



Determinants Influencing Calving to Service Interval in Danish Holstein Cows

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ABSTRACT

The calving-to-first-service interval is crucial for dairy herd productivity. However, factors influencing this reproductive parameter remain understudied in modern dairy systems. The present study aimed to investigate the determinants of the calving-to-first-service interval in Danish Holstein cows, focusing on parity, age at first insemination, age at first calving, gestation length, gender of the born calf, and calving season. Data from 699 cows, managed on a single farm in Southwest Denmark, were collected and analyzed to identify risk factors associated with the calving-to-first-service interval. The mean calving-to-the-first-service interval was 87.6 ± 23.7 days. The present results indicated that parity, age at first calving, and calving season are key modifiable factors that affected the calving-to-first-service interval of the investigated Danish Holstein cows. The final multivariate linear regression model, which explained 13.7% of the variation in the calving-to-first-service interval, identified parity, age at first calving, and the gender of the born calf as significant risk factors for the interval. Parity and age at first calving were negatively related to the calving-to-first-service interval. Male calves were linked to an increased calving-to-first-service interval. Gestation length and age at the first artificial insemination indicated no association with the calving-to-first-service interval. Focusing on key factors such as parity, age at first calving, and gender of the born calf can improve reproductive success and profitability in dairy herds.

Keywords: Age, Calving, First calving, Gender, Parity, Service interval

INTRODUCTION

The reproductive efficiency of dairy cows is a key factor influencing the profitability and sustainability of dairy farms (De Vries and Marcondes, 2020). Among different reproductive performance parameters, the calving-to-service interval (CSI) serves as a crucial indicator of a cow's readiness for rebreeding post-parturition (Recce et al., 2021; Temesgen et al., 2022). The CSI can significantly impact the calving interval, influencing milk production cycles and herd productivity (Hossain et al., 2021). A long CSI can delay subsequent pregnancies, reduce the number of calves produced over a cow's lifetime, and potentially lower milk production (Bonato and dos Santos, 2012; Hossain et al., 2021; Temesgen et al., 2022). Conversely, a short CSI can help in maintaining a consistent milk supply, thereby supporting the economic viability of the dairy operation (Hossain et al., 2021). Improving reproductive efficiency is crucial in dairy cows because delays in returning to reproductive activity not only reduce lifetime calf production but also disrupt lactation cycles, causing economic losses (Wiltbank et al., 2011; Dung et al., 2025). Understanding the factors that influence CSI provides valuable insights for developing management strategies that enhance reproductive performance and sustain long-term herd productivity (Dung et al., 2025).

In dairy herds, the CSI can be affected by a range of factors, including cow's parity (Kim et al., 2009; Ali et al., 2013; Hossain et al., 2021), age at first artificial insemination (AFAI; Ali et al., 2013), age at first calving (AFC; Watanabe et al., 2017, Softic et al., 2020), gestation length (Motlagh et al., 2013; Atashi et al., 2019; Senbeta et al., 2024), and environmental factors such as season of calving (Kim et al., 2009). Younger cows or cows in their first parity may display different CSI patterns compared to older, multiparous cows (Softic et al., 2020). Furthermore, the interaction between the age at AFAI and calving season may influence the CSI through their combined effects on ovarian activity, estrous expression, and the effectiveness of reproductive management strategies (Lima et al., 2010; Temesgen et al., 2022). Understanding these relationships is crucial for developing effective reproductive management strategies that optimize herd fertility and increase milk production yield.

The present study aimed to identify the influencing factors of the CSI in Danish Holstein cows, considering parity, AFC, gestation length, gender of the born calf, and calving season, using a multivariate approach.

ORIGINAL ARTICLE
Received: July 05, 2025
Revised: August 09, 2025
Accepted: September 10, 2025
Published: September 30, 2025

MATERIALS AND METHODS

Ethical approval

The present study was conducted utilizing farm records of Danish Holstein cows on Holdan Argo farm, located at Tyrholmvej 7, 6230 Røddekro, Denmark. It did not incorporate any experimental manipulation or direct intervention with live animals. All data collection and analysis were performed in strict accordance with established ethical standards governing the use of animal production records in research at the Vietnam National University of Agriculture.

