolic disorders, and improved estrous detection efficiency.

The present results highlighted the substantial impact of BCS on conception rate in dairy cows raised within smallholder farms in Northern Vietnam, where feeding strategies, heat load, and housing conditions vary considerably among households. The decreased performance in cows with higher BCS might be attributed to metabolic factors such as impaired insulin sensitivity, increased adipose-derived inflammation, and reduced estrous expression, all of which have been associated with diminished reproductive efficiency in dairy cows (Qiao et al., 2024). Since this pattern differs from findings in temperate regions, the present results should be interpreted with caution. Cows with moderate to high BCS might be more susceptible to the adverse effects of environmental conditions, such as heat stress, which could negatively influence ovarian function and estrous expression (Ettema and Santos, 2004). Heat stress might explain the differences observed, as cows with greater body fat tend to dissipate heat less effectively and have higher temperatures in tropical settings (Becker et al., 2020). In tropical or subtropical environments such as Northern Vietnam, cows with lower BCS may experience less thermal changes and maintain more proper metabolic and endocrine conditions (Jayawardana et al., 2022), thereby facilitating timely ovulation and successful fertilization. The present findings contrast with those of Kim and Jeong (2018) in non-tropical regions, where a BCS below 3.0 was associated with lower conception rates. These differences highlighted the critical influence of the regional environment, indicating that the current BCS threshold might not be directly suitable for tropical environmental settings.

The current results demonstrated that the month of service considerably influenced conception rates, with higher success observed in December-January compared to September-October. This seasonal variation suggested that environmental factors, such as temperature and humidity, could affect reproductive physiology (Becker et al., 2020). Supporting this finding, Nguyen-Kien et al. (2017) indicated that conception rates in cows in Vietnam were significantly higher during the cooler months (December–January), when the temperature-humidity index (THI) was at its lowest (76.6-78.6), and substantially lower in September-October, when THI remained elevated (approximately 81.3). The reduced heat stress during December to January might have enhanced estrus expression, improved sperm survival within the female reproductive tract, and promoted optimal uterine conditions. Specifically, a lower THI helps adequate blood flow to the uterus, stabilizes uterine pH, and maintains the endometrium activity, thereby promoting early embryo development and implantation. Previous studies have provided strong evidence supporting the impacts of seasonal factors on fertility. Higher conception rates are correlated with later months in the breeding season, a relationship driven by improved weather conditions (Fenlon et al., 2017). Similarly, high temperature and humidity during warmer months increase THI, leading to decreased estrous expression and impaired oocyte quality (Ferreira et al., 2011). Additionally, conception rates differ notably across seasons, highlighting the vital role of climatic conditions in reproductive performance (Ettema and Santos, 2004; Kuhn et al., 2006). These findings highlighted that environmental stress, especially heat load indicated by THI, can influence reproductive efficiency.

The present findings indicated that cows aged 8-12 months at first AI had lower conception rates, likely due to insufficient uterine and endocrine maturity. Delaying the first AI until at least 16 months might enhance reproductive success. This approach provided economic benefits by reducing the number of inseminations, lowering breeding costs, and ensuring heifers gain adequate body size for the recommended age at first calving. These findings align with those reported by Wathes et al. (2008), Cooke et al. (2013), and Zavadilová and Stípková (2013), suggesting that the optimal age for first calving is 24-25 months. This corresponds to a first AI at approximately 15-16 months, thereby ensuring maximal reproductive efficiency through proper heifer development. Consistent with the present findings, Boulton et al. (2017) reported that first calving before 24 months has been shown to support fertility, reduce rearing costs, and shorten the generation interval. Exposure to multiple estrous cycles before the first AI has been linked to higher estrus expression and improved conception rates (Galvão et al., 2004; Pereira et al., 2014). Ooi et al. (2023) demonstrated that high fertility was associated with improved reproductive performance, including increased submission rates, higher conception to first service, and earlier calving, thereby supporting genetic selection for improved fertility.

CONCLUSION

The present study demonstrated that parity and age at first AI are the most influential factors affecting conception rates in Holstein × Lai Sind crossbred cows under smallholder conditions in Northern Vietnam. Heifers exhibited the highest fertility, while cows in parity 2, parity 4-8, and those older than six years had reduced conception rates. Delaying the first AI to 16 months or later significantly improved fertility, emphasizing the importance of reproductive maturity before breeding. The current results highlighted that optimizing parity management and age at the first AI can enhance reproductive performance in tropical dairy systems. Implementing this approach in Northern Vietnam could boost fertility rates and enhance herd productivity, emphasizing the value of structured heifer breeding programs. However, these findings are based on smallholder conditions in Northern Vietnam and should be validated under different management systems and regions before broader application.

DECLARATIONS

Ethical considerations

The authors declared that this manuscript is original, has not been published elsewhere, and is not under consideration by any other journal. All authors have read and approved the manuscript, confirming adherence to ethical standards concerning authorship, research integrity, data accuracy, and publication consent. No artificial intelligence was used for preparing and writing the current study.

Authors' contributions

Bui Van Dung, Nguyen Van Thanh, and Nguyen Hoai Nam conceived and designed the study. Data collection and statistical analyses were conducted by Bui Van Dung, Nguyen Van Thanh, Do Thi Kim Lanh, and Nguyen Hoai Nam. The first draft of the manuscript was written by Bui Van Dung. All authors contributed to data interpretation, critically revised the manuscript, and approved the final edition for publication.

Availability of data and materials

All data and materials supporting the present study are available from the corresponding author upon reasonable request.

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Competing interests

The author declared no conflict of interest.

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