


Possible effect of artificial insemination and multiple ovulation embryo transfer on the health and survival of Holstein-Friesian calves

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RESEARCH ARTICLE



ABSTRACT

Reproductive technologies like artificial insemination (AI) and multiple-ovulation embryo transfer (MOET) are extensively used in cattle breeding, but their effects on the health and growth of calves remain unclear. This study evaluated the effects of AI and MOET on morbidity, survival and weaning weight in Holstein-Friesian heifer calves. A total of 366 calves (AI: $n = 314$; MOET: $n = 52$) were followed for 91 days from birth. Morbidity was classified based on the incidence and severity of diarrhoeal and respiratory disease. Weaning weights were contrasted with disease incidence. Statistical analyses included chi-square tests for categorical data and ANOVA for continuous data ($P < 0.05$). Survival and overall morbidity rates were not influenced greatly by reproductive technology. The prevalence of diarrhoeal disease was similar for MOET and AI groups ($P > 0.05$), but respiratory disease was greater and more severe in MOET calves ($P < 0.05$). Morbidity negatively impacted weaning weight in AI calves ($P < 0.05$) but had no significant impact on MOET calves. Season and colostrum quality significantly influenced survival, with greater survival rates noted for autumn-born calves and calves that received high-quality colostrum ($>25\%$ IgG) ($P < 0.05$). While reproductive technology had little effect on survival, MOET calves showed a heightened susceptibility to respiratory illnesses, highlighting the importance of targeted health management.

KEYWORDS

Holstein-Friesian calves, artificial insemination, embryo transfer, growth performance, respiratory diseases, colostrum quality

INTRODUCTION

Climate change poses significant challenges for dairy calf production in Hungary. Hutch-reared calves had greater fatality rates due to heat stress brought on by rising temperatures, especially when daily mean temperatures above 22 °C (Jurkovich et al., 2024). Pregnancy-related heat exposure lowers birth weight; the second and third trimesters are particularly vulnerable (Hajdu-Hajdu, 2021). Cow welfare, fodder quality and grassland production are all impacted by global warming, with high-yielding animals being particularly vulnerable (Gaully-Ammer, 2020). Environmental elements like temperature, humidity and air quality have an impact on the bovine respiratory disease complex (BRDC). While high temperatures and low humidity lead to dust generation, which exacerbates respiratory problems, low temperatures mixed with high humidity and wind speed increase morbidity and death

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(Sáfar et al., 2023). Continuous monitoring of environmental factors and improved housing conditions can help mitigate these challenges and reduce the impact of climate change on dairy calf health and production.

Calf mortality is a significant challenge in dairy production, with rates varying widely between herds and countries. In Norwegian dairy herds, the mortality rate during the first year of life was reported at 7.8% (Gulliksen et al., 2009), while a Korean study indicated a rate of 10.7% (Hur et al., 2013). According to Bogdan (2024), mortality rates should remain below 5%, with morbidity rates under 8%. The highest mortality occurs within the first few weeks of life, primarily due to digestive and respiratory diseases (Hur et al., 2013; Gulliksen et al., 2009). Risk factors for increased mortality include calving difficulties, twin births, winter seasons, larger herd sizes and group housing (Gulliksen et al., 2009; Zucali et al., 2013). Practical shortcomings, such as delayed colostrum feeding and insufficient milk intake, also elevate the risk (Zucali et al., 2013). Improving environmental conditions and focusing on early detection and treatment of at-risk calves are crucial to reducing mortality rates, enhancing animal welfare and boosting productivity (Uetake, 2012).

Studies comparing calves born from multiple-ovulation embryo transfer (MOET) and artificial insemination (AI) show mixed health and survival outcomes. While no significant differences have been observed in passive immunity transfer, morbidity or mortality between MOET and AI calves (Pimenta-Oliveira et al., 2011), several studies have reported increased birth weights and higher mortality rates during the early life stages in ET calves (Bonilla et al., 2014). The gestation length was longer in frozen MOET embryos compared to fresh MOET or AI embryos. Embryo survival rates varied between studies; one reported similar survival rates of 45–47% for AI and MOET, while another found an average survival rate of 41% for MOET-induced twin pregnancies.

Studies have shown that calves born via MOET experience lower pre-weaning mortality, improved regulation of fertility genes and enhanced reproductive performance. MOET heifers were less likely to be culled during their first year of life and showed better reproductive performance during their first lactation compared to AI heifers. Fertility-related genes were upregulated in MOET calves (Abdel et al., 2023). However, King et al. (1985) found no differences in disease susceptibility or survival rates between ET and non-ET calves.