Study population

The present study utilized data from 699 Danish Holstein cows, which had previously been used to investigate the factors associated with conception rate (Nam et al., 2025). The investigated cows ranged from first to fourth parity. All cows were born from January 2017 to September 2021. The animals were milked twice daily, with the first milking session starting at 3:00 a.m. and concluding at 11:00 a.m., and the second milking session starting at 3:00 p.m. and ending at 10:30 p.m. The documented calving interval was 394 days. On average, each cow produced about 36 kg of milk per day. The cows were housed indoors and fed alfalfa hay throughout the year. Estrus detection was carried out using pedometers, and insemination with frozen-thawed semen was performed once per estrus cycle. Vaccinations against *Escherichia coli*, Coronavirus, and Rotavirus (Bovigen® Scour, Virbac, Ireland) were given before the cows were dried off.

Data collection

Data of the investigated cows were collected from the dairy management system (Denmark), which was kept on the farm's computer. The essential sample size was calculated using GPower 3.1.9.7 (Germany), based on a linear multiple regression model (fixed model, R^2 deviation from zero; Faul et al., 2009). An effect size of 0.053 was estimated based on a priori $R^2 = 0.05$. Based on a power analysis ($\alpha = 0.05$, power = 0.95) for a model with six predictors, a sample size of 400 was determined to be sufficient. The collected information included birth date, AFAI (days), AFC (days), as well as the insemination date leading to the previous pregnancy, dates of the most recent calving, first service after that calving, and the gender of the born calf at the most recent calving. Based on this information, the collected data were used to calculate the following parameters, including AFAI, AFC, gestation length of the most recent calving (days), and the most recent CSI (days). Data were collected from August to December 2023.

Based on parity number, cows were divided into four categories, including Parity 1, 2, 3, and 4, as these categories naturally represented the distribution of the dataset. The length of the most recent gestation was classified into ≤ 275 days, 276-280 days, and 281-292 days. The AFAI was grouped into three categories, including 365-400 days, 400-430 days, and over 430 days. The AFC was categorized into five groups, including 646-670 days, 671-700 days, 701-730 days, 731-760 days, and over 760 days. The classification of gestation length into three categories, namely short, regular, and long gestation lengths, is based on the mean value of 277 days. The grouping of AFAI and AFC was conducted using monthly intervals to investigate whether variations of approximately one month in these reproductive events exerted an influence on CSI. The calving months were divided into four seasons, including winter (December-March), spring (April-May), summer (June-August), and autumn (September-November). Roughly, the average monthly temperature spanned from 1.96 to 4.75°C in winter, 8.99 to 13.16°C in spring, 16.39 to 18.45°C in summer, and 6.56 to 14.99°C in autumn.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, Version 22.0. The association between the CSI and different independent variables, including parity, gender of the born calf, gestation length, AFAI, AFC, and calving season, was assessed using the Kruskal-Wallis and Mann-Whitney U tests. For significant results, post hoc analyses were conducted using Dunn's test, with the Bonferroni correction applied. Subsequently, a linear regression analysis was employed to construct the most explanatory model for the CSI, which served as the dependent variable. The independent variables included parity, gender of the born calf, AFAI, AFC, recent gestation length, and calving season. The modeling process involved initial univariate analyses to evaluate the individual influence of each factor, followed by multivariate analyses using forward stepwise selection to build the optimal model. The variance inflation factor (VIF) was calculated to check for multicollinearity (Kim, 2019). For all analyses, including one-way ANOVA and Student's t-test, a p-value of less than 0.05 was considered statistically significant.