A biotechnology called multiple-ovulation embryo transfer (MOET) speeds up the process of genetic selection and increases the involvement of female cows in the process of herd genetic improvement (Bonilla et al., 2014). Atypical placental development (Siqueira et al., 2009), increased foetal development, decreased embryo implantation efficiency and, most importantly, a larger birth weight (Bonilla et al., 2014) are some of the technique's disadvantages.

Large offspring syndrome (LOS) is a foetal overgrowth condition in bovine animals, often observed in offspring conceived using reproductive technologies like MOET and

IVF. It causes transcript abundance changes in genes involved in cell-cell junctions, transport, RNA processing and stress (Li et al., 2019). Overgrowth can cause abnormal organ enlargement, leading to metabolic changes (Chen et al., 2013). High birth weight may increase daily gain and height, but also increase dystocia, leading to neonatal asphyxia, metabolic and respiratory acidosis, reduced immunoglobulin absorption, increased disease susceptibility and death (Johanson-Berger, 2003; Schild et al., 2020; Weiller et al., 2024).

This study aims to investigate the effect of technological parameters—including reproduction methods (MOET vs. AI), seasonal variations and management practices—on calf health. Attention is focused on the differences between MOET and AI calves in terms of health status, as previous studies have reported conflicting outcomes regarding morbidity, survival rates and immune function (Bonilla et al., 2014; Weiller et al., 2024). By analysing these factors, the study seeks to provide a deeper understanding of the impact of reproductive technologies on calf well-being and overall herd productivity. The aim of the present study is to investigate the possible role of assisted reproductive techniques on the health status of newborn calves with special regard on the changing climate and environment conditions in Hungary.

MATERIALS AND METHODS

The data were collected from a dairy farm in southeastern Hungary. The study was conducted on Holstein-Friesian calves born between January 1, 2023, and December 31, 2023, with data collection continuing until April 2024. A total of 982 calves were born during the study period, of which 366 pure-bred Holstein-Friesian heifers were selected for the analysis. The remaining calves were Holstein-Friesian bull calves or F1 cross-bred calves sired by Charolais-Excellent bulls, which were excluded from the study.

Stillborn calves and those that died within the first 24 h of birth were also excluded. Of the 366 heifer calves studied, 300 were born through artificial insemination (AI) and 66 were the result of multiple-ovulation embryo transfer (MOET). The calves were weighed at birth using a digital scale (Lot number: AM02-da-22) and the weight data were recorded.

Each calf was fed 4 L of pasteurised colostrum via tube feeding within 1 h of birth. The quality of the colostrum was evaluated using a refractometer and the data were recorded for each individual. Only colostrum with a Brix value of 20% or higher was used; colostrum below this threshold was excluded.

All calves were vaccinated with BovilisBovipast RSP and BioBos IBR marker inactivated vaccines. Their health status was continuously monitored and the data were recorded in the farm database. For every health issue and treatment administered, the following information was required to be documented by the producer: the date of treatment, the suspected disease, the calf's rectal temperature and the type of treatment applied.

Treatments were recorded only in cases of suspected disease and not as part of preventive measures. Long-acting antibiotic treatments were considered separate events if at least 7 days elapsed between treatments. For other medications, a minimum interval of 3 days was required between treatments to register them as new events.

The diseases and their definitions observed in the herd are summarised in Table 1.

Gastrointestinal diseases

The calves' stool consistency was visually assessed twice daily from birth until weaning. The stool consistency was evaluated using a scale ranging from 0 to 3 (Larson et al., 1977; Renaud et al., 2020), as shown in Table 2.

The primary pathogens isolated from calves with diarrhoea included rotavirus, coronavirus, *Escherichia coli* and *Cryptosporidium parvum*. The *E. coli* isolates were not analysed further.

Respiratory diseases

The severity of respiratory diseases in Holstein-Friesian calves was assessed using standardised scoring systems. A theoretical scoring system proposed four levels of clinical symptoms and treatment needs (Coghe et al., 1999). McGuirk and Peek (2014) developed a system assigning

severity scores based on clinical parameters, such as rectal temperature, coughing and nasal discharge. A composite respiratory score of 5 or higher, with at least two abnormal parameters, indicated respiratory disease.

Windeyer et al. (2017) adapted the Wisconsin Scoring System to categorise calves as 'Sick', 'Infected' or 'Healthy.' Behavioural changes, such as reduced activity and feeding time, were also found to correlate with clinical respiratory scores and disease severity (White et al., 2012). Table 3 summarises the classification of respiratory disease severity based on these findings.

Nasal swab samples were collected from calves to diagnose respiratory diseases. Pathogens isolated included *Pasteurella multocida*, *E. coli*, *Mannheimia haemolytica* and β -haemolytic streptococci.

Statistical analysis

The frequency and severity of diseases (respiratory and gastrointestinal) between the two groups (MOET and AI) were statistically analysed using IBM SPSS Statistics 26.0. The morbidity of respiratory and diarrhoeal diseases was evaluated using the Independent Samples Test. The severity of diseases was analysed using the Chi square test and the influence of disease frequency on weight gain was assessed using ANOVA. While the variance of weight gain across disease frequency was homogeneous, it was tested with Levene test, in case of significant difference ($P < 0.05$), a Tukey test was used for pairwise comparison.

Mortality rates were visualised using Kaplan-Meier survival curves in IBM SPSS Statistics 26.0. Survival time was defined as the number of days from birth until weaning. The weaning period was set between 70 and 90 days. Survival was marked as 'completed' if the calf reached 91 days, while calves weaned earlier were labelled as 'censored.'

Table 1. Diseases and case definitions

Disease	Definition
Respiratory disease	Increased resting respiratory rate, abnormal lung sounds, or laboured breathing with fever (>39.5 °C) and additional signs such as cough, nasal discharge, depression, reduced appetite, or rough coat
Diarrhoea	Stool consistency looser than normal for ≥ 2 days
Colic	Abdominal cramping, restlessness, or signs of discomfort
Bloating	Rumen distension causing discomfort \pm respiratory distress
Joint inflammation	Swelling of one or more joints causing lameness \pm fever
Navel infection	Enlargement or foul-smelling discharge of the navel caused by infection

Table 2. Stool consistency assessment (based on Larson et al., 1977; Renaud et al., 2020)

Score	Status	Stool consistency	Treatment
0	Normal	Solid but not hard, may deform	None
1	Soft	Lacks form, slightly spreads	None or electrolyte replacement
2	Liquid	Easily spreads	Electrolytes and antibiotics
3	Watery	Liquid, splashes	Electrolytes and antibiotics

Table 3. Classification of respiratory disease severity (based on White et al., 2012; Windeyer et al., 2017)

Grade	Status	Infection type	Symptoms	Treatment
0	Healthy	Subclinical disease	None	None
1	Infected	Compensated clinical disease	Nasal discharge, increased respiratory rate, fever	Antibiotics
2	Sick	Non-compensated clinical disease	Nasal discharge, cough, lethargy, anorexia, fever	Antibiotics and anti-inflammatories
3	Sick	Irreversible clinical disease	Cough, lung sounds, fibrosis, stunted growth	None (culling)

RESULTS AND DISCUSSION

Seasonal effects

During the study period, 2.5% of the calves ($n = 9$) either died or were removed from the farm, before reaching 91 days of age. Conversely, 97.5% of the calves ($n = 357$) survived the critical rearing period.

Mortality rates varied seasonally, with higher rates observed during the summer and winter, compared to the spring and autumn periods (Jurkovich et al., 2024). Some breeds experience increased mortality rates in winter (Hossain et al., 2013; Selvan et al., 2019), whereas others are reported to have higher mortality during summer or rainy seasons (Selvan et al., 2019). Season played a significant role as a factor influencing survival and mortality in our study, too. The majority of calvings occurred in winter ($n = 120$), with a mortality rate of 2.5%. Fewer births were recorded in spring ($n = 79$) and summer ($n = 101$), with mortality rates of 3.5% and 3%, respectively. The lowest number of calvings occurred in autumn and no calf mortalities (0%) were recorded during this season, showing a significant difference ($P < 0.05$) in calf survival by season (Fig. 1).

Colostrum quality

The survival rate of the calves is shown as a function of age under different colostrum quality levels (measured by Brix %) (Fig. 2).

Differences in survival were observed between the two groups. Calves that received colostrum with a Brix percentage of $\geq 26\%$ (green curve) had a higher survival rate compared to those receiving colostrum with a Brix percentage between 20 and 25% (blue curve). Feeding higher-quality colostrum (with a higher Brix %) has been shown to positively influence calf survival. This finding is supported by the results of Dardillat et al. (1978) and Armengol and Fraile (2016), who both observed improved survival rates in calves receiving colostrum of superior quality.

The correlation coefficient between colostrum quality and the number of treatments administered for illnesses was -0.09 . This indicates that there is a slight, statistically not significant inverse relationship between the two variables.

Morbidity and survival based on reproductive techniques

The survival rate of calves born through artificial insemination (AI) was 98% ($n = 294$). Among the examined calves born through in vivo multiple ovulation embryo transfer (MOET) ($n = 66$), 95.5% survived until weaning, as shown by the Kaplan-Meier curve in Fig. 3.