RESULTS

Calving to service interval in different cow groups

Parity

The CSI decreased significantly from Parity 1 to Parity 3 ($p < 0.05$; Table 1). The mean CSI in the first parity cows was 96.3 ± 19.4 days, and that in the parities 2 and 3 was 86.2 ± 21.8 and 72.2 ± 25.1 days, respectively. Cows in their Parity 4 exhibited a CSI of 96.9 ± 43.9 days, which did not differ significantly from that observed in cows of parities 1, 2, and 3 ($p > 0.05$). The findings related to Parity 4 should be interpreted carefully because of the small sample size.

Gestation length

The gestation length did not significantly impact CSI ($p > 0.05$; Table 1). Cows with gestation lengths ≤ 275 days had a CSI of 85.7 ± 23.1 days, while those with gestation lengths between 276 and 280 days had a CSI of 89.4 ± 24.4 days. For cows with gestation lengths of 281 and 292 days, the CSI was 86.7 ± 24.9 days.

Gender of the born calf

There was no significant difference in the CSI based on the gender of the born calf ($p > 0.05$; Table 1). Cows that gave birth to heifers had a CSI of 87.7 ± 22.0 days, while those that delivered bull calves had a CSI of 87.5 ± 26.8 days.

Age at first artificial insemination

The AFAI did not influence CSI ($p > 0.05$; Table 1). Cows inseminated initially between 365 and 400 days exhibited a CSI of 86.7 ± 23.6 days. Those inseminated within the 400 to 430-day period had a CSI of 87.0 ± 24.2 days, which was not statistically different from the cows inseminated after 430 days (CSI: 92.2 ± 21.2 days; $p > 0.05$).

Age at first calving

Cows with AFC over 760 days exhibited a shorter CSI (81.3 ± 28.6 days) in comparison to cows with AFC ranging from 671 to 700 days (87.7 ± 21.6 ; $p < 0.05$) and cows with AFC ranging from 701 to 730 days (89.6 ± 23.5 ; $p < 0.05$). Pairwise comparisons among the other groups revealed no statistically significant differences ($p > 0.05$; Table 1).

Calving season

The calving season influenced CSI. Cows calving in winter had a significantly longer CSI (89.1 ± 24.9) than those calving in spring, summer, and autumn (87.3 ± 22.9 , 84.4 ± 17.5 , and 86.5 ± 27.8 days, respectively; $p < 0.05$).

Table 1. Calving to service interval of different cow categories in Danish Holstein cows, Holdan Argo farm, Røddekro, Denmark in 2023

Investigated parameters	Calving to service interval (day)
Parity	
1 (n = 284)	96.3 ± 19.4^a
2 (n = 265)	86.2 ± 21.8^b
3 (n = 142)	72.2 ± 25.1^c
4 (n = 8)	95.9 ± 43.9^{abc}
Gestation length (day)	
≤ 275 days (n = 216)	85.7 ± 23.1
276-280 days (n = 316)	89.4 ± 23.4
281-292 days (n = 167)	86.7 ± 24.9
Gender of the calf	
Heifer (n = 466)	87.7 ± 22.0
Bull (n = 233)	87.5 ± 26.8
Age at first artificial insemination (day)	
365-400 days (n = 219)	86.7 ± 23.6
401-430 days (n = 387)	87.0 ± 24.2
> 430 days (n = 93)	92.2 ± 21.2
Age at first calving	
646-670 days (n = 66)	88.8 ± 21.7^a
671-700 days (n = 270)	87.7 ± 21.6^a
701-730 days (n = 194)	89.6 ± 23.5^a
731-760 days (n = 85)	88.1 ± 26.1^{ab}
> 760 days (n = 84)	81.3 ± 28.6^b
Season of calving	
Winter (n = 377)	89.1 ± 24.9^a
Spring (n = 104)	87.3 ± 22.9^b
Summer (n = 139)	84.4 ± 17.5^b
Autumn (n = 79)	86.5 ± 27.8^b