Considering mortality among AI-born calves ($n = 5$), the first 40 days were critical, with losses recorded at $d = 6$, $d = 13$, $d = 28$, $d = 33$ and $d = 39$. Additionally, one mortality occurred at the end of the feeding period ($d = 70$). For MOET-born calves ($n = 3$), no particularly critical

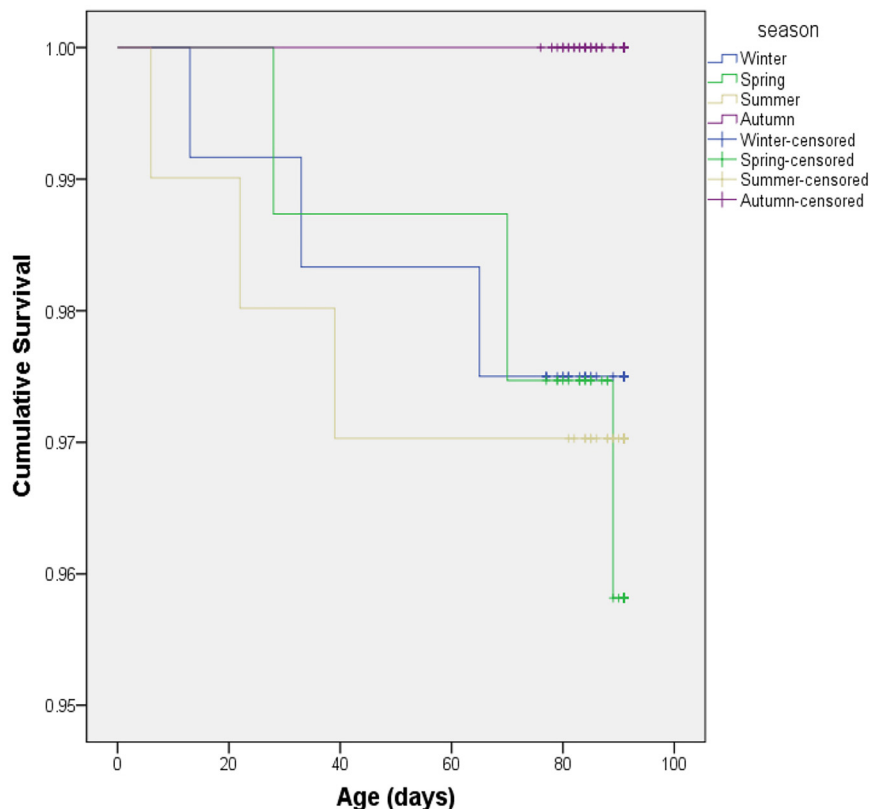


Fig. 1. Kaplan-Meier curve showing the cumulative survival of Holstein-Friesian heifer calves born in 2023 by season (winter, spring, summer, autumn) until weaning (91 days)

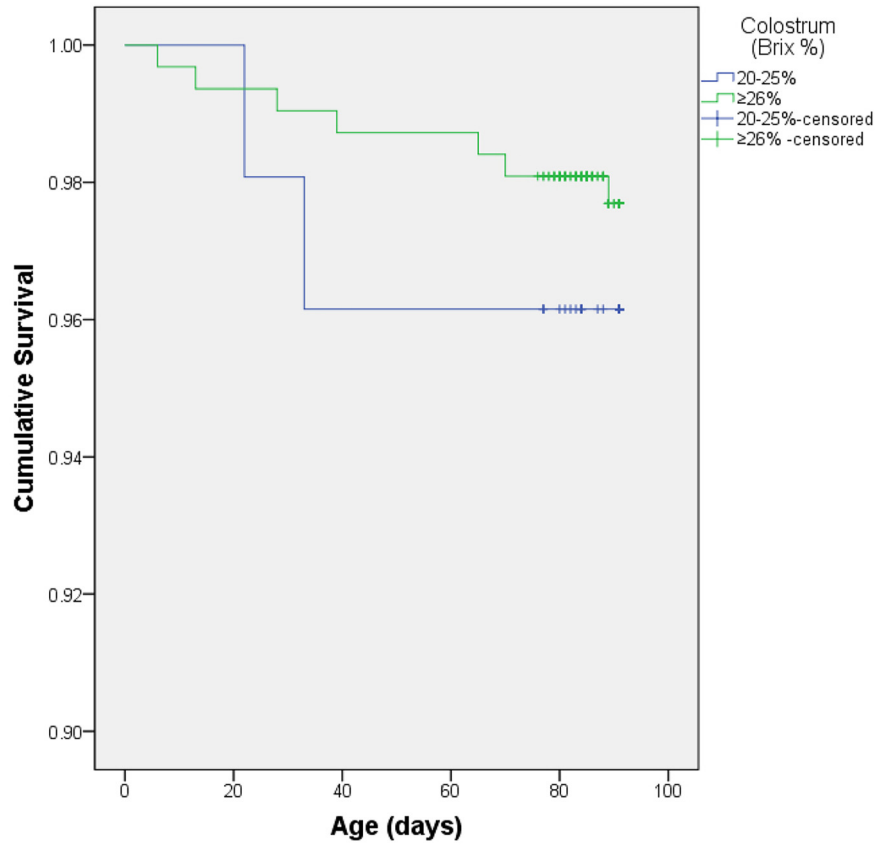


Fig. 2. The effect of different quality colostrum on the survival of Holstein-Friesian calves

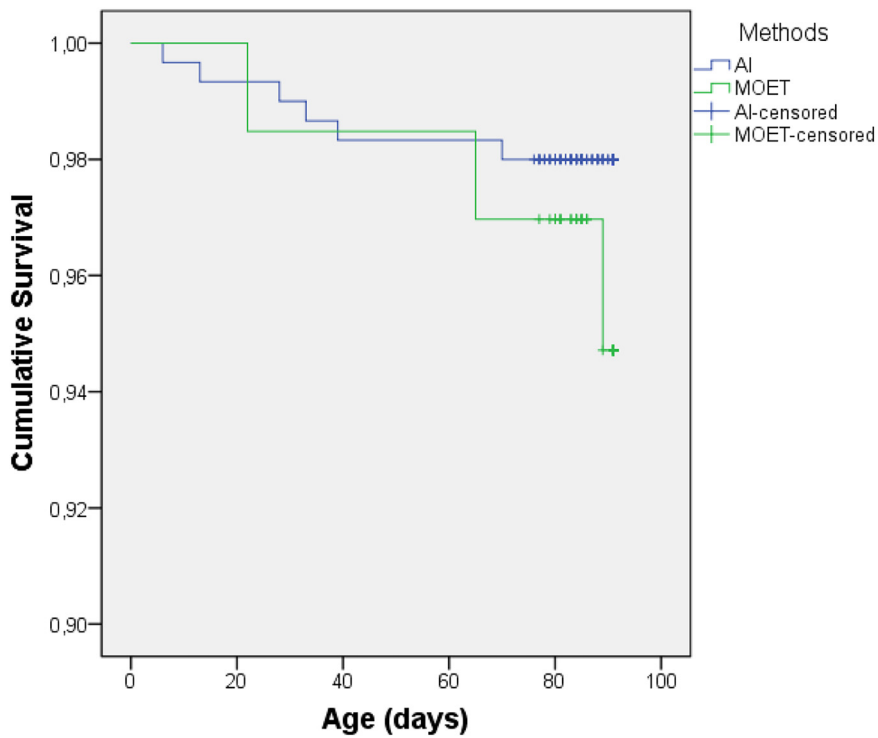


Fig. 3. Kaplan-Meier curve illustrating the cumulative survival of Holstein-Friesian heifer calves born via embryo transfer (MOET) and artificial insemination (AI) in 2023 until 91 days of age

period was identified during the feeding phase, with losses occurring at $d = 22$, $d = 65$, and $d = 89$. There was no statistically significant difference ($P > 0.05$) in mortality between the in vivo MOET and AI groups.

Birth weight and morbidity

No significant difference ($P > 0.05$) was found between the two groups in terms of birth weight (Table 4). Birth weight had no impact on subsequent morbidity or survival in either AI- or MOET-born calves. Additionally, birth weight did not influence the number of later illnesses.

The morbidity analysis of the study population ($n = 366$) revealed that 71.6% ($n = 262$) of the calves remained healthy throughout the observation period without requiring medical intervention (Fig. 4). Conversely, 28.4% ($n = 104$) of the calves had at least one illness episode necessitating veterinary treatment. Splitting this group, 24.9% ($n = 91$) experienced a single episode, while 3.3% ($n = 12$) had two separate

instances of illness. Only one calf (0.3%) required treatment for three distinct episodes during the 91-day period.

When comparing morbidity rates by reproductive technique, 63.6% ($n = 42$) of the 66 MOET-born calves remained healthy, while 30.3% ($n = 20$) required antibiotic treatment for a single illness episode. Only 6.1% ($n = 4$) of the MOET-born calves experienced two illness episodes and none had more than two.

Among the 300 AI-born calves, 73.3% ($n = 220$) showed no signs of illness, while 23.7% ($n = 71$) required treatment for a single illness episode. Additionally, 2.7% ($n = 8$) experienced two separate illness episodes and 0.3% ($n = 1$) required antibiotic therapy for three illness episodes.

Although morbidity rates were slightly higher among MOET-born calves compared to AI-born calves, severe cases remained rare in both groups. These findings suggest that while MOET-born calves may be more susceptible to illness, the overall impact on calf survival and health outcomes was minimal.

The most common causes of mortality included respiratory diseases, general weakness and gastrointestinal infections (Hossain et al., 2013; Selvan et al., 2019). We specifically examined the two most frequent illnesses (respiratory disease and diarrhoea) across both groups (MOET and AI).