Data is presented as mean and standard deviation. ^{a,b,c} Means within a column with different superscript letters differ significantly ($p < 0.05$). For post-hoc comparisons, the P-values of all the comparisons were: Parity 1 compared to Parity 2,3,4 ($p < 0.001$; < 0.001 , and 0.247), Parity 2 compared to Parity 3, 4 ($p < 0.001$ and 0.880), Parity 3 compared to Parity 4 ($p = 0.521$), Gestation length (GL) ≤ 275 days compared to GL= 276-280 and 281-292 days ($p = 0.105$ and 1.000), GL= 276-280 days compared to GL= 281-292 days ($p = 0.771$), Male calf compared to Female calf ($p = 0.679$), Age at first artificial insemination (AFAI) = 365-400 days compared to AFAI = 401-430 and > 430 days ($p = 1.000$ and 0.243), AFAI = 401-430 days compared to AFAI > 430 days ($p = 0.147$), Age at first calving (AFC) = 646-670 days compared to AFC = 671-700, 701-730, 731-760, and >760 days ($p = 1.000$, 1.000 , 1.000 , and 0.007), AFC = 671-700 days compared to AFC = 701-730, 731-760, and > 760 days ($p = 1.000$, 1.000 , and 0.007), AFC = 701-730 days compared to AFC = 731-760, and > 760 days ($p = 1.000$ and 0.002), AFC = 731-760 days compared to AFC >760 days ($p = 0.099$), Winter compared to Spring, Summer and Autumn ($p < 0.001$, < 0.001 , < 0.001), Spring compared to Summer and Autumn ($p = 1.000$ and 1.000), Summer compared to Autumn ($p = 1.000$).

Associated factor with calving to service interval in Danish Holstein cows

The multivariate analysis identified the most illustrative model, comprising parity, AFC, and gender of the born calf (Table 2). This model accounted for 13.7% of the variance in the CSI among the investigated Danish Holstein cows. Parity was negatively correlated with CSI (coefficient = -11.7, 95% CI = -13.9 to -9.5; $p < 0.05$). Additionally, AFC demonstrated a significant negative association with the CSI (coefficient = -2.1, 95% CI = -3.6 to -0.7; $p < 0.05$). In the multivariate analysis, the gender of the born calf exhibited a significant relationship with CSI (coefficient = 5.4, 95% CI = 1.8 to 9.0; $p < 0.05$), with a longer CSI observed in cows calving a bull. The VIF ranged from 1.012 to 1.103, indicating the absence of multicollinearity among the model variables.

Table 2. Multivariate analysis of factors associated with calving to service interval in 699 Danish Holstein cows, Holdan Argo farm, Røddekro, Denmark in 2023

Variables	Coefficients/95% confidence interval	P-value	VIF
Constant	113.1 (107.0-119.1)	<0.001	
Parity	-11.7 (-13.9 - [-9.5])	<0.001	1.103
Age at first calving	-2.1 (-3.6 - [-0.7])	0.003	1.012
Gender of the calf	5.4 (1.8-9.0)	0.004	1.090

VIF: Variance inflation factor. The final model explained 13.7% of the variation in calving to first service interval in the investigated animals.

DISCUSSION

The current results revealed that parity, AFC, and gender of the born calf were the most crucial risk factors for CSI in Danish Holstein cows.

Parity effects

Multiparous cows typically exhibit a more stable endocrine-metabolic adaptation around calving compared to primiparous cows, which often encounter greater metabolic imbalance and extended periods of anestrus (Briano et al., 2024). Kim et al. (2009) noted that calving intervals shortened with increasing parity, with the highest reproductive efficiency observed in second-parity cows. This trend aligned with findings of Baek et al. (1998), who documented the shortest calving intervals in second-parity cows, indicating a peak in reproductive performance at this stage. Moreover, Briano et al. (2024) identified prolonged postpartum anestrus in primiparous cows, contributing to their extended calving intervals. These findings collectively highlighted the impact of parity on reproductive efficiency, emphasizing the extended intervals necessary for primiparous cows to resume cyclicity and complete subsequent reproductive processes after calving. This trend may be partially attributable to a reduced dystocia rate in older cows, which facilitates uterine recovery and reduced calving intervals (Gaafar et al., 2011). Nevertheless, older cows face an increased risk of conditions such as dystocia, retained placenta, metritis, and metabolic disorders (Rohmah et al., 2023), which may extend the CSI in higher-parity cows (Motlagh et al., 2013). Several studies indicated that the prevalence of these complications tends to increase remarkably only after the fourth parity, which could explain the observed rise in CSI after this point (Gabr et al., 2005). Inflammatory conditions such as subclinical mastitis can delay postpartum recovery and prolong the resumption of reproductive activity (Bacha and Regassa, 2010). Ghasemian and Asri-Rezaei (2024) reported elevated inflammatory markers such as serum amyloid A, interleukins IL-6 and IL-8 in cows with mastitis caused by *Escherichia coli* and *Staphylococcus aureus*, underscoring the role of systemic inflammation in reproductive inefficiency. The current findings suggest that postpartum multiparous cows generally return to estrus more rapidly than primiparous cows, likely attributable to physiological adaptations and improved reproductive efficiency acquired through successive calvings. Moreover, negative energy balance remains an important challenge in primiparous cows, particularly during their second lactation, as indicated by lower plasma glucose and higher β -hydroxybutyrate and urea levels (Cattaneo et al., 2023). These metabolic markers reflected increased body reserve mobilization and metabolic adaptation, which may contribute to prolonged postpartum anestrus and delayed reproductive recovery in younger cows.

Age at first calving

The negative association between AFC and CSI aligns with the findings of Mufti et al. (2010) and Khan et al. (2015). Cows that calved at a younger age (below 700 days or 23 months) displayed more substantial variation in lifespan compared to those calving between 671 and 823 days (22 to 27 months). The negative association between AFC and CSI highlighted the benefit of calving at a more mature age to support optimal body condition and reproductive health throughout a cow's life. Heifer's calving are more skeletally and metabolically developed, which lowers dystocia

risk, decreases postpartum disease, and eases negative energy balance. These benefits help accelerate uterine involution and restore ovarian cycles more quickly, resulting in a shorter CSI. On the other hand, early calving has been linked to reduced longevity (Haworth *et al.*, 2008). In addition, Nilforooshan and Edriss (2004) found a small positive phenotypic correlation between AFC and lifetime in Iranian Holsteins, implying that delaying calving may help improve reproductive maturity and overall health, potentially extending productive lifespan.

Gender effects

Numerous studies have shown that the gestation period in dairy cows tends to be longer for pregnancies resulting in male calves compared to those resulting in female calves (Silva *et al.*, 1992; Vieira-Neto *et al.*, 2017). This discrepancy may be attributed to fetal weight, as male calves generally exhibit a higher birth weight than female calves, which could potentially result in an extended gestation period (Atashi and Asaadi, 2019). Extended gestation may influence maternal health by elevating the risk of complications and prolonging recovery, thereby extending the interval from calving to the first breeding (Mwangi *et al.*, 2025). Cows that give birth to female calves generally recover their uterine function faster and return to estrus sooner than those birthing male calves (Atashi and Asaadi, 2019). This difference may be due to the larger size of male calves, which results in a longer gestation period and impacts uterine recovery after calving (Atashi and Asaadi, 2019). Additionally, serum estrogen levels are remarkably higher in cows giving birth to female calves than in those birthing male calves (Ibrahim *et al.*, 2017). Estrogen is essential for postpartum uterine recovery, as it stimulates uterine muscle and endometrial activity (Sheldon and Dobson, 2004). Studies conducted by Gad *et al.* (2017) and Rezende *et al.* (2020) confirmed that the gender of the born calf substantially influences uterine characteristics, including lumen diameter, curvature, and involution rate. Specifically, cows giving birth to female calves indicated a shorter uterine involution period, while those with male calves experienced a slower return to estrus and delayed uterine involution (Atashi and Asaadi, 2019). Additionally, the higher rate of dystocia linked to male calves, mainly due to their larger birth weight and fetopelvic disproportion, further prolongs the CSI by exacerbating postpartum complications and delaying uterine recovery (De Amicis *et al.*, 2018; Tsaousioti *et al.*, 2024). Therefore, calf gender is a crucial factor influencing the CSI, with cows giving birth to male calves having longer CSIs.