Among AI-born calves, diarrhoea occurred in 48 cases (16%) while respiratory illness was reported in 10 cases (3%) during the feeding period.

Among MOET-born calves, diarrhoea was observed in 13 cases (20%) and respiratory illness was recorded in 8 cases (12%) (Fig. 5).

The prevalence of diarrhoeal disease did not differ significantly ($P > 0.05$) between calves born from multiple ovulation embryo transfer (MOET) and those born via

Table 4. The effect of birth weight on the frequency of later illnesses

AI calves birth weight (kg)				
	N	Mean	Minimum	Maximum
0	208	38.73	26.0	53.0
1	65	37.76	27.0	49.0
2	8	36.88	26.0	44.0
Total	281	38.46	26.0	53.0
MOET calves birth weight (kg)				
	N	Mean	Minimum	Maximum
0	41	38.26	28.0	45.0
1	19	37.76	32.0	50.0
2	4	38.75	32.0	45.0
Total	64	38.14	28.0	50.0

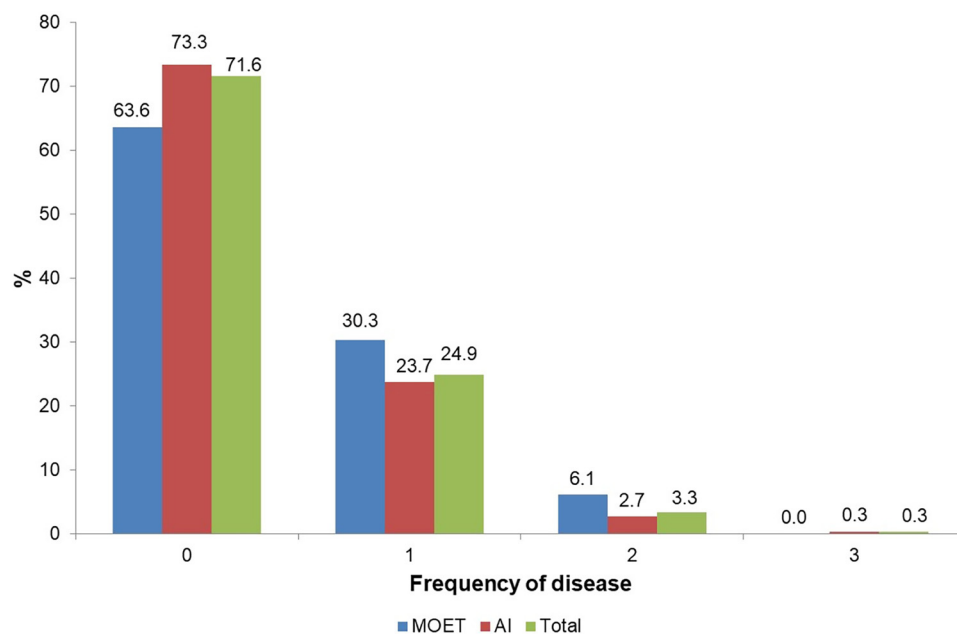


Fig. 4. Frequency of diseases in Holstein-Friesian heifer calves born in 2023, categorised by reproductive technique (MOET, AI)

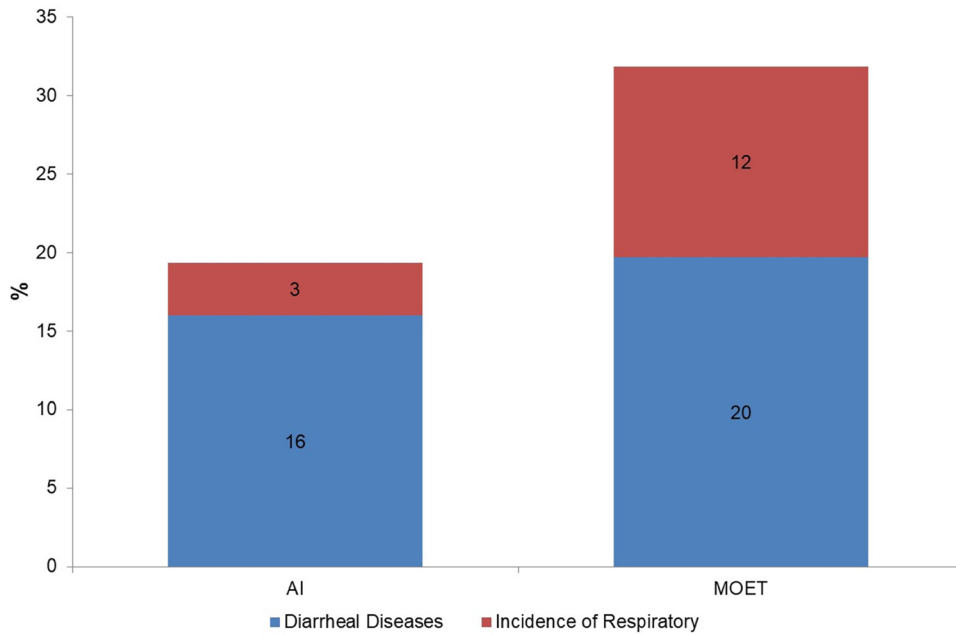


Fig. 5. Incidence of respiratory and diarrheal diseases in MOET- and AI-born Holstein-Friesian heifer calves