Seasonal effects

The prolonged CSI observed in cows that calve during winter may be associated with increased metabolic demands during colder periods, potentially hindering postpartum recovery and delaying the estrous cycles. Exposure to low temperatures increases energy requirements (Roland *et al.*, 2016; Fu *et al.*, 2022; Wang *et al.*, 2023), reduces feed utilization efficiency (Mota-Rojas *et al.*, 2021), and disrupts hormonal balance (Fu *et al.*, 2022; Lezama-García *et al.*, 2022; Wang *et al.*, 2023), thereby potentially extending CSI. Findings concerning beef cattle indicated that the calving season has considerable effects on the duration of postpartum anestrus. Specifically, cows calving during winter months (November to April) remained in anestrus for an average of 70.8 days, which was nearly twice as long as those calving during the summer months (May to October), with an average of 35.9 days (Peters and Riley, 1982). These findings were consistent with previous studies, which have demonstrated that cows calving in summer generally exhibit shorter CSIs compared to their counterparts calving in winter or spring (Santos *et al.*, 2009). Echevarria *et al.* (2001) further emphasized the impact of calving season on CSI, with notable differences across different parity groups and seasonal calving cohorts. The seasonal fluctuations in CSI highlighted the importance of implementing targeted management practices to address reproductive challenges that occur during specific periods. Providing enhanced nutritional and reproductive support to winter-calving cows may reduce the duration of CSIs, thereby fostering improved reproductive outcomes and overall herd productivity.

Although the present study identified parity, AFC, and calf gender as the most critical determinants of CSI, other potentially influential factors, such as nutritional status, postpartum diseases, and management practices, were not assessed. Nevertheless, the present findings provided valuable insights into the key factors associated with CSI and contributed to a more comprehensive understanding of reproductive performance in dairy cattle.

CONCLUSION

The present study highlighted the significant effects of parity, AFC, and gender of the born calf at the CSI in Danish Holstein cows. The current findings indicated a significant reduction in CSI as parity increases to the third parity. However, CSI increased in cows with the fourth parity, indicating an age-related reversal in CSI trends beyond the third parity. These results suggested that reproductive management practices in Danish Holstein cows may be optimized by prioritizing an appropriate AFC and carefully monitoring multiparous cows to enhance reproductive outcomes. The present findings provided valuable insights into enhancing breeding and postpartum management strategies, which could

ultimately boost herd fertility and productivity in Holstein dairy systems. Future studies should incorporate nutritional status, body condition during the transition, common postpartum diseases such as metritis and mastitis, as well as metabolic disorders, along with management practices including heat detection efficiency, insemination timing, and housing systems, to provide a more comprehensive understanding of CSI determinants.

DECLARATIONS

Acknowledgments

The authors express their gratitude to Mr. Jacobus A.M. Broeders for his consent to the collection and publication of the herd's data.

Authors' contributions

Bui Van Dung prepared the initial draft of the manuscript. Le Thi Le Thuong collected data. Nguyen Hoai Nam analyzed data and designed the study. All authors reviewed and approved the final edition of the manuscript.

Funding

The present study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests

The authors declared no conflicts of interest.

Availability of data and materials

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

Ethical considerations

The authors declare that this manuscript is original and not under consideration for publication elsewhere. All ethical considerations, including consent to publish, research misconduct, data fabrication, and redundancy, have been thoroughly reviewed and approved by all authors.

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