artificial insemination (AI) (Fig. 5). During the 91-day feeding period, 221 (74%) AI-born calves and 42 (64%) MOET-born calves remained asymptomatic.

Mild (Grade 1) alterations in faecal consistency were observed in 8 AI-born calves and 3 MOET-born calves (Table 2, Fig. 6). Moderate diarrhoea (Grade 2) was diagnosed in 31 AI-born calves (14%) and 9 MOET-born calves (10%). Severe diarrhoea (Grade 3), characterized by significant changes in faecal consistency, was observed in 8 AI-born calves and 1 MOET-born calf.

There was no statistically significant difference ($P > 0.05$) between the two groups regarding the verity or progression

of diarrhoeal disease. Similarly, the incidence of respiratory disease did not differ significantly ($P > 0.05$) between AI- and MOET-born calves. These findings suggest that reproductive technique does not appear to influence the verity or occurrence of gastrointestinal illnesses in Holstein-Friesian heifer calves (Fig. 7).

Mild (grade 1) respiratory illness was observed exclusively in the MOET group, affecting 2 calves (3%). Moderate (grade 2) respiratory disease occurred in 10 AI-born (3%) and 5 MOET-born (8%) calves during the rearing period ($d = 91$). Severe, irreversible (grade 3) respiratory disease was recorded in one MOET-born calf, which was later culled (Fig. 7).

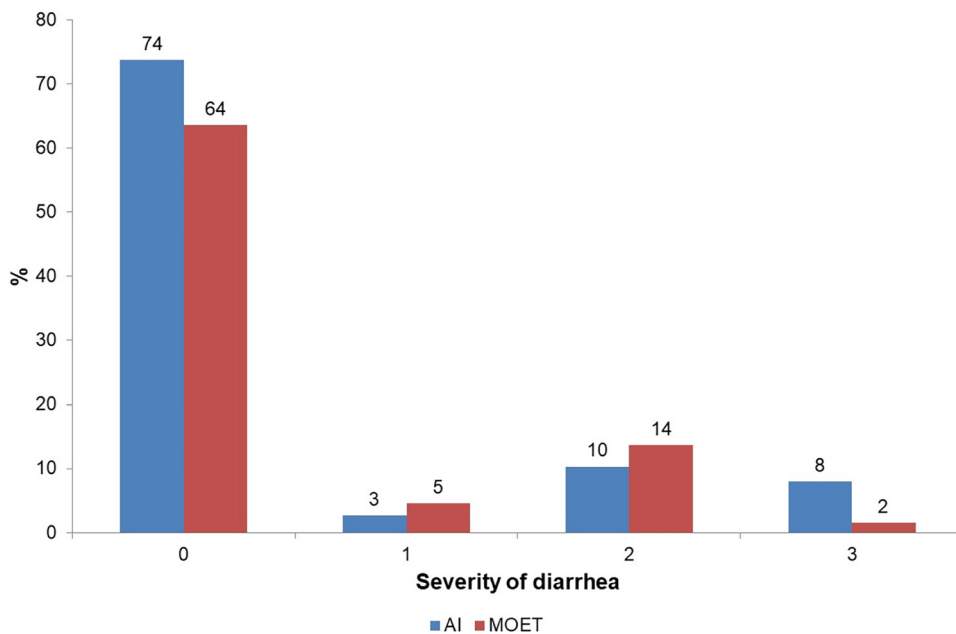


Fig. 6. Severity of diarrhoeal cases in AI- and MOET-born Holstein-Friesian heifer calves

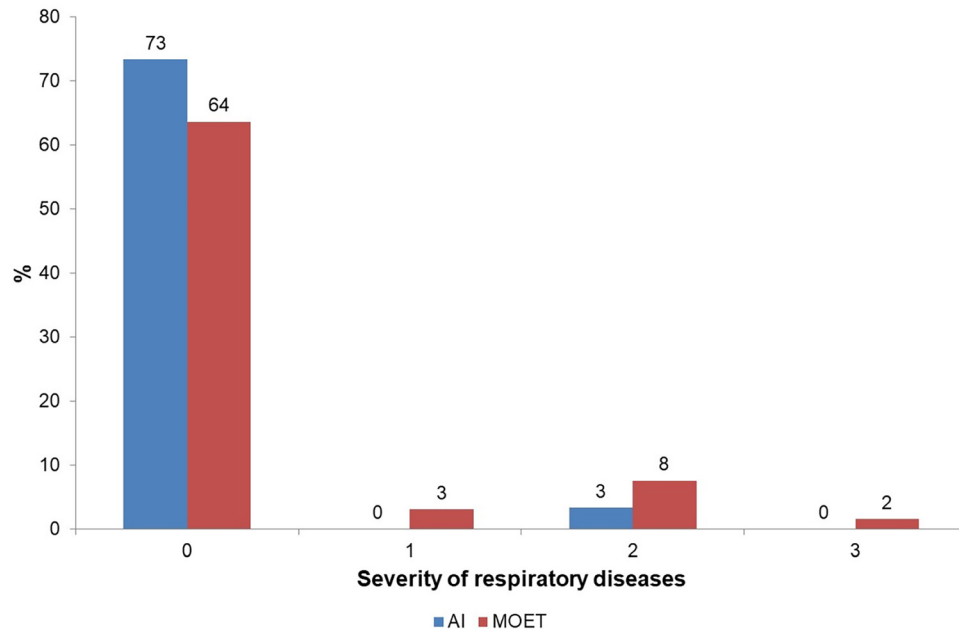


Fig. 7. Severity grading of respiratory diseases in AI and MOET Holstein-Friesian heifer calves

The majority of calves in both groups fell into the 0-severity category. However, more MOET-born calves were represented in the higher severity categories (grades 1, 2 and 3). The difference in severity distribution between the AI and MOET groups was statistically significant ($P = 0.001$). This suggests that MOET-born calves had a higher tendency for more severe respiratory illness than their AI-born counterparts.

All calves were weighed at weaning and their weights were compared with morbidity frequency (Table 5). Regarding AI-born calves, the number of illness episodes significantly affected weaning weight ($P < 0.05$). Even a single illness episode led to a measurable reduction in weaning weight ($P = 0.014$) and calves that required treatment two or more times showed the most pronounced weight differences compared to their healthy counterparts ($P < 0.05$).

In contrast, for MOET-born calves, morbidity did not have a statistically significant impact on weaning weight ($P > 0.05$). However, MOET calves treated twice or more showed weight differences compared to both healthy and single-episode cases, though this result was not statistically significant. Similar findings were reported by Donovan et al. (1998), who demonstrated the negative impact of illness on weight gain.

Table 5. Weaning weight (mean (\pm standard deviation)) by morbidity

Disease frequency	AI weight (kg)	MOET weight (kg)
0	129.06 \pm 20.619 ^a	124.10 \pm 20.564 ^a
1	120.72 \pm 19.568 ^b	121.45 \pm 19.399 ^a
2 \leq	111.50 \pm 14.938 ^b	101.75 \pm 3.500 ^a

^{a,b}: Different letters show significant ($P < 0.05$) differences

CONCLUSIONS

The findings of this study indicate that calf survival was significantly influenced by environmental factors, particularly seasonality and colostrum quality. Autumn-born calves exhibited a higher survival rate, potentially due to more favourable climatic conditions, which may reduce pathogen load and improve thermoregulation in neonatal calves. Furthermore, colostrum quality emerged as a critical determinant of survival, as calves receiving high-quality colostrum (>25% IgG concentration) demonstrated superior resilience against early-life diseases. These findings align with previous research (Donovan et al., 1998), emphasising the pivotal role of passive immunity in neonatal health.

In contrast, reproductive technologies (AI vs. MOET) did not exert a statistically significant impact on overall survival rates. However, morbidity patterns differed between groups. While diarrhoeal disease prevalence was similar across AI- and MOET-born calves, respiratory disease occurrence was significantly higher in the MOET group ($P = 0.001$). Additionally, MOET-born calves exhibited more severe respiratory symptoms, suggesting a potential underlying physiological or immunological difference between the groups.

The impact of illness on growth performance was also evident. In AI-born calves, even a single illness episode significantly reduced weaning weight ($P = 0.014$), with the most substantial impact observed in calves that experienced multiple illnesses ($P < 0.05$). However, in MOET-born calves, the effect of disease on weaning weight was not statistically significant, implying potential compensatory growth mechanisms or metabolic differences.

Overall, while reproductive technology did not directly influence survival or disease prevalence, MOET-born calves

exhibited greater susceptibility to respiratory disease, with a more severe disease course. This highlights the importance of environmental and management strategies in mitigating respiratory risks in MOET-born calves. These findings suggest that environmental conditions, passive immunity and health management play a greater role in calf viability than reproductive technology itself.

Declaration of interest: The authors declare that there is no conflict of interest.

